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Subclinical *Propionibacterium acnes* Infection Estimation in the Intervertebral Disc (SPInE-ID): Protocol for a Prospective Cohort

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ABSTRACT

Introduction

Low back pain is a frequent condition in the population, as well as vertebral endplates abnormalities. Subclinical infection caused by low-virulence pathogen can possibly lead to vertebral endplate abnormalities, detected in magnetic resonance imaging (MRI) studies, and differentiation between infection and endplate changes may be difficult. Subclinical infection of the disc can also be associated with increasing low back pain and Propionibacterium acnes has been related to sciatica. Main purpose of this study is to identify if the presence of an infection pathogen in the intervertebral disc is real or if it is intraoperative contamination, and if it correlates to endplate abnormalities.

Methods and analysis

An open prospective cohort study will be performed at a single center. Subjects between 18 and 65 years of age; both genders; with diagnose of lumbar disc herniation undergoing open decompression surgery (microdiscectomy) will be included. Excised herniated disc fragment, muscle and ligamentum flavum samples will be collected during surgery and immediately sent to microbiology for tissue culture and search for pathogens. Score questionnaires for pain, function and quality of life will be applied before surgery and at 1, 3, 6 and 12 months time-points. A new MRI will be performed 12 months after surgery for analysis of Modic changes and baseline comparison. Primary endpoint is real rate of disc infection in symptomatic patients with degenerative disc disease. Secondary endpoints will be low back pain, quality of life, function, Modic incidence and volume.

Ethics and dissemination

This study is going to be submitted to our Institutional Review Board and will only begin after its approval. Patients accepting to participate will sign an Informed Consent Form before entering the study. Results will be published in a peer reviewed medical journal and presented in medical conferences independently of study findings.

Registration ClinicalTrials.gov NCT0315876

INTRODUCTION

Low back pain is a frequent condition in the population, as well as vertebral endplates abnormalities, described by Modic et al.[1,2], that affect up to 6% of the general population, and, up to 46% of patients with low back pain[3]. Modic type I changes are described as vertebral bone marrow edema related to acute low back pain[4]. When Modic changes are detected, chances of one presenting unspecific low back pain are 4.5 times higher[1,2].

Subclinical infection caused by low-virulence pathogen can possibly lead to vertebral endplate abnormalities, detected in magnetic resonance imaging (MRI) studies, and differentiation between infection and Modic changes may be difficult[5,6]. Subclinical infection can also be associated with increasing low back pain[7]. Albert et al[8] reported 61 patients who had undergone surgical treatment for lumbar disc herniation where 46% of cases had a positive culture. The same authors also reported that 80% of patients with a positive culture for anaerobic pathogens presented Modic type I changes at the adjacent vertebra after a two-year follow-up, against 44% of patients with negative culture. Some studies demonstrated the presence of low-virulence pathogens in intervertebral disc tissue cultures[6–10], most commonly reported to *Propionibacterium acnes*.

Chronic low back pain and Modic type I changes have been treated with antibiotics for up to 100 days with superior outcomes compared to sham treatment[7]. Patients were treated with amoxillin/clavulanate (500mg/125mg)[7] based on the study where sciatica is associated with *Propionibacterium acnes*[8].

However, Carricajo et al[11] suggest that the presence of *P. acnes* in the intervertebral discs is due to either external surgical or laboratory contamination. These authors detected positive disc culture in only 3.7% of cases out of 54 patients. Furthermore, same authors demonstrated that samples of spinal muscle and ligamentum *flavum* collected intraoperatively at the end of procedure had positive cultures in 14.8% of cases with a negative disc culture.

In agreement to that study, Rigal et al[12] analyzed a sample of 313 patients submitted to video assisted or retroperitoneal anterior approach and found only six cases of positive cultures. No correlation between infection and degeneration of the intervertebral disc was found.

Still, Rollason et al[13], in a study of genotype characterization, observed that *P. acnes* cultured from disc samples surgically taken from 64 patients with disc herniation were different from those usually found on skin, suggesting that this pathogen could be related to low back pain.

A systematic review performed by Urquhart et al[14] concluded, that there is moderate evidence that a relationship between positive culture with Modic type I changes and low back pain exists, although there was low evidence for relationship of cause. For that, authors concluded that new studies should be made to determine whether pathogens in the disc are originated from external contamination or if they are truly involved in the development of chronic back pain.

HYPOTHESIS

Lumbar disc herniation is related to subclinical infection of the intervertebral disc

Null hypothesis: incidence of subclinical infection is the same as incidence of cases without infection in patients with lumbar disc herniation treated with surgery.

OBJECTIVES

Main purpose of this study is to identify if the presence of an infection pathogen in the intervertebral disc is real or if it is intraoperative contamination.

Secondary objectives are to analyze clinical prognostic factors in patients and diagnosis of infection.The study also proposes to analyze the relationship between radiological changes (Modic I and II) and infection.

JUSTIFICATION AND CIENTIFIC CHALLENGES

In case there is a confirmation that lumbar disc herniation is associated with subclinical pathogens as well as Modic changes with unspecific chronic back pain, it can change the way this affection is treated and improve treatment costs and outcomes.

Previously published studies that reported a strong correlation between *P. acnes* and low back pain and/or disc herniation are almost all from the same study group[5–8]. Few studies questioned their results, and those who did presented a small number of patients, and no adequate statistical methodology[11].

For this prospective cohort study, we previously calculated minimum number of subjects needed for adequate statistical analysis. The project addresses, besides a specific culturing for *P. acnes*, molecular analysis and clinical outcomes follow-up in a single study, which provides a complexity and significance that were not achieved in previously published studies on this matter.

METHODS AND ANALYSIS

This study protocol is registered at *Clinicaltrials.gov* under NCT0315876.
https://clinicaltrials.gov/ct2/show/NCT03158766?term=NCT03158766&rank=1

Study design:

An open prospective cohort study will be performed at a single center, (Hospital Israelita Albert Einstein – HIAE) taking 1 year for recruiting, and ending 1 year after inclusion of last patient. Patients' data will be collected with a specific form created for this study. Patients will be summoned for a new magnetic resonance image of the lumbar spine one year after their surgical procedure.

All included patients will go through further treatment of ten sessions of postoperative physical therapy. They will be instructed to maintain learned exercises in their residences. Pain medications will not be controlled and will follow attending physician prescriptions.

Population:

Patients will be consecutively included in the study.

- **Inclusion criteria:** Subjects between 18 and 65 years of age; both genders; with diagnose of lumbar disc herniation undergoing open decompression surgery (microdiscectomy). Patients willing and able to go through all phases of clinical investigation and rehabilitation will be included. An Informed Consent Form (ICF) must be signed.

- **Exclusion criteria:** Patients with previous lumbar disc surgery at the same level at any point of life; patients undergoing chemotherapy; patients with any immune deficiency; patients previously submitted to disc injection and/or discography; patients submitted to previous endoscopic disc surgery; patients with fusion performed at the same stage of decompression surgery; patients with any other infection within the last six months or usage of antibiotics within the last two months; patients with incomplete specific form or data; decline to participate or sign the ICF.

Patient enrollment in the study:

Evaluation of patient eligibility will be carried out by the main investigator or by a co-investigator, both study coordinator that will perform an interview with candidate patient about his willing to participate in the study. They will be responsible to confirm inclusion and exclusion criteria; and, if patient accepts his participation, the investigator will explain all study details and read along the Informed Consent Form, when any questioning on the objective of the study, involved procedures, risks/benefits, confidentiality, will be resolved.

Patients accepting their participation will date and sign the ICF. A copy of the form will be attached to patients' medical record, and another will be provided to the patient. After ICF is properly signed, patient will undergo an interview to complete initial demographic data and pretreatment forms.

If patient is unable to sign the written ICF, the investigator will orientate and vocally explain the study and patient will provide an oral consent in the presence of a witness that will sign the ICF.

Patient recruitment will be carried out for 24 months, when 95 patients shall be included (details of estimated *n* reported at sample size determination).

Patient allocation

Patients will undergo surgery according to surgeons' preference. Attending surgeons will determine chosen operative technique according to their experience and preference.

Blinding

Patients will not have access to the results of tissue cultures for pathogens, as well as the attending physician.

The radiologist that will analyze imaging studies of performed magnetic resonance will also be blinded to patients' data or laboratory culture results. A blinded investigator will analyze pain and function scores.

Early stopping of participation in the study

Patients will be excluded from the study when:

- Withdrawn of ICF
- Diseased
- Patient selection flaw - incompatible eligibility criteria
- Lost to follow-up
- If patient presents clinical symptoms of infection such as severe lumbar or radicular pain, fever with no other detected foci, abnormal ESR, CRP, leucogram, and, altered imaging studies that leads to interruption of blinding of the results of culture exams.

For each eventually excluded case, reasons and circumstances for withdrawn will be detailed. Patients' data collected until determinate point of the study will be included at final analysis.

Selected endpoints

Primary endpoint

- **Real rate of intervertebral disc infection**

The main objective of this study considers that the intervertebral disc is infected by any type of low virulence pathogen, which leads to Modic changes and chronic low back pain. Thus, calculation of the incidence of infection in lumbar disc herniations will be performed.

1. **Incidence of infection rate (IIR)** will be calculated as follows:

$$IIR = \frac{\text{(number of detected infections)}}{\text{(total number of included patients)}}$$

Secondary endpoint

- **Low back pain**

Intensity of low back pain and limitation for daily activities of patients with and without infection will be analyzed through the Numeric Rating Score (NRS) system applied at time of patient recruitment and 1, 3, 6 and 12 months after surgical procedure. Minimal clinically important difference will be considered as an increase of 30% of baseline lumbar pain at first postoperative month, due to possible bias of postoperative pain due to surgical manipulation as well as pain due to the disc herniation itself.

NRS and the visual analogue scale (VAS) have good correlation and are equally sensitive to quantify postoperative pain[15]. Compared to VAS, NRS is easier to manage and codify, furthermore, less mistakes occur during data insertion[16]. Otherwise, it is easier to complete[16] and preferred by patients[17].

- **Quality of life**

Quality of life at the end of one year for both infected and uninfected groups, with and without Modic changes, will be analyzed through the validated Portuguese version of the EuroQol (EQ-5D) questionnaire. This measurement tool will be applied at timing of patient recruitment, and 1, 3, 6 and 12 months after surgery.

EQ-5D is a self-completing standardized tool containing 5 items (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Compared to the Short-Form 36 (SF-36) questionnaire, EQ-5D is a shorter and faster form to analyze.

- **Function**

Function will be quantified through the Portuguese version of the Oswestry Disability Index (ODI) for lumbar pain that will be

applied at time of recruitment and 1, 3, 6 and 12 months after surgery.

- **Modic incidence**

Insurgent Modic changes in patients will be analyzed one year after surgery, as well as its relationship with presence or absence of infection.

Incidence of Modic (IM) changes will be calculated for the infection group (IM infec) and for the total group (IM total) as follows:

$$\text{IM infec} = \frac{\text{(number of Modic changes in infected patients after 1 year)}}{\text{(total number of infections)}}$$

$$\text{IM total} = \frac{\text{number of Modic changes at final 1 year follow-up}}{\text{total number of patients}}$$

- **Volume and size of Modic changes, additional imaging analysis**

Quantification of sizing will be done by two radiologists with expertise in musculoskeletal diagnosis. All images will be analyzed in sagittal T1, T2, and FAT-T2 weight sequences of the lumbar spine in DICOM format. Modic volume will be measured according to Wang et al[18]. Three sagittal slices of the lumbar spine will be considered: midsagittal slice; left pedicle parasagittal slice; and right pedicle parasagittal slice. The parameters examined to quantify Modic changes will include measures of ratios of the region affected by Modic changes to the entire corresponding vertebral body, including maximal width ratio, maximal height ratio, and area ratio. Vertebral body changes will be classified accordingly to Modic chages type I, II, and III[1,2]. Soft tissues around the vertebra, such as disc, muscles, and ligaments will also be analyzed. Data will be collected for presence of vertebral or disc edema, and presence of disc hydration or not. Disc degeneration will be collected as: normal; degeneration with height preservation; and, degeneration with loss of height. Preop and 12-month postop acquired MRI studies will be compared. Both superior and inferior disc endplates will be evaluated and compared. Preoperative study will be taken as baseline for comparative purposes.

- **Adverse effects**

Fail of surgical treatment (recurrence, instability, need for reoperation, etc.); need for additional physical therapy sessions; superficial infection; drainage; deep venous thrombosis; and, any

other possible adverse event that may show up will be included as well.

Study stages

Sample collection:

Included patients will undergo standard fashion general anesthesia and prepped with clorhexidine solution. Intravenous antibiotics prophylaxis will be administered within first hour before skin incision, according to standard protocol of HIAE Infection Control Committee published at the hospital Pharmaceutics Manual.

Preoperative blood sample will be collected for leucogram, ESR, and CRP. Same laboratory will be repeated at 1, 6 and 12 months time-point.

The excised herniated disc fragment will be immediately sent to microbiology laboratory analysis in a universal sterile container (screw cap tube) in no more than 30 minutes to be processed as follows. Same process will be applied to samples of deep muscle and ligamentum flavum at the end of the surgical procedure. Three cultures of the intervertebral disc will be done, as well as three of the ligamentum flavum and three of the multifidus muscle for each patient. Flowchart of this stage is described in Figure1.

Search for pathogens:

Herniated intervertebral disc will be split in three equally sized fragments of over 2x2x5 mm and squashed in a laminar flow cabinet until homogeneous material is achieved. Same process will be carried out for the ligamentum flavum and multifidus muscle samples, although split in three fragments. Tissues will be cultured in specific growth medium and incubated according to the respective culture:

Similar criteria used by the Infectious Diseases Society of America (IDSA) to detect joint replacement infection will be adopted, which is the recommendation on at least two positive tissue cultures by the same pathogen to confirm diagnosis of infection[19]. This same criterion was already adopted in a study to characterize isolated pathogens in herniated intervertebral discs[14].

- **A - Aerobic culture**

For aerobic cultures, samples will be cultured in 5% sheep blood agar, chocolate agar and MacConkey agar plate, and will be placed in a 35°C incubator (CO2 atmosphere) for 5 days. If a positive bacteria culture is detected in the plate, the colony will be identified by MALDI TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) Microflex LT (Bruker Daltonics/BD). Sensitivity profile will be performed when needed, according to CLSI (Clinical Laboratory Standards Institute) recommendations.

- **B - Anaerobic culture**

For anaerobic culture, tissue will be cultured in a Thioglicolate tube and incubated in a 35°C incubator for up to 21 days. If turbidity occurs at the Thioglicolate medium, material will be cultured in anaerobic blood agar and incubation at 35°C will be done in an anaerobic atmosphere. After growing of colonies, identification will

be done by MALDI TOF and, when necessary, a sensitivity test to antibiotics will be performed according to CLSI recommendations.

- **C - Histological analysis**

Anatomic pathology analysis of the other fragment of the herniated disc (2x2x5 mm) will be done. Sample will be transported in a universal container with tamponated formalin (10%), followed by dehydration in alcohol diafanized in xylol and inclusion in paraffin (60-65°C), which will be stained in hematoxylin-eosin (HE) and GRAM staining.

- **HE:** Histological cuts of 4µm will be performed, followed by clearing with xylol for 10 minutes twice, embedding with alcohol under increasing concentrations and stained with hematoxylin for 5 minutes, running water for 5 minutes, eosin for 1 minute and running water for 2 minutes followed by assembly of glass microscope slide with Entellan®.
- **GRAM:** Another slide will be embedded with crystal violet for 1 minute, running water for 1 minute followed by lugol for another 1 minute and additional wash with running water. Unstaining of the slide will be done with alcohol 95% for 10 seconds followed by running water and then, stain with fuchsine for 30 seconds, running water wash again, drying and slide assembly with Entellan®.

- **D – Molecular analysis of pathogens**

Positive cultures that present aerobic or anaerobic pathogens culturing, will be isolated and stowed refrigerated in -80°C freezer for posterior molecular analysis.

Molecular typing will be performed through Pulsed Field Gel Electrophoresis technique of isolated samples according to the protocol described by Oprica et al.[20] using Spe-I restriction enzyme and Bionumerics software for analysis of results.

Questionnaires

Scores for pain (NRS), function (ODI), and quality of life (EQ-5D) will be self assessed and applied before surgery and at 1, 3, 6 and 12 months time-points. All questionnaires will be collected by a employee not involved in the study. Follow-up clinic visits will be at 1, 3, 6 and 12 months after surgical procedure, with acceptance deviation of 7, 14, 21, and 28 days, respectively.

Imaging studies

Magnetic resonance imaging studies will be performed in Siemens or General Electric 1.5T devices. Studies in 3.0T devices will not be done due to higher frequency of artifacts generated by chemical shift, which would modify measurements of Modic changes.

The following sequences will be used:

- Sagittal cut: Fast Spin-Echo T1 and T2 weighted with fat suppression or STIR instead of T2, according to our institution protocol established for all exams
- Coronal cut: same sequence imaging will be used for measurements.

At follow-up, contrasted MRI studies of patients will be done as an institution established protocol for postoperative patients, otherwise, it will not interfere with the study protocol.

Lumbar disc herniation will be diagnosed through MRI in eligible patients. Included patients will be submitted to new MRI 12 months after surgery.

Two radiologists with expertise in musculoskeletal MRI will independently classify and perform measurements, and divergences will be solved blindly by common opinion.

Confounding variables

Data on the following confounding variables will be collected: age, gender, alcohol intake, smoking, body mass index (BMI), spinal injections with corticoid within 6 months before surgery, usage of oral corticoids up to 3 months before surgery, diabetes.

Since this information may change over time, data will be considered at time of last assessment before surgery.

A – Alcohol intake: categorized as – none or sporadically (<1 glass/day); light intake (1-2 glass/day); moderate/heavy intake (3 or more glasses/day); and, not assessed[21].

B – Smoking: categorized as – ex-smokers; smoker; non-smoker; and, not assessed[22].

C – BMI: categorized as – underweight (<18.5); normal weight (18.5-25); overweight (25-30); obese (>30); not assessed or not available[23].

ETHICS AND DISSEMINATION

This study is going to be submitted to our Institutional Review Board (IRB) and will only begin after its approval. Patients accepting to participate will sign an Informed Consent Form before entering the study. Results will be published in a peer reviewed medical journal and presented in medical conferences independently of study findings.

Some authors suggest that subclinical infection of the intervertebral disc is one of the causes of chronic low back pain unresponsive to treatment, besides promoting Modic type I changes on MRI. Although there are studies reporting relative success on treatment of well-selected patients, there is still uncertainty that these patients were really infected or not.

We hope to define the accurate incidence of subclinical infection of the intervertebral disc with disc herniation and provide data and possibly an answer to this present gap in the literature.

STATISTICAL PLANNING

Rate of subclinical infection (or Modic change) will be obtained by the ratio between number of positive cultures from surgical samples and total number of patients, and estimates will follow 95% confidence intervals. After infection cases are identified, we will investigate if there is an association between detected infection and patient outcomes by logistic regression models for Modic changes, ordinal logistic regression for Modic volume and size, and linear regression or general linear models for numeric outcomes, such as: low back pain, quality of life and function. All models will

consider confounding variables such as: smoking and alcohol intake, diabetes, corticosteroids injection, BMI, gender, and age. Results will be presented as effects estimates such as odds ratio or mean ratio, 95% confidence intervals and *p* values. Study dropout cases, for any reason, will be considered for final analysis.

Sample size calculation

Sample size was calculated to estimate of incidence of subclinical infection in patients with lumbar disc herniation. Considering that the rate of infection lies around 46%[3], we need to observe a minimum of 95 patients to achieve a 95% confidence interval with 10% absolute accuracy.

The necessary sample size to analyze secondary endpoints will depend on the observed rate of cases with subclinical infection in our study sample. If the observed rate is too small, an increase in the number of included patients will be needed. To better evaluate this, the sample size calculation will be revisited by the time we have reached half of initially planned sample size (48 patients).

CHRONOGRAM

At the end of the protocol (Table 1 and 2)

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COMPETING INTERESTS STATEMENT

Authors have no potential conflict of interests.

AUTHORS CONTRIBUTIONS

Nelson Astur – Spine surgeon – Conception and design of the work, data collection, data analysis and interpretation, drafting the article

Delio E. Martins – Spine surgeon – Conception and design of the work, data collection, data analysis and interpretation, critical revision of the article, final approval of the version to be published

Marcelo Wajchenberg – Spine surgeon – Data collection, critical revision of the article, final approval of the version to be published

Mario Ferreti – Orthopedic surgeon – Critical revision of the article, final approval of the version to be published

Fernando G. Menezes – Infectologist – Conception and design of the work, final approval of the version to be published

Andre M. Doi – Microbiologist – Conception and design of the work, data collection, data analysis and interpretation

Laercio A. Rosemberg – Radiologist – Design of the work, data analysis and interpretation, data collection

Durval C. B. Santos – Radiologist – Design of the work, data analysis and interpretation, data collection

Alexandre S. Yutaka – Spine surgeon – Data collection, critical revision of the article

Luciano M. R. Rodrigues – Spine surgeon – Data collection, critical revision of the article

Arthur W. Poetscher – Spine surgeon – Data collection, critical revision of the article

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Mario Lenza – Orthopedic surgeon – Conception of the work, critical revision of the article, final approval of the version to be published

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Figure legends

Figure 1: Flowchart of collected clinical samples that will be sent to culture analysis.

Table 1: Study chronogram

Month / Year	IRB approval	Recruiting	Last cases follow-up	Data analysis	Data interpretation	Final report	Submission to peer-reviewed indexed journal
Jan 2017 to Feb 2017	X						
Mar 2017 to Mar 2018		X	X				
Mar 2017 to Mar 2019			X				
Nov 2018 to Feb 2019				X			
Feb 2019 to Apr 2019				X	X		
Apr 2019 to Jun 2019					X	X	
Jun to Dec 2019							X

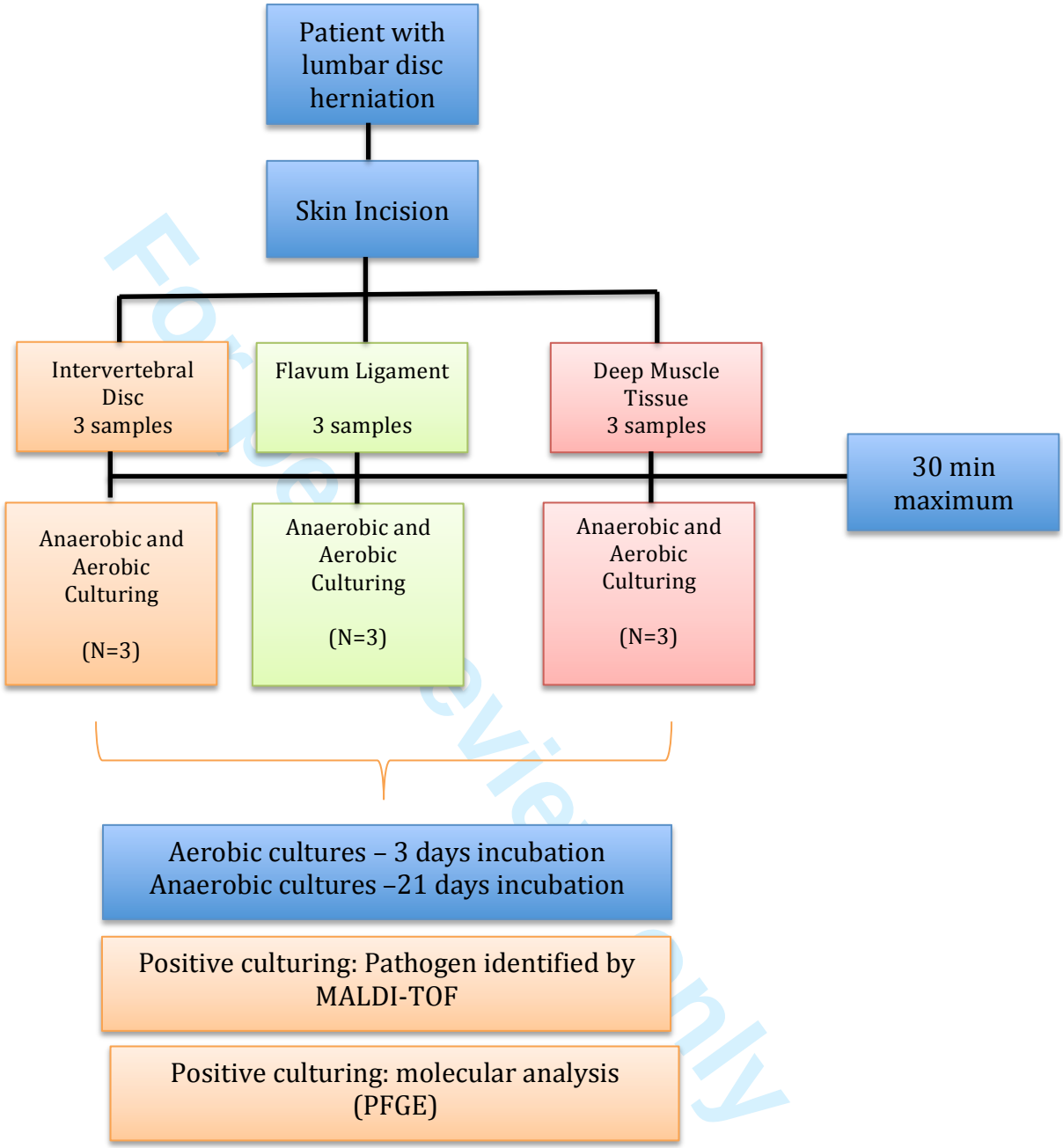
Table 2: Chronogram of included patients

	Screening/ Recruiting	Postop 4 weeks ± 7 days	Postop 3 months ± 14 days	Postop 6 months ± 21 days	Postop 12 months ± 30 days
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
ICF	X				
Check including and excluding criteria	X				
Collect demographic data	X				
Investigate medical history / Complete enrollment form	X				
Lab screening, ESR, CRP, Leucogram	X	X		X	X
Apply NRS tool	X	X	X	X	X
Apply Oswestry questionnaire	X	X	X	X	X
Apply EQ-5D questionnaire	X	X	X	X	X
Physical therapy		X			
Magnetic resonance imaging					X

ICF = Informed Consent Form; ESR = erythrocyte sedimentation rate; CRP = C reactive protein; NRS = Numeric rating scale; EQ-5D = European quality of life 5 dimensions.

Financing planning: Approved by FAPESP

	Description	Final cost (R$)
1	Molecular typing necessary material	22.471,59
2	Antimicrobial sensitivity testing necessary material	3.300,00
3	Strain storage necessary material	3.702,00
4	Laboratory culture necessary material	44.462,00
5	Laboratory waste disposal material	550,00
6	Bacterial strain identification material	11.247,90
7	Technical reserve – Additional benefits	16.000,00
8	Technical reserve – budget for direct project infrastructure	12.860,02
		114.593,51



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **pg1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **attached to submission**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **pg2**
Objectives	3	State specific objectives, including any prespecified hypotheses **pg3**
Methods		
Study design	4	Present key elements of study design early in the paper **pg3**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **pg3-4**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **pg3-4** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **N/A** *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **pg5-6**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **pg7-8**
Bias	9	Describe any efforts to address potential sources of bias **pg 9**
Study size	10	Explain how the study size was arrived at **pg 10**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **pg 9**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **pg 9**
		(*b*) Describe any methods used to examine subgroups and interactions **pg 9**
		(*c*) Explain how missing data were addressed **pg 4**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **pg 4** *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results PROTOCOL OF STUDY – NO RESULTS AVAILABLE		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(b) Give reasons for non-participation at each stage **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(c) Consider use of a flow diagram **PROTOCOL OF STUDY – NO RESULTS AVAILABLE (Table 2)**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(b) Indicate number of participants with missing data for each variable of interest **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
Discussion PROTOCOL OF STUDY – NO DISCUSSION BASED ON RESULTS AVAILABLE		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **pg 1 and pg 16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Subclinical Propionibacterium Acnes Infection Estimation in the Intervertebral Disc (SPInE-ID): Protocol for a Prospective Cohort.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2017-017930.R1
Article Type:	Protocol
Date Submitted by the Author:	23-Aug-2017
Complete List of Authors:	Astur, Nelson; Hospital Israelita Albert Einstein, Programa Locomotor Martins, Delio; Hospital Israelita Albert Einstein, Programa Locomotor Wajchenberg, Marcelo; Hospital Israelita Albert Einstein, Programa Locomotor Ferreti, Mario; Hospital Israelita Albert Einstein, Programa Locomotor Menezes, Fernando; Hospital Israelita Albert Einstein, Serviço de Controle de Infecção Hospitalar Doi, Andre; Hospital Israelita Albert Einstein, Setor de Microbiologia do Laboratório Clínico Rosemberg, Laercio; Hospital Israelita Albert Einstein, Departamento de Diagnostico por Imagem Santos, Durval; Hospital Israelita Albert Einstein, Departamento de Diagnostico por Imagem Iutaka, Alexandre; Hospital Israelita Albert Einstein, Programa Locomotor Rodrigues, Luciano; Hospital Israelita Albert Einstein, Programa Locomotor Martino, Marines; Hospital Israelita Albert Einstein, Serviço de Microbiologia do Laboratorio Clinico Pagura, Jorge; Hospital Israelita Albert Einstein, Programa Locomotor Kihara Filho, Eduardo; Hospital Israelita Albert Einstein, Programa Locomotor Lenza, Mario; Hospital Israelita Albert Einstein, Programa Locomotor
Primary Subject Heading:	Surgery
Secondary Subject Heading:	Infectious diseases, Radiology and imaging
Keywords:	Spine < ORTHOPAEDIC & TRAUMA SURGERY, Magnetic resonance imaging < RADIOLOGY & IMAGING, Diagnostic microbiology < INFECTIOUS DISEASES, Propionibacterium acnes, Low back pain

Subclinical *Propionibacterium acnes* Infection Estimation in the Intervertebral Disc (SPInE-ID): Protocol for a Prospective Cohort

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Competing interests statement: Authors have no potential conflict of interests.

Ethical approval: CAAE 65102617.2.0000.0071 / Hospital approval 2998-17

ABSTRACT

Introduction

Low back pain and vertebral endplate abnormalities are common conditions within the population. Subclinical infection caused by indolent pathogens can potentially lead to these findings, with differentiation between them notably challenging from a clinical perspective. Progressive infection of the intervertebral disc has been extensively associated with increasing low back pain, with Propionibacterium acnes specifically implicated with relation to sciatica. The main purpose of this study is to identify if the presence of an infective pathogen within the intervertebral disc is primary or is a result of intraoperative contamination, and whether this correlates to low back pain.

Methods and analysis

An open prospective cohort study will be performed. Subjects included within the study will be between the ages of 18 to 65 years, and have a diagnosis of lumbar disc herniation requiring open decompression surgery. Excised herniated disc fragments, muscle and ligamentum flavum samples will be collected during surgery and sent to microbiology for tissue culture and pathogen identification Score questionnaires for pain, functionality and quality of life will be given preoperatively and at 1, 3, 6 and 12 months postoperatively. A MRI will be performed 12 months after surgery for analysis of Modic changes and baseline comparison. The primary endpoint is the rate of disc infection in symptomatic patients with degenerative disc disease. The secondary endpoints will be performance scores, Modic incidence and volume.

Ethics and dissemination

This study was approved by our Institutional Review Board and was only initiated after it (CAAE 65102617.2.0000.0071). Patients agreeing to participate will sign an Informed Consent Form before entering the study. Results will be published in a peer reviewed medical journal irrespective of study findings. If shown to be the case, this would have profound effects on the way physicians treat chronic low back pain, even impacting health costs.

Registration ClinicalTrials.gov NCT0315876

INTRODUCTION

Low back pain and vertebral endplate abnormalities are common conditions within the population, with Modic et al.[1,2] reporting endplate abnormality rates of up to 6% within the population and 46% of patients complaining of low back pain[3]. Modic type I changes are described as vertebral bone marrow edema related to acute low back pain[4]. When Modic changes are detected, there is a 4.5 times higher incidence of nonspecific low back pain on presentation[1,2].

Subclinical infection caused by low-virulence pathogens can potentially lead to vertebral endplate abnormalities, which are identified through magnetic resonance imaging (MRI). Differentiation between subclinical infection and Modic changes may be notably difficult, given the paucity of examination findings[5,6]. Additionally, subclinical infections can be associated with increasing low back pain[7]. Albert et al.[8] reported 61 patients who had undergone surgical treatment for lumbar disc herniation, with 46% of cases showing a positive culture. The same authors also reported that 80% of the patients with a positive culture for anaerobic pathogens presented with Modic type I changes at the adjacent vertebra after a two-year follow-up. This is in stark contrast to only 44% of patients with negative culture. Some studies demonstrated the presence of low-virulence pathogens in intervertebral disc tissue cultures[6–10], with the most common causative organism reported as *Propionibacterium acnes*.

Chronic low back pain and Modic type I changes have been treated with antibiotics for up to 100 days with superior outcomes in comparison to placebo treatment according to Albert et al[7]. In this study, patients were treated with amoxicillin/clavulanate (500mg/125mg)[7] following another study where *Propionibacterium acnes* was linked to sciatica[8]. However, Carricajo et al[11] suggest that the presence of *P. acnes* in the intervertebral discs is due to either external surgical or laboratory contamination. Within their study, they detected positive disc cultures in only 3.7% of 54 patients. Further, the same group demonstrated that samples of spinal muscle and ligamentum *flavum* had positive cultures in 14.8% of cases that had negative disc cultures. Reinforcing the findings of this study, Rigal et al[12] analysed a sample of 313 patients undergoing video-assisted or retroperitoneal anterior approach, and found only six cases of positive cultures. No correlation between infection and degeneration of the intervertebral disc was found. Contrastingly, Rollason et al[13], in a study of genotype characterization, observed that *P. acnes* cultured from disc samples surgically resected from 64 patients with disc herniation were different from those normally colonizing the skin, suggesting that this variant of the *P. acnes* bacterium could be related to low back pain. A systematic review performed by Urquhart et al[14] concluded that there is moderate evidence of a relationship between positive *P. acnes* cultures with Modic type I changes and low back pain, although the evidence was not substantive. The group concluded that new studies should be conducted to determine whether pathogens within the vertebral disc arise from external contamination or if they are truly implicated in the development of chronic back pain (low back pain for at least three months)..

HYPOTHESIS AND OBJECTIVES

We hypothesise that lumbar disc herniation is related to subclinical infection of the intervertebral disc

Our primary end point of this study is to identify whether the presence of a pathogen within the intervertebral disc is primary or if it is a result of intraoperative contamination.

The secondary end points are to analyse clinical prognostic factors in patients and the diagnosis of infection. The study also proposes to analyse the relationship between radiological changes (Modic I and II) and infection.

JUSTIFICATION AND SCIENTIFIC CHALLENGES

If there is a confirmation of a relationship between subclinical pathogens, lumbar disc herniation and Modic changes with nonspecific chronic back pain, this will change the way this disease process is managed and improve treatment costs and patient outcomes.

Previously published studies that report a strong correlation between *P. acnes* and low back pain and/or disc herniation are almost entirely from the same study group[5–8]. Few studies have questioned their results, and those that have, only presented small sample groups and inadequate statistical methodologies[11]. For this prospective cohort study, we previously calculated the minimum number of subjects needed for adequate statistical analysis. Aside from outlining a specific culturing method for *P. acnes*, this project addresses molecular analysis and clinical outcomes follow up in a single study. This provides a complexity and significance that have not been achieved in previously published studies on the topic.

METHODS AND ANALYSIS

This study protocol is registered at *Clinicaltrials.gov* under NCT0315876.
https://clinicaltrials.gov/ct2/show/NCT03158766?term=NCT03158766&rank=1

Institutional Review Board approval (CAAE 65102617.2.0000.0071)

Study design:

An open prospective cohort study will be performed at a single center, (Hospital Israelita Albert Einstein – HIAE) taking 1 year for recruiting, and ending 1 year after inclusion of last patient. Patients' data will be collected with a specific form created for this study. Patients will be summoned for a new magnetic resonance image of the lumbar spine one year after their surgical procedure.

All included patients will go through further treatment of ten sessions of postoperative physical therapy. They will be instructed to maintain learned exercises in their residences. Pain medications will not be controlled and will follow attending physician prescriptions.

Population:

Patients will be consecutively included in the study.

- **Inclusion criteria:** Subjects between 18 and 65 years of age; both genders; diagnosis of lumbar disc herniation and are undergoing open decompression surgery (microdiscectomy). Indication for surgery is sciatica caused by disc herniation compression of a lumbar nerve root failing conservative treatment for at least six weeks or on-going neurological deficit. Patients with history of previous spinal injection will not be excluded from the study. Patients willing and able to go through all phases of clinical investigation will be included. An Informed Consent Form (ICF) must be signed.
- **Exclusion criteria:** Patients with previous lumbar disc surgery at the same level at any point of life; patients undergoing chemotherapy; patients with any immune deficiency; patients previously submitted to disc injection and/or discography; patients submitted to previous endoscopic disc surgery; patients with fusion performed at the same stage of decompression surgery; patients with any other infection within the last six months or usage of antibiotics within the last two months; patients with incomplete specific form or data; decline to participate or sign the ICF.

Patient enrollment in the study:

Evaluation of patient eligibility will be carried out by the main investigator or by a co-investigator. Both study coordinators will perform an interview with a candidate patient about his/her willingness to participate in the study. They will be responsible for confirming their eligibility with relation to the inclusion and exclusion criteria; and, if the patient accepts, the investigator will explain all study details and read along the ICF. Any questions regarding the objectives of the study, involved procedures, risks/benefits, and confidentiality will be resolved. Patients accepting to participation will date and sign the ICF. A copy of the form will be attached to the patient's medical record, and another will be provided to the patients themselves. After the ICF is properly signed, the patient will undergo an interview to complete the initial demographic data and pre-treatment forms. If the patient is unable to sign the written ICF, the investigator will vocally explain the study and the patient will provide oral consent in the presence of a witness that will sign the ICF. Patient recruitment will be carried out for 24 months, so that 95 patients shall be included (details of estimated *n* reported at sample size determination).

Patient allocation

Patients will undergo surgery according to surgeons' preference. Attending surgeons will determine chosen operative technique according to their experience and preference.

Blinding

Neither the patient or attending physician will have access to the results of tissue cultures. The radiologist that will analyse the imaging studies of performed MRIs will also be blinded to the patient data and laboratory results. A blinded investigator will analyse pain and function scores.

Early stopping of participation in the study

Patients will be excluded from the study upon:

- Withdrawal of ICF
- Death
- Patient selection flaw identified (incompatible eligibility criteria)
- Lost to follow-up
- Patient presents with clinical symptoms of infection, inclusive of severe lumbar or radicular pain, fever with no other detected foci, abnormal ESR/CRP/leucogram, altered imaging studies that leads to interruption of the blinding of the results of culture exams.

For each excluded case, the reason and circumstance for the withdrawal will be detailed. Patient data collected until that point of the study will be included at final analysis.

Selected endpoints

Primary endpoint

- **Rate of intervertebral disc infection**

The primary objective of this study was to consider the incidence of intervertebral disc infection by any type of low virulence pathogen, with consequent Modic changes and chronic low back pain. Thus, the calculation of the incidence of infection in lumbar disc herniations will be performed.

1. **Incidence of infection rate (IIR)** will be calculated as follows:

$$IIR = \frac{\text{(number of detected infections)}}{\text{(total number of included patients)}}$$

Secondary endpoint

- **Low back pain**

Intensity of low back pain and limitation for daily activities of patients with and without infection will be analysed through the Numeric Rating Score (NRS) system applied at time of patient recruitment and 1, 3, 6 and 12 months after surgical procedure (Table 1). The clinically significant threshold will be considered to be an increase of 30% of baseline lumbar pain at the first postoperative month. NRS and the visual analogue scale (VAS) have good correlation and are equally sensitive to quantify postoperative pain[15]. Compared to VAS, NRS is easier to manage and codify, furthermore, less mistakes occur during data insertion[16]. Otherwise, it is easier to complete[16] and preferred by patients[17].

- **Quality of life**

Quality of life at the end of one year for both infected and uninfected groups, with and without Modic changes, will be analysed through the validated Portuguese version of the

EuroQol (EQ-5D) questionnaire. This measurement tool will be applied at timing of patient recruitment, and 1, 3, 6 and 12 months after surgery.
EQ-5D is a self-completing standardized tool containing 5 items (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Compared to the Short-Form 36 (SF-36) questionnaire, EQ-5D is a shorter and faster form to analyse.

- **Function**

Function will be quantified through the Portuguese version of the Oswestry Disability Index (ODI) for lumbar pain that will be applied at time of recruitment and 1, 3, 6 and 12 months after surgery.

- **Modic incidence**

Insurgent Modic changes in patients will be analysed one year after surgery, as well as its relationship with presence or absence of infection.
Incidence of Modic (IM) changes will be calculated for the infection group (IM infec) and for the total group (IM total) as follows:

$$\text{IM infec} = \frac{\text{(number of Modic changes in infected patients after 1 year)}}{\text{(total number of infections)}}$$

$$\text{IM total} = \frac{\text{number of Modic changes at final 1 year follow-up}}{\text{total number of patients}}$$

- **Volume and size of Modic changes, additional imaging analysis**

Quantification of sizing will be done by two radiologists with expertise in musculoskeletal diagnosis. All images will be analysed in sagittal T1, T2, and FAT-T2 weight sequences of the lumbar spine in DICOM format. Modic volume will be measured according to Wang et al[18]. Three sagittal slices of the lumbar spine will be considered: midsagittal slice; left pedicle parasagittal slice; and right pedicle parasagittal slice. The parameters examined to quantify Modic changes will include measures of ratios of the region affected by Modic changes to the entire corresponding vertebral body, including maximal width ratio, maximal height ratio, and area ratio. Vertebral body changes will be classified accordingly to Modic chages type I, II, and III[1,2]. Soft tissues around the vertebra, such as disc,

muscles, and ligaments will also be analysed. Data will be collected for presence of vertebral or disc edema, and presence of disc hydration or not. Disc degeneration will be collected as: normal; degeneration with height preservation; and, degeneration with loss of height. Preop and 12-month postop acquired MRI studies will be compared. Both superior and inferior disc endplates will be evaluated and compared. Preoperative study will be taken as baseline for comparative purposes.

- **Adverse effects**

Fail of surgical treatment (recurrence, instability, need for reoperation, etc.); need for additional physical therapy sessions; superficial infection; drainage; deep venous thrombosis; and, any other possible adverse event that may show up will be included as well.

Study stages

Sample collection:

Included patients will undergo standard fashion general anesthesia and prepped with chlorhexidine solution. Intravenous antibiotics prophylaxis will be administered within first hour before skin incision, according to standard protocol of HIAE Infection Control Committee published at the hospital Pharmaceutics Manual.

Preoperative blood sample will be collected for leucogram, ESR, and CRP. Same laboratory will be repeated at 1, 6 and 12 months time-point (Table 1).

The excised herniated disc fragment will be immediately sent to microbiology laboratory analysis in a universal sterile container (screw cap tube) in no more than 30 minutes to be processed as follows. The same process will be applied to samples of deep muscle and ligamentum flavum at the end of the surgical procedure. Three cultures of the intervertebral disc will be done, as well as three of the ligamentum flavum and three of the multifidus muscle for each patient. The flowchart of this stage is described in Figure1. Search for pathogens protocol is similar to the one proposed by Levy et al[19], although due to the avascular nature of the intervertebral disc, we will inoculate the sonication fluid (after sample concentration) in blood cultures bottles (automated system) to improve the recovery of pathogens and reduce contamination.

Search for pathogens:

The herniated intervertebral disc will be split equally into three fragments of 2x2x5 mm and flattened in a laminar flow cabinet until a homogenous material is achieved. The same process will be carried out for the samples of ligamentum flavum and multifidus. Tissues will be cultured in specific growth medium and incubated according to the respective culture:

Similar criteria used by the Infectious Diseases Society of America (IDSA) to detect joint replacement infection will be adopted, which is the recommendation on at least two positive tissue cultures by the same pathogen to

confirm diagnosis of infection[20]. This same criterion was already adopted in a study to characterize isolated pathogens in herniated intervertebral discs[14].

- **A - Aerobic culture**

For aerobic cultures, samples will be cultured in 5% sheep blood agar, chocolate agar and MacConkey agar plate, and will be placed in a 35°C incubator (CO2 atmosphere) for 5 days. If a positive bacteria culture is detected in the plate, the colony will be identified by MALDI TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) Microflex LT (Bruker Daltonics/BD). Sensitivity profile will be performed when needed, according to CLSI (Clinical Laboratory Standards Institute) recommendations.

- **B - Anaerobic culture**

For anaerobic culture, tissue will be cultured in a Thioglicolate tube and incubated in a 35°C incubator for up to 14 days. If turbidity occurs at the Thioglicolate medium, material will be cultured in anaerobic blood agar and incubation at 35°C will be done in an anaerobic atmosphere. After growing of colonies, identification will be done by MALDI TOF and, when necessary, a sensitivity test to antibiotics will be performed according to CLSI recommendations.

- **C - Histological analysis**

Anatomic pathology analysis of the remaining fragment of the herniated disc (2x2x5mm) will be completed. The sample will be transported in a universal container with tamponated formalin (10%), followed by dehydration in alcohol diafanized in xylol and inclusion in paraffin (60-65°C), which will be stained in hematoxylin-eosin (HE) and GRAM staining.

  - **HE:** Histological cuts of 4µm will be performed, followed by clearing with xylol for 10 minutes twice, embedding with alcohol under increasing concentrations and stained with hematoxylin for 5 minutes, running water for 5 minutes, eosin for 1 minute and running water for 2 minutes followed by assembly of glass microscope slide with Entellan®.
  - **GRAM:** Another slide will be embedded with crystal violet for 1 minute, running water for 1 minute followed by lugol for another 1 minute and additional wash with running water. Unstaining of the slide will be done with alcohol 95% for 10 seconds followed by running water and then, stain with fuchsine for 30 seconds, running water wash again, drying and slide assembly with Entellan®.

- **D – Molecular analysis of pathogens**

Positive cultures that present aerobic or anaerobic pathogens culturing, will be isolated and stowed refrigerated in -80°C freezer for posterior molecular analysis.

Molecular typing will be performed through Pulsed Field Gel Electrophoresis technique of isolated samples according to the protocol described by Oprica et al.[21] using Spe-I restriction enzyme and Bionumerics software for analysis of results.

Questionnaires

Scores for pain (NRS), function (ODI), and quality of life (EQ-5D) will be self assessed and applied before surgery and at 1, 3, 6 and 12 months time-points. All questionnaires will be collected by a employee not involved in the study. Follow-up clinic visits will be at 1, 3, 6 and 12 months after surgical procedure, with acceptance deviation of 7, 14, 21, and 28 days, respectively (Table 1).

Imaging studies

Magnetic resonance imaging studies will be performed in Siemens or General Electric 1.5T devices. Studies in 3.0T devices will not be done due to higher frequency of artifacts generated by chemical shift, which would modify measurements of Modic changes.

The following sequences will be used:

- Sagittal cut: Fast Spin-Echo T1 and T2 weighted with fat suppression or STIR instead of T2, according to our institution protocol established for all exams
- Coronal cut: same sequence imaging will be used for measurements.

At follow-up, contrasted MRI studies of patients will be done as an institution established protocol for postoperative patients, otherwise, it will not interfere with the study protocol.

Lumbar disc herniation will be diagnosed through MRI in eligible patients. Included patients will be submitted to new MRI 12 months after surgery.

Two radiologists with expertise in musculoskeletal MRI will independently classify and perform measurements, and divergences will be solved blindly by common opinion.

Confounding variables

Data on the following confounding variables will be collected: age, gender, alcohol intake, smoking, body mass index (BMI), spinal injections with corticoid within 6 months before surgery, usage of oral corticoids up to 3 months before surgery, diabetes.

Since this information may change over time, data will be considered at time of last assessment before surgery.

A – Alcohol intake: categorized as – none or sporadically (<1 glass/day); light intake (1-2 glass/day); moderate/heavy intake (3 or more glasses/day); and, not assessed[22].
B – Smoking: categorized as – ex-smokers; smoker; non-smoker; and, not assessed[23].
C – BMI: categorized as – underweight (<18.5); normal weight (18.5-25); overweight (25-30); obese (>30); not assessed or not available[24].

ETHICS AND DISSEMINATION

This study started only after approval of our Institutional Review Board (IRB) (CAAE 65102617.2.0000.0071). Patients agreeing to participate will sign an Informed Consent Form before entering the study. Results will be published in a peer-reviewed medical journal and presented in medical conferences independently of study findings.

Some authors suggest that subclinical infection of the intervertebral disc is one of the causes of chronic low back pain unresponsive to treatment, besides promoting Modic type I changes on MRI. Although there are studies reporting relative success on treatment of well-selected patients, there is still uncertainty as to whether these patients were actually infected or not.

We hope to define the accurate incidence of subclinical infection of the intervertebral disc with disc herniation and provide data and possibly an answer to this present gap in the literature.

STATISTICAL PLANNING

Rate of subclinical infection (or Modic change) will be obtained by the ratio between number of positive cultures from surgical samples and total number of patients, and estimates will follow 95% confidence intervals. After infection cases are identified, we will investigate if there is an association between detected infection and patient outcomes by logistic regression models for Modic changes, ordinal logistic regression for Modic volume and size, and linear regression or general linear models for numeric outcomes, such as: low back pain, quality of life and function. All models will consider confounding variables such as: smoking and alcohol intake, diabetes, corticosteroids injection, BMI, gender, and age. Results will be presented as effects estimates such as odds ratio or mean ratio, 95% confidence intervals and *p* values. Study dropout cases, for any reason, will be considered for final analysis.

Sample size calculation

Sample size was calculating to estimate the incidence of subclinical infection in patients with lumbar disc herniation. Considering that the rate of infection lies around 46%[3], we need to observe a minimum of 95 patients to achieve a 95% confidence interval with 10% absolute accuracy.

The necessary sample size required for analysis of the secondary endpoints will depend on the observed rate of cases with subclinical infection in our study sample. If the observed rate is too small, an increase in the number of included patients will be needed. To better evaluate this, the sample size calculation will be revisited by the time we have reached half of initially planned sample size (48 patients).

CHRONOGRAM

At the end of the protocol (Table 1)

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COMPETING INTERESTS STATEMENT

Authors have no potential conflict of interests.

AUTHORS CONTRIBUTIONS

Nelson Astur – Spine surgeon – Conception and design of the work, data collection, data analysis and interpretation, drafting the article

Delio E. Martins – Spine surgeon – Conception and design of the work, data collection, data analysis and interpretation, critical revision of the article, final approval of the version to be published

Marcelo Wajchenberg – Spine surgeon – Data collection, critical revision of the article, final approval of the version to be published

Mario Ferreti – Orthopedic surgeon – Critical revision of the article, final approval of the version to be published

Fernando G. Menezes – Infectologist – Conception and design of the work, final approval of the version to be published

Andre M. Doi – Microbiologist – Conception and design of the work, data collection, data analysis and interpretation

Laercio A. Rosemberg – Radiologist – Design of the work, data analysis and interpretation, data collection

Durval C. B. Santos – Radiologist – Design of the work, data analysis and interpretation, data collection

Alexandre S. Yutaka – Spine surgeon – Data collection, critical revision of the article

Luciano M. R. Rodrigues – Spine surgeon – Data collection, critical revision of the article

Marines D. V. Martino – Microbiologist – Data collection, critical revision

Jorge R. Pagura - Spine surgeon – Data collection, critical revision of the article

Eduardo N. Kihara Filho - Radiologist – Design of the work, data analysis and interpretation, data collection

Mario Lenza – Orthopedic surgeon – Conception of the work, critical revision of the article, final approval of the version to be published

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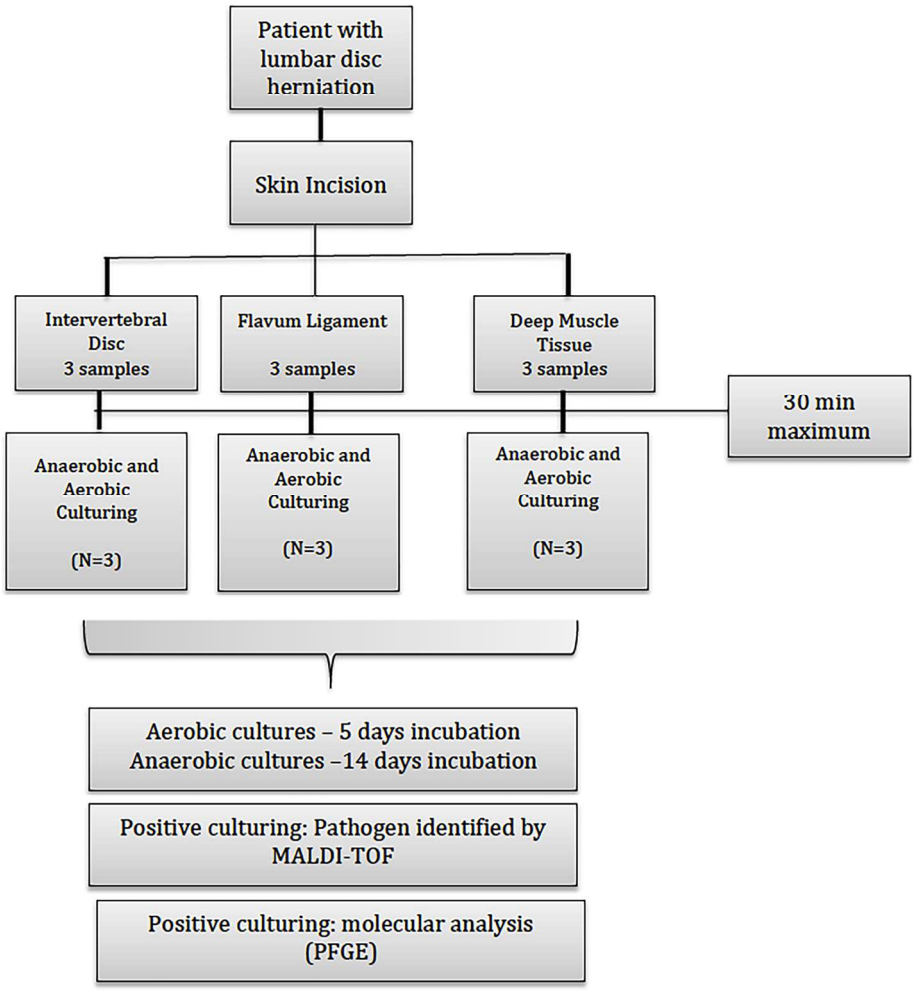
Figure legends

Figure 1: Flowchart of collected clinical samples that will be sent to culture analysis.

Table 1: Chronogram of included patients

	Screening/ Recruiting	Postop 4 weeks ± 7 days	Postop 3 months ± 14 days	Postop 6 months ± 21 days	Postop 12 months ± 30 days
	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
ICF	X				
Check including and excluding criteria	X				
Collect demographic data	X				
Investigate medical history / Complete enrollment form	X				
Lab screening, ESR, CRP, Leucogram	X	X		X	X
Apply NRS tool	X	X	X	X	X
Apply Oswestry questionnaire	X	X	X	X	X
Apply EQ-5D questionnaire	X	X	X	X	X
Physical therapy		X			
Magnetic resonance imaging					X

ICF = Informed Consent Form; ESR = erythrocyte sedimentation rate; CRP = C reactive protein; NRS = Numeric rating scale; EQ-5D = European quality of life 5 dimensions.



Flowchart of collected clinical samples that will be sent to culture analysis.

150x150mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **pg1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **attached to submission**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **pg2**
Objectives	3	State specific objectives, including any prespecified hypotheses **pg3**
Methods		
Study design	4	Present key elements of study design early in the paper **pg3**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **pg3-4**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up **pg3-4** *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed **N/A** *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **pg5-6**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **pg7-8**
Bias	9	Describe any efforts to address potential sources of bias **pg 9**
Study size	10	Explain how the study size was arrived at **pg 10**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why **pg 9**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **pg 9**
		(*b*) Describe any methods used to examine subgroups and interactions **pg 9**
		(*c*) Explain how missing data were addressed **pg 4**
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed **pg 4** *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results PROTOCOL OF STUDY – NO RESULTS AVAILABLE		
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(b) Give reasons for non-participation at each stage **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(c) Consider use of a flow diagram **PROTOCOL OF STUDY – NO RESULTS AVAILABLE (Table 2)**
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(b) Indicate number of participants with missing data for each variable of interest **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount) **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses **PROTOCOL OF STUDY – NO RESULTS AVAILABLE**
Discussion PROTOCOL OF STUDY – NO DISCUSSION BASED ON RESULTS AVAILABLE		
Key results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based **pg 1 and pg 16**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at

http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.