PROTOCOL

Prevention of post mEniscectomy osteoarthritis: from new Animal model to patient pRofiLing (PEARL)

Protocol Code: PNRR-MAD-2022-12375978

Version No. (Date): V 2.0 13.12.2024

Sponsor (Institution): Humanitas Research Hospital - Via Manzoni 56, Rozzano (Milano), Italy

Coordinating Investigator: Elizaveta Kon - Phone: 0282247523 - e-mail: elizaveta.kon@humanitas.it

Confidentiality Statement
The information contained in this document, especially unpublished data, is provided to you in confidence as an investigator, potential investigator or consultant. It is understood that this information will not be disclosed to others than the applicable Competent Ethics Committee(s) and Regulatory Authority(ies) without written authorization from Istituto Clinico Humanitas except to the extent necessary to obtain informed consent from those who will participate in the study.

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COORDINATING INVESTIGATOR

I have approved this Protocol entitled "Prevention of post mEniscectomy osteoarthritis: from new Animal model to patient pRofiLing (PEARL)" and I agree to conduct the study as detailed herein and according to the current version of the World Medical Association Declaration of Helsinki, Good Clinical Practice guideline and applicable regulatory requirements. I will provide all study personnel under my supervision with all information needed to perform the study and I will inform them about their responsibilities and obligations.

Printed name	Elizaveta Kon
Role	PI
Department	Center of Functional and Biological reconstruction of the Knee, Humanitas Research Hospital, Milan, Italy
Signature	
Date	13/12/2024

STATISTICIAN

I have approved this Protocol entitled "Prevention of post mEniscectomy osteoarthritis: from new Animal model to patient pRofiLing (PEARL)"

Printed name	Emanuela Morenghi
Signature	
Date	13/12/2024

CENTRE SIGNATURE – PRINCIPAL INVESTIGATOR

I have read this Protocol Amendment relevant to the study entitled "Prevention of post mEniscectomy osteoarthritis: from new Animal model to patient pRofiLing (PEARL)" and I agree to conduct the study as detailed herein and in compliance with guidelines for Good Clinical Practice and applicable regulatory requirements. I will provide all study personnel under my supervision with all information provided by the Coordinating Investigator/Sponsor and I will inform them about their responsibilities and obligations.

Printed name	Elizaveta Kon
Role	PI
Department	Center of Functional and Biological reconstruction of the Knee, Humanitas Research Hospital, Milan, Italy
Signature	
Date	13/12/2024

History of substantial amendment

Protocol Sections	Starting Protocol Version	Amended Protocol Version	Reason for changes
Study assessment (section 7)	V 1.0 28/11/2024	V 2.0 13/12/2024	This change is done in order to make MRI procedures easier for both clinicians and patients of all the centres involved in the clinical study. This change does not modify the study's objectives, endpoint and statistical analysis described in the original version of the study protocol.
Case report forms (section 9.2)	V 1.0 28/11/2024	V 2.0 13/12/2024	The eCRF platform to be used as registry has been updated to REDCAP given that it is more customizable. This change does not modify the study's objectives, endpoint and statistical analysis described in the original version of the study protocol.

Abbreviations

CA	Competent Authority
EC	Ethics Committee
eCRF	electronic Case report form
GCP	Good Clinical Practice
OA	Osteoarthritis

1. SUMMARY

Study title		Prevention of post meniscectomy osteoarthritis: from new animal model to patient profiling	
Study code	PNRR-MAD-2022-12375978	**Acronym**	PEARL
Version and date		V 2.0 13.12.2024	
Sponsor (Institution)		Humanitas Research Hospital - Via Manzoni 56, Rozzano (Milano), Italy	
Coordinating Investigator		Elizaveta Kon - Phone: 0282247523 - e-mail: elizaveta.kon@humanitas.it	
Study indication		Degenerative meniscus and osteoarthritis of the knee	
Study population		100 patients affected by degenerative meniscal lesion	
Background and rationale		Degenerative meniscal tears are the most common type of meniscal lesion and their key role in knee osteoarthritis induction and progression has been extensively demonstrated. Huge efforts have been made to identify therapeutic strategies able to slow down if not reverse the progression from meniscal degeneration to knee osteoarthritis, but we believe that one problem still needs to be addressed: the lack of evidence of clinical, morphological, radiological parameters and miRNA markers linked to different degrees of risk of OA progression after meniscectomy. Having identified this deficient area, our project is aimed to the identification of potential markers of patients with a high risk of OA progression after meniscectomy.	
Study objectives		The objective of the study is to describe the morphological, clinical and mirnomic characteristics of patients treated for degenerative meniscal lesions according to the current standard of care. The completely characterized cohort of patients enrolled in the study for which clinical, imaging, morphological	

	assessment and high-throughput transcriptomic analysis will be used to generate a unique registry (PEARL) for this specific patients population and will be used to evaluate potential specific pre-treatment markers and risk factors related to negative outcome in the long term.
Study endpoints/outcomes	The PEARL Patient registry will allow monitoring the possible progression of post-meniscectomy OA and to define specific pre-treatment markers and risk factors related this negative outcome. In particular, the presence of this registry will permit evaluating the onset of post-meniscectomy OA not only within the study period, but it will represent an asset for long term evaluations and monitoring. In the long term, above the study time limit, this tool will allow to develop an algorithm for the discrimination of patients more likely to suffer from postmeniscectomy OA and therefore to help in the therapeutic choice by reinforcing the indication of meniscectomy in a patient with a non-progressing profile or, on the other hand, suggesting to switch to a different, possibly non-surgical treatment in a patient with an OA progressing profile.
Study design	Data collection from patients included in the observational clinical study will be achieved by the following activities: A total of 100 patients with degenerative meniscal tears will be enrolled in the observational study and treated according to the current standard of care which consists in an arthroscopic partial meniscectomy in all the three clinical operative Unit involved in the project. Demographic, anamnestic and clinical data (age, gender, BMI, habits, co-morbidities, pharmacological treatments) of the patients included in the observational clinical study will be collected. Clinical routine assessments will be performed before surgery to assess the overall condition of the joint. A morphological profiling based on the MRI will be performed on patients prior or after the surgery since no change in knee morphology is expected to occur. Clinical routine assessments will be performed also after treatment to check for possible joint changes after the surgery. Before surgery, peripheral blood samples from each patient enrolled in the observational study will be collected. Total blood count will be registered and

	included in the set of the patients' data. Total RNA will be purified from the blood samples and integrity of each RNA sample will be evaluated to esclude possible degradation during the procedure. Total RNA will be preamplified to perform the qRT-PCR-based analysis sifting a well-described set of 754 miRNAs panel known for their involvement in several joint and blood cell types' homeostasis and activation. The most abundantly expressed and relevant miRNAs will be identified in the whole set of samples and included in the set of the patients' data. Combination and interpretation of the clinical, morphological, imaging and biomarker -omics data will follow in order to identify the patient characteristics that are more associated to the development of post-meniscectomy OA, following these steps: A patient registry (PEARL registry) containing all the anamnestic, clinical, radiological and morphological data, as well as the blood count and the most relevant and abundant miRNAs obtained through the transcriptomic analyses carried out during the study, will be generated. Patients will be clinically and radiologically monitored at short- and long-term to evaluate the possible onset of OA post-meniscectomy. Any possible relevant clinical data and adverse events encountered during the patient's clinical path will be tracked. Patients will be assigned to categories based on the PEARL registry data and their clinical outcome post-meniscectomy. This will help identifying possible risk factors and pre-treatment markers associated to the susceptibility of patients to develop post-meniscectomy OA. An algorithm that will use the information gathered in the previous steps will be developed. This tool will allow to identify patients more likely to suffer from post-meniscectomy OA and therefore to help in the therapeutic choice. The constant updating of the register over time beyond the project duration will allow to obtain increasingly reliable and consolidated information to further strength the predictivity of the algorithm for choosing the best treatment based on the patients' characteristics.
Intervention	- Screening visit: Clinical scores (KOOS, IKDC, VAS, EQ-5D5L), MRI of the index knee + X-ray of the index knee and X-ray of the pelvis and lower limbs (optional), orthopedic examination

	- Surgery day: Blood tests + arthroscopic partial meniscectomy - 6 months follow-up visit: Clinical scores (KOOS, IKDC, VAS, EQ-5D5L), MRI of the index knee + X-ray of the index knee and X-ray of the pelvis and lower limbs -12 months follow-up visit: Clinical scores (KOOS, IKDC, VAS, EQ-5D5L), orthopedic examination
Eligibility criteria	**INCLUSION CRITERIA** 1. Signed Informed Consent Form; 2. Male or female patients ≥18 years of age at time of screening; 3. Patients physically and mentally able to comply with all aspects of the study, including the requirements for follow-up visits; 4. Patients suffering from symptomatic medial and/or lateral degenerative meniscal lesion identified at MRI with a surgical indication of arthroscopic partial meniscectomy; 5. In case of bilateral degenerative meniscus, patients with no or mild pain in the contralateral knee, defined as a score < 2 on a 0-10 numerical rating scale (NRS); 6. Patients who, before the last 3 months, have undergone and failed at least one prior conservative OA treatment (NSAIDs, physiotherapy, hydrokinesitherapy); 7. Body mass index (BMI) ≤ 40 kg/m2 **EXCLUSION CRITERIA** 1. Presence of clinically observed active infection in the index knee joint or skin disease/breakdown or infection in the area of the planned injection site of the index knee; 2. Patients who experienced traumatic injury at the index knee within 6 months prior to the procedure; 3. Patients with a history of invasive malignancies (except non- melanoma skin cancer), unless treated with curative intent and with no clinical signs or symptoms of the malignancy for 5 years; 4. Patients with known systemic disorders or any systemic inflammatory condition such as rheumatoid arthritis; 5. Patients with known metabolic bone diseases such as those affecting calcium metabolism; 6. Patients with a BMI greater than 40 kg/m2; 7. Patients who abuse of the following substances: alcohol, recreational drugs; 8. Major surgery (e.g. osteotomy) of the index knee within 12 months prior to screening;

	9. Minor surgery (e.g. shaving or arthroscopy) of the index knee within 6 months prior to screening; 10. Use of systemic immunosuppressants within 6 weeks prior to screening; 11. Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and treatment effects of the study injection.
Study procedures	Patient demographic, anamnestic, clinical, radiological and morphological data will be collected and reported in the database PEARL, assigning an individual ID to each patient. The most relevant and abundant miRNAs obtained through the transcriptomic analyses will be included too. Information about age, gender, body mass index, type of meniscal lesion, type of injury (if any), time of symptoms onset, general knee condition, previous surgical and/or pharmacological treatments, co-morbidities will be included too. Subjective knee evaluations of patients will be collected by using internationally accepted PROMs (Patient Reported Outcome Measures) among which IKDC (International Knee Documentation Committee) Objective Score, KOOS (Knee Injury and Osteoarthritis Outcome Score), VAS (Visual Analogue Score) Pain and EQ-5D-5L. All these data will be collected during the pre-surgical visit and on follow up. Two MRIs are planned for the study, during screening visit and at 6 months follow-up visit; at least one of the two MRIs will be performed with a 3D protocol to assess the bone component. Therefore,radiological and morphological data will include standard x-ray and MRI protocol implemented by metadata of 3D MRI protocol (near-isotropic 0.83mm T2 SPACE and near-isotropic 0.63mm proton density SPACE) when needed. Accurate 3D models of femur bone and cartilage, tibia bone and cartilage and menisci will be reconstructed from (near-)isotropic high-resolution 3D MRI scans. For morphology analysis, a 3D DESS MRI sequence will be complemented with modern clinical sequences for diagnostic purposes. Using the reconstructed 3D models, advanced landmark-based and statistical shape analyses will be performed. The extracted shape features from these analyses will then serve as input for conventional statistical algorithms or state-of-the-art machine learning techniques. Finally, the addition of standing full-leg radiographs gives more insights in

	the biomechanics of the complete lower-limb from hip to toe. Alignment of the femur with respect to the tibia will be evaluated in both coronal and sagittal plane. These alignment variables will then be used as input for conventional statistics and machine learning algorithms both separately and combined with the knee-specific shape features (cf. previous paragraph). Data related to the high-throughput transcriptomic analyses will include the most expressed blood miRNAs for each patients. Data will be generated on the Applied Biosystems' QuantStudio' 12K Flex RealTime PCR System using Expression Suite Software. Quantitation will be assessed by the 'relative threshold' method (Crt method) that sets a threshold for each amplification individually that is based on the shape of the amplification curve. Data normalization will be performed with RefFinder tool that evaluates and screens stable candidates from extensive experimental datasets. After publication, the raw data related to in vitro analyses as well as pre-clinical and clinical anonimazed data will be made available through an open access database.
Number of patients (planned)	100 patients
Investigational sites (planned)	Single-centre study □ Multi-centre study X 3 Italian centers involved: • IRCCS Istituto Clinico Humanitas - Centro per la ricostruzione funzionale e biologica del ginocchio (ORT3) - Via Manzoni, 56, 20089 – Rozzano (MI) • IRCCS Ospedale Galeazzi-Sant'Ambrogio - Via Cristina Belgioioso, 173, 20157 - Milano (MI) • Azienda ospedaliero-universitaria di Sassari, Viale S. Pietro, 43/B, 07100, Sassari (SS)
Sample sizes and considerations	Sample size has been determined based on the high-throughput miRNomic experiments. A model developed by MD Anderson Cancer Center of the University of Texas was used to estimate the sample size per group needed to identify micro-RNAs differentially expressed in patients who develop post-meniscectomy OA and those who did not. The model assumes equal variances between groups and log-normal distribution per each transcript. A total of 754 transcripts will be tested using micro-array based experiments. The acceptable number of false positive

	was set at 1, with a desired fold change of 2 between transcripts in the different groups, and power equal to 99%. A standard deviation of 0.7 was considered realistic for transcripts that are expressed at moderate levels. These parameters allowed to estimate that 31 sample (patients) per group would be required. Given the hypothesis that 40% of patients do not develop post-meniscectomy OA, to observe a minimum of 31 subjects in the least represented group with a probability of 97.5%, a total of 100 patients should be included in the study according to the binomial distribution.
Study timetable	The timing of analysis data of the study will be as follows: 1) Enrollment and treatment of 100 patients with symptomatic degenerative meniscal lesions undergoing partial arthroscopic meniscectomy: 0-12 months. 2) Transcriptomic analysis will be conducted on blood samples collected at the time of surgery from the patients enrolled in the observational clinical study. The final data will be available within six months after the last enrollment. 3)Follow up evaluation: all the patients will be evaluated at 6 and 12 months. The PEARL registry will permit to eventually clinically and radiologically evaluate the patients also beyond the project duration to monitor the possible onset of post-meniscectomy OA also beyond the one year time limit. Their evaluation after the end of the project will strengthen the findings of the study with longer-term evaluation.
GCP statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki and applicable guidelines as well as all national legal and regulatory requirements.

2. STUDY FLOW-CHART

			Follow up	
	VISIT 1 Screening	**VISIT 2 baseline**	**VISIT 3**	**VISIT 4**
	within 90	Day 0	6 months	12 months

	days prior Visit 2			
Interval windows			± 4 weeks	± 4 weeks
Signed ICF	X			
Eligibility	X	X		
Demographics	X			
Weight, height	X			
Physical exam	X		X	X
Medical history	X			
Prior and concomitant treatments and medications	X	X	X	X
Questionnaires	X		X	X
Knee assessment	X		X	X
X-Ray	X*		X	
MRI	X*		X	
Surgical operation		X		
Blood sampling		X		
Adverse events	X	X	X	X

*: optional; at least one MRI (either at V1 or at V3) should be implement with 3D MRI sequences

3. BACKGROUND

Degenerative meniscal tears are the most common type of meniscal lesions, consisting of nearly 30% of all tears with a peak incidence in patients aged 41 to 70 years. It has been reported that a third of asymptomatic patients aged 50 to 59 years and 56% of patients aged 60 to 90 years had a meniscal tear on MRI.
The role of meniscal degeneration in knee osteoarthritis onset and progression is well established and demonstrated by several retrospective studies. Till now, there is a complete lack of knowledge on why some patients quickly develop postmeniscectomy OA and other patients are clinically silent for many years. Preliminary studies have demonstrated that the knee morphotype might be critical for the post-meniscectomy OA development and progression, but no biological markers that can predict the OA progression have been identified so far.

4. RATIONALE

Degenerative meniscal lesions are a common finding in many patients, even young adults. They are mainly treated surgically, and a relevant percentage of patients develop post-meniscectomy osteoarthritis. Up to date, there is a complete lack of knowledge about the reason why some patients quickly develop post-meniscectomy OA and other patients are clinically silent for many years. We believe that the reasons why some patients remain asymptomatic after meniscectomy and others experience the onset and progression of OA rely on a combination of different parameters, among which morphotype and biological predisposition play the main role and could therefore be used as predictors of outcome. Thus, the hypothesis of this project relies on the possibility to find specific characteristics and biomarkers allowing to discriminate between patients with "progressing" and "non-progressing" profile towards post-meniscectomy OA. Allocating patients with degenerative meniscal lesions into a specific patient profile would improve the indications for their treatment, knowing in advance which patients are at risk to develop post-meniscectomy OA before the surgery is performed. In this regard, the generation of a specific registry of patients that includes, beyond the conventional

parameters, the morphotypes and the miRNA profile, has to be seen as a powerful tool to greatly improve the knowledge in this field and to reduce unnecessary and not effective treatments with the evident ethical and economic advantages. The significance of the project is reinforced not only by the data that will be collected within the study period but also by the possibility to continue exploiting the registry data to constantly update outcome measures and thus improving the predictive power of the PEARL project.

5. STUDY OBJECTIVES

5.1 Objectives

The objective of the study is to describe the morphological, clinical and mirnomic characteristics of patients treated for degenerative meniscal lesions according to the current standard of care.
The clinical, radiological and morphological parameters will be collected for all the patients. In particular, anamnestic data (age, gender, BMI, habits, co-morbidities, pharmacological treatments) and radiological data (X-ray and MRI) will be collected. Preliminary studies have demonstrated that specific knee morphotypes might be important for the post- meniscectomy OA development and progression, but definite evidence is still not available.
In addition, blood samples for each patient will be harvested and analyzed by high-throughput transcriptomic techniques. In particular, the analysis will be addressed to identify the miRNAs profile that represents the most innovative and detailed approach to discover new possible diagnostic markers.
Isolation of miRNAs from blood samples will be performed with commercially available kits according to the manufacturer's protocol. miRNAs quality and integrity will be assessed and they will be analyzed after reverse transcription (RT) and preamplification, followed by real-time RT-PCR analysis with the QuantStudio OpenArray® Platform (Applied Biosystem).
The completely characterized cohort of patients enrolled in the study for which clinical, imaging, morphological assessment and high-throughput transcriptomic analysis will be available, will be used to generate a unique registry for this specific patients' population (PEARL Patient Registry). A patient registry is an organized system that use observational methods to collect uniform data on a population defined by a particular disease and/or treatment and that is followed over time. In our case, the PEARL Patient registry will allow monitoring the possible progression of post-meniscectomy OA and to possibly identify and describe specific pre-treatment markers linked to a higher frequency of post-meniscectomy OA onset. Further future evaluation and analysis, beyond the specific study objectives, will evaluate if those markers are actually to be considered as risk factors of post-meniscectomy OA onset and progression.
In particular, the presence of this registry will permit evaluating the onset of post-meniscectomy OA not only within the study period, but it will represent an asset for long term evaluations and monitoring.
In the long term, this tool will allow to develop an algorithm for the discrimination of patients more likely to suffer from post- meniscectomy OA and therefore to help in the therapeutic choice by reinforcing the indication of meniscectomy in a patient with a non-progressing profile or, on the other hand, suggesting to switch to a different, possibly non-surgical treatment in a patient with a progressing profile.

- ***5.1.1 Primary objective***

To monitor the possible progression of post-meniscectomy OA one year after the treatment, describing the morphological, clinical and miRNA profile of patients undergoing arthroscopic meniscectomy for degenerative meniscal lesions.

- ***5.1.2 Secondary objectives***

1) To develop the PEARL Patient registry
2) To define specific pre-treatment markers and risk factors related to negative outcome in the long term.

6. STUDY POPULATION

6.1 Inclusion criteria

100 subjects fulfilling all of the following inclusion criteria are eligible for the study:

1. Signed Informed Consent Form;
2. Male or female patients ≥18 years of age at time of screening;
3. Patients physically and mentally able to comply with all aspects of the study, including the requirements for follow-up visits;
4. Patients suffering from symptomatic medial and/or lateral degenerative meniscal lesion identified at MRI with a surgical indication of arthroscopic partial meniscectomy;
5. In case of bilateral degenerative meniscus, patients with no or mild pain in the contralateral knee, defined as a score < 2 on a 0-10 numerical rating scale (NRS);
6. Patients who, before the last 3 months, have undergone and failed at least one prior conservative OA treatment (NSAIDs, physiotherapy, hydrokinesitherapy);
7. Body mass index (BMI) ≤ 40 kg/m2

6.2 Exclusion criteria

The presence of any one of the following exclusion criteria will lead to the exclusion of the subject:

1. Presence of clinically observed active infection in the index knee joint or skin disease/breakdown or infection in the area of the planned injection site of the index knee
2. Patients who experienced traumatic injury at the index knee within 6 months prior to the procedure;
3. Patients with a history of invasive malignancies (except non- melanoma skin cancer), unless treated with curative intent and with no clinical signs or symptoms of the malignancy for 5 years;
4. Patients with known systemic disorders or any systemic inflammatory condition such as rheumatoid arthritis;
5. Patients with known metabolic bone diseases such as those affecting calcium metabolism;
6. Patients with a BMI greater than 40 kg/m2,
7. Patients who abuse of the following substances: alcohol, recreational drugs;
8. Major surgery (e.g. osteotomy) of the index knee within 12 months prior to screening;
9. Minor surgery (e.g. shaving or arthroscopy) of the index knee within 6 months prior to screening;
10. Use of systemic immunosuppressants within 6 weeks prior to screening;
11. Any documented clinically significant degree of cognitive impairment or other condition, finding, or psychiatric illness at screening which, in the opinion of the investigator, could compromise patient safety or interfere with the assessment of the safety and treatment effects of the study injection;

6.3 Recruitment

Patients diagnosed with symptomatic degenerative meniscal lesions will be preselected for the study and then addressed to the screening visit by experienced orthopaedic surgeons from the three centers involved in the study. At the screening visit the eligible patients will be subjected to inclusion and exclusion criteria

evaluation and, if still eligible, they will be eventually proposed to take part to the observational clinical study.

7. STUDY ASSESSMENT

At the screening visit, the investigator will inform the patient about the study and all the trial procedures. Patient will be given the information sheet to read and will have time to ask questions on the study. Patient will decide freely whether to participate or not. If he decides to participate, he will be asked to sign the ICF. The original signed ICF will be retained in the investigator site file and a copy in original will be provided to the participant. Individuals will be free to decline further participation without giving reasons.

The investigator will ensure that each participant meets all the inclusion and exclusion criteria. During this visit that should be performed within 90 days prior baseline visit, the investigator will:
1. Assign Patient Number after informed consent signature
2. Collect patient's demographic characteristics, weight and height
3. Perform physical examination
4. Record Medical history
5. Record prior and concomitant treatments and medications
6. Perform MRI and X-ray of the index knee and full weight-bearing X-Rays of the lower limbs (optional)
7. Administer to the patient the following questionnaires

I. KOOS subscales
II. IKDC questionnaire
III. NRS score
IV. EQ5D5L

8. Determine eligibility according to inclusion/exclusion criteria
9. Record any AE starting from the informed consent signature

In case of bilateral symptomatic knees, it is recommended that the most symptomatic knee be chosen for treatment in the study, as long as the inclusion/exclusion criteria are met. The knee that does not enter the study can be treated according to general practice.
If during Visit 2 (Baseline Visit/Procedure Visit (arthroscopy), the knee chosen is found to not meet the inclusion/exclusion criteria, the patient will be determined to be intra-operatively ineligible and not be included in this study.

7.1 Screening Visit

Screening visit: orthopaedic visit,clinical scores (KOOS, IKDC, VAS, EQ-5D5L), MRI of the index knee + X-ray of the index knee and X-ray of the pelvis and lower limbs (optional).

7.2 Baseline

Meniscectomy surgery and collection of blood samples

7.3 Follow Up

Follow-up at 6 months: orthopaedic visit, clinical scores (KOOS, IKDC, VAS, EQ-5D5L), MRI of the index knee + X-ray of the index knee and X-ray of the pelvis and lower limbs

7.3 Follow Up

Follow-up at 12 months: orthopaedic visit, clinical scores (KOOS, IKDC, VAS, EQ-5D5L)

8. STATISTICAL CONSIDERATIONS

8.1 Sample size

Sample size has been determined based on the high-throughput miRNomic experiments. A model developed by MD Anderson Cancer Center of the University of Texas was used to estimate the sample size per group needed to identify micro-RNAs differentially expressed in patients who develop post-meniscectomy OA and those who did not. The model assumes equal variances between groups and log-normal distribution per each transcript. A total of 754 transcripts will be tested using micro-array based experiments. The acceptable number of false positive was set at 1, with a desired fold change of 2 between transcripts in the different groups, and power equal to 99%. A standard deviation of 0.7 was considered realistic for transcripts that are expressed at moderate levels. These parameters allowed to estimate that 31 sample (patients) per group would be required. Given the hypothesis that 40% of patients do not develop post-meniscectomy OA, to observe a minimum of 31 subjects in the least represented group with a probability of 97.5%, a total of 100 patients should be included in the study according to the binomial distribution.

8.2 Analysis

Normal distribution of continuous variables will be tested by Shapiro-Wilk test. According to the result of this test, the comparisons between groups will be performed using parametric (Student t test, one-way ANOVA models) or nonparametric tests (Mann-Whitney, Wilcoxon test, Kruskal-Wallis test). Two-way ANOVA models will be used to test the contemporary effect of two variables on the parameters of interest. Differences in categorical variables will be tested by Fisher's exact test or Chi-squared test if appropriate. Appropriate regression models will be selected to test the influence of multiple covariates on continuous variables of interest. As well, logistic regression models will be developed to test the influence of several covariates on dichotomous variables. Multilevel models will be used to take into account within patient correlation in repeated measures analysis. Concerning the miRNomics analysis, the Expression Suite Software (Thermo Fisher Scientific) will be used to process miRNA expression data. CRT values > 28 will be considered as absence of amplification. The global mean of shared EV-miRNAs will be used to normalize expression data. miRNA abundance between samples will be determined using relative quantification 2 delta CRT. P values <0.05 will be considered statistically significant.

9. QUALITY ASSURANCE AND CONTROL

Quality Assurance and Quality Control systems based on written SOPs are in place at the Sponsor site.

9.1 Data handling and record keeping / archiving

The investigator must keep the documents on file for at least 7 years after completion or discontinuation of the study. After that period, the documents may be destroyed, subject to local regulations. Before proceeding to documents' destruction, sites must inform the Coordinating Investigator/delegate in writing.
Should the investigator wish to assign the study records to another party or move them to another location, the Coordinating Investigator/delegate must be notified in advance.

9.2 Case Report Forms

After having signed the informed consent form (ICF) and privacy form, patients' clinical, demographic and radiological data will be recorded during each visit in the visit report. Any data relevant to the study protocol will be recorded in REDCAP specifically designed from IT department of ICH, which are protected by a password and who will be accessed only by study staff.
The investigator will ensure the accuracy, completeness and timeliness of the data reported in the files. Appropriate coded identification will be used.

9.3 Source documents

Source data will be available at the site to document the existence of the study participants. Source data include the original documents relating to the study, as well as the medical treatment and medical history of the participant.

10. CONFIDENTIALITY OF PATIENT RECORDS

The investigator assures that patients' anonymity should be maintained and that their identities are protected from unauthorized parties. Particular attention should be paid whenever patient data are supplied to third parties and may be autonomously processed.
The investigator should keep in a confidential way a patient identification log recording both patient code and name. The investigator should also maintain patients' written consent forms, in strict confidence (i.e. not for submission to the Coordinating Investigator).
Any investigator and/or research staff member who has a conflict of interest with this study (such as patent ownership, royalties, or financial gain greater than the minimum allowable by their institution) must fully disclose the nature of the conflict of interest.

11. ETHICAL CONSIDERATIONS

The responsible investigator ensures that this study is conducted in agreement with this protocol, the Good Clinical Practice, the current version of Declaration of Helsinki and the applicable regulations.
The protocol and any amendments are subject to review and approval by the competent Independent Ethics Committee(s) ("IEC").

12. INFORMED CONSENT

All patients should be informed of the aims of the study. They should be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for study purposes by authorized individuals other than their treating physician.
It should be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she wants. This does not prejudice the patient's subsequent care. Documented informed consent must be obtained for all patients included in the study before any study related procedure is performed. The written informed consent form should be signed and personally dated by the patient or by the patient's legally acceptable representative, and by the Investigator who has provided the study information.

13. DATA OWNERSHIP

Istituto Clinico Humanitas is the owner of the data resulting from the study. All centers and investigators participating in the study should be made aware of such circumstance and not to disseminate information or data without the prior written consent by Istituto Clinico Humanitas.

14. PUBLICATION POLICY

After completion of the study, the Coordinating Investigator prepares a draft manuscript containing final results of the study on the basis of the statistical analysis. The manuscript is delivered to the co-authors for comments and then sent to a scientific journal for publication.
All publications, abstracts, presentations, manuscripts and slides - issued by the Investigators of the collaborative sites and including data from the present study- should be submitted to and reviewed by the Coordinator Investigator at least 3 (three) weeks in advance the planned date for the submission to the scientific journal.

15. FUNDING AND SUPPORT

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