PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Subclinical Propionibacterium Acnes Infection Estimation in the Intervertebral Disc (SPInE-ID): Protocol for a Prospective Cohort.
AUTHORS	Astur, Nelson; Martins, Delio; Wajchenberg, Marcelo; Ferreti, Mario; Menezes, Fernando; Doi, Andre; Rosemberg, Laercio; Santos, Durval; Iutaka, Alexandre; Rodrigues, Luciano; Martino, Marines; Pagura, Jorge; Kihara Filho, Eduardo; Lenza, Mario

VERSION 1 – REVIEW

REVIEWER	Dr Prashanth J Rao Prince of Wales Private Hospital, University of New South Wales Sydney Australia
REVIEW RETURNED	18-Jun-2017

GENERAL COMMENTS	At present, the standard of English is insufficient for publication. Summary: the study I poised to answer an important question of if disc infection is associated with back or radicular pain. The authors identified several issues with the current literature but fail to clarify the reason for the study. Several investigations are being performed but the reason for each hasn't been discussed. Abstract: Fails to clarify the gist of the study. Introduction: English editing is needed : Unspecific= nonspecific Differentiation between infection and modic changes: what does this mean? Doesn't make sense Null hypothesis doesn't make sense either.What does this mean ? CIENTIFIC CHALLENGES : wrong spelling Methods: Inclusion criteria: What will be the indication for surgery? Is it radiculopathy and if so what is the timeframe for conservative management? Is transforaminal or epidural injections a contraindication for the study? What is the definition of real infection?

	What is the purpose of collecting the ligamentum and muscle sample ? What is the role of histopathology? How do you determine contamination? What is the definition of chronic back pain? I presume discectomy is performed for radiculopathy and not back pain. If so how would you use this cohort for comparing back pain and infection . What is the rationale for 3 days of incubation only for aerobic cultures?while it is 21 days for anaerobic cultures. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Ofer Levy Royal Berkshire Hospital and Berkshire Independent Hospital, Reading, United Kingdom
REVIEW RETURNED	29-Jun-2017

GENERAL COMMENTS	Overall, good protocol. A few methodological comments that should be addressed: In order to decide between real infection and contamination, the authors should take more specimen ((5 to 7 specimen) and regard as infection only if more than 50% are positive. They should use advanced techniques as well as broth as described by Levy et al. (Propionibacterium acnes: an underestimated etiology in the pathogenesis of osteoarthritis? J Shoulder Elbow Surg (2013) 22, 505-511). They should take as well control samples of swabs from the theatre air.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name
Dr Prashanth J Rao

Institution and Country
Prince of Wales Private Hospital, University of New South Wales
Sydney
Australia

Please state any competing interests or state 'None declared':
None declared.

Please leave your comments for the authors below

Comment: At present, the standard of English is insufficient for publication.

Response: English has been reviewed throughout whole manuscript.

Summary: the study I poised to answer an important question of if disc infection is associated with back or radicular pain. The authors identified several issues with the current literature but fail to clarify the reason for the study. Several investigations are being performed but the reason for each hasn't been discussed.

Response: We agree. Although there is a trend in the literature to justify low back pain due to subclinical disc infection, most of them have weak methodologies and are all from the same study group. Furthermore, this same group was the only to find such high rates of disc infection related to low back pain, suggesting treatment with antibiotics. Additional research from other groups failed to identify such infection and also had methodological issues putting their results into question. Based on all this previous reports, we developed a very strict and sound methodology to detect disc infection and differentiate it from a surgical contamination. We have also included molecular analysis of samples to confirm if pathogens are from the disc ("real infection") or contamination from skin pathogens.

Abstract:
Comment: Fails to clarify the gist of the study.

Response: Abstract has been restructured and clarified for a better understanding. We reformulated study objectives and also added an impact of possible results.

Introduction:
English editing is needed :
Unspecific= nonspecific

Comment: CIENTIFIC CHALLENGES : wrong spelling

Response: English has been reviewed throughout whole manuscript.

Comment: Null hypothesis doesn't make sense either. What does this mean ?

Response: Null hypothesis means equality of endpoints, showing no diference between comparisons. We have reformulated hypothesis section and merged it to objectives.

Comment: Differentiation between infection and modic changes: what does this mean? Doesn't make sense.

Response: MRI findings are not always clear and precise to differentiate inflammatory Modic changes and infection imaging changes such as discitis. When diagnosis is not clear, a biopsy is often required. We have clarified the sentence related to this comment for an easier understanding.

Methods
Comment: Inclusion criteria: What will be the indication for surgery? Is it radiculopathy and if so what is the timeframe for conservative management?

Response: Indication for surgery is sciatica caused by a nerve root compressed by a vertebral disc herniation failing conservative treatment for at least six weeks or presenting neurological deficit. This has been included into the manuscript.

Comment: Is transforaminal or epidural injections a contraindication for the study?

Response: No. Previous spinal injections are not a exclusion criteria. This has been clarified into the text.

Comment: What is the definition of real infection? What is the purpose of collecting the ligamentum and muscle sample?

Response: Real infection is a positive disc culture without muscle or ligamentum positive cultures, meaning it is not a surgical contamination. Muscle and ligamentum samples were also collected in different surgical time points to define whether a positive culture represents surgery contamination of tissues (muscle and/or ligamentum cultures for pathogens are positive) or a real disc infection (when only disc sample results in a positive culture). For better understanding, we have replaced the term "real infection" to "disc infection" opposed to "disc contamination".

Comment: What is the role of histopathology?

Response: Histopathology could possibly indicate signs of tissue damage and inflammation, reinforcing the infective etiology.

Comment: How do you determine contamination?

Response: When muscle and/or ligamentum sample cultures are positive for same pathogens. We are analyzing both deep and superficial tissues to determine contamination.

Comment: What is the definition of chronic back pain?

Response: A low back pain for at least three months (12 weeks). This has been included into the text.

Comment: I presume discectomy is performed for radiculopathy and not back pain. If so how would you use this cohort for comparing back pain and infection.

Response: The only ethical way to collect disc samples to identify a possible disc infection related to degenerative disc disease is by surgically removing it during a discectomy procedure. Furthermore, degenerative disc disease that leaded this cohort to a disc herniation is also related to low back pain.

Comment: What is the rationale for 3 days of incubation only for aerobic cultures? While it is 21 days for anaerobic cultures.

Response: The incubation time for aerobic cultures follow the recommendations of IDSA and ASM guidelines*. For anaerobic cultures we corrected the information at the protocol. Anaerobic cultures will be incubated for 14 days.

Reference: *Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson RB Jr, Bourbeau P, Carroll KC, Kehl SC, Dunne WM, Robinson-Dunn B, Schwartzman JD, Chapin KC, Snyder JW, Forbes BA, Patel R, Rosenblatt JE, Pritt BS. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). Clin Infect Dis. 2013

Reviewer: 2

Reviewer Name
Ofer Levy

Institution and Country
Royal Berkshire Hospital and Berkshire Independent Hospital, Reading, United Kingdom

Please state any competing interests or state ‘None declared’:
None declared

Please leave your comments for the authors below
Overall, good protocol.
A few methodological comments that should be addressed:

Comment: In order to decide between real infection and contamination, the authors should take more specimen ((5 to 7 specimen) and regard as infection only if more than 50% are positive.

Response: In order to follow the recommendation of the reviewer we will collect 3 fragments of the disc to perform anaerobic and aerobic cultures. Despite we agree that 5 to 7 specimens should be more appropriate, we have limited budget resources to process all this amount.

Comment: They should use advanced techniques as well as broth as described by Levy et al. (Propionibacterium acnes: an underestimated etiology in the pathogenesis of osteoarthritis? J Shoulder Elbow Surg (2013) 22, 505-511).

Response: We will use a similar protocol to that published by Levy et al. However once the vertebral disc is an avascular tissue, we are going to inoculate the sonication fluid (after sample concentration) in blood cultures bottles (automated system) to improve the recovery of pathogens and reduce contamination.

All procedures will be performed in a class 2 laminar flow safety cabinet. The laboratory is certified by College of American Pathologists and Joint Commission. We have referenced Levy et al study for better understanding and provided further details.

Comment: They should take as well control samples of swabs from the theatre air.

Response: Air quality in Operating Rooms and Surgical Suites is controlled and monitored by an experienced company. This is protocoled by our hospital administration and infection control. Otherwise, patient's muscle is exposed to air during the whole surgical procedure. A positive muscle culture would point a possible contamination of surgical site, opposed to disc infection. Furthermore, if any pathogen is cultured, pulsed field technique will be performed to determine if there was any external contamination.