24/10 2012

Does nuclear tissue infected with bacteria following disc herniations lead to Modic changes in the adjacent vertebrae?

Hanne B. Albert, PT, MPH, Ph.D.*
Peter Lambert, B.Sc, Ph.D., D.Sc.**
Jess Rollason. BSc, Ph.D.**
Joan Solgaard Sorensen, MD.
Tony Worthington, FIBMS, CSci, Ph.D. FHEA**
Mogens Bach Pedersen, MD***,
Hanne Schack Nørgaard, RN***
Ann Vernallis, Ph.D.**
Frederik Busch, MD***
Claus Manniche, MD, Dr Med Sci*
Tom Elliott, BM, BS, B.MedSci, BTech, PhD, MRCPFRCPath, D.Sc **

* Research Department, Spine Centre of Southern Denmark, Hospital Lillebaelt, Middelfart, Institute of Regional Health Services Research, University of Southern Denmark, Clinical Locomotion Science Network.

** The School of Life and Health Sciences at Aston University, Birmingham, UK & University Hospital NHS Trust, Birmingham, UK

*** The Mølholm Private Hospital, Vejle, Denmark

ABSTRACT

Background

Modic changes (MC) (bone oedema) in vertebrae are observed in 6 % of the general population and in 35-40 % of people with low back pain. These changes are strongly associated with low back pain.

There are probably two pathogenetic mechanisms which cause MC. A mechanical cause, where degeneration in the disc results in micro fractures in the endplates result in oedema or an infective cause. Indeed several studies on nuclear tissue from herniated discs have demonstrated the presence of low virulent anaerobic microorganisms, predominantly *Propionibacterium acnes, in 7 to 53% of patients.* At the time of a herniation these low virulent anaerobic bacteria may enter the disc and give rise to an insidious infection. Local inflammation in the adjacent bone may be a secondary effect due to cytokine and propionic acid production. A small cohort study has also shown a positive response to treatment with antibiotics of patients with MC after a lumbar disc herniation.

Aims:

To investigate the prevalence of infected herniated nucleus material in lumbar disc herniations and to determine if patients with an infected disc are more likely to develop MC (bone oedema) in the adjacent vertebrae after the disc herniation.

Results

Sixty one patients were included, mean age 46.4 years (SD 9.7), 27% female. All patients were immuno-competent. No patients had received a previous epidural steroid injection or undergone back surgery. In total, microbiological cultures were positive in 28 (46%) patients. Anaerobic cultures were positive in 26 (43%) patients, and of these 4 (7%) had dual microbial infections, containing both one aerobic and one anaerobic culture. No tissue specimens had more than two types of bacteria identified. Two (3%) cultures only had aerobic bacteria isolated.

In the discs with a nucleus with anaerobic bacteria, 80% developed new MC in the vertebrae adjacent to the previous disc herniation. In contrast, none of those with aerobic bacteria and only 44% of patients with negative cultures developed new MC. The association between an anaerobic culture and new MCs is highly statistically significant ($p=0.0038$), with an odds ratio of 5.60 (95% CI 1.51 to 21.95).

Conclusion

In this study, 46 % of the 61 patients with a lumbar disc herniation were found to have extruded nuclear tissue which contained microorganisms. The predominant microorganism isolated was the bacterium *Propionibacterium acnes.* These findings support the theory that the occurrence of Modic Changes (MCs) Type 1 in the vertebrae adjacent to a previously herniated disc may be due to oedema surrounding an infected disc. The discs that were infected with anaerobic bacteria were much more likely ($p<0.0038$) to develop MCs in the adjacent vertebrae than those in which no bacteria were found or those in which aerobic bacteria were found.

Key words. Bacterial infection, Modic changes, endplate changes, *Propionibacterium acnes*, lumbar disc herniation

BACKGROUND

Modic changes (MC) are bone oedema in vertebrae and have been shown to be both commonly observed and associated with low back pain [1,2]. A recent systematic review showed that the prevalence for any type of MC in patients with chronic non-specific low back pain (CLBP) was 46% as opposed to 6% in the general population [1]. A positive association between MC (bone oedema) and non-specific LBP was found in 70% of studies with odds ratios ranging from 2.0 to 19.9 [1]. The significance of these findings should not be underestimated as CLBP is universally acknowledged to be extremely difficult to reliably attribute to specific pathoanatomical causes [3].

MC are only visible on magnetic resonance imaging (MRI) and three types have been identified (Types 1, 2 and 3) [4]. MC Type 1 consists of disruption and fissuring of the endplate, micro fractures of the trabeculae, extracellular water and vascularised tissue within the adjacent marrow. MC Type 2 consists of disruption and fissuring of the endplate, micro fractures of the trabeculae, and replacement of the haematopoietic elements of the marrow by yellow fat. MC Type 3 are sclerotic bone [4]. The temporal development of MC is uncertain, but the time span is years. Several studies have examined the reliability of reporting MC and all report excellent inter- and intra-observer reliability [5,6]. In comparative studies, MC have demonstrated a higher reliability than other MRI findings, such as disc herniations [7,8].

There are two possible pathogenetic mechanisms resulting in MC. These are:

(i) A mechanical cause, where degeneration of the disc results in the loss of soft nuclear material and reduced disc height and hydrostatic pressure. This increases the shear forces on the endplates with the result that micro fractures may occur. The observed MC could be caused by oedema that is secondary to the fractures and subsequent inflammation, or the result of an inflammatory process associated with a toxic stimulus from the nucleus pulposus that permeates through the fractures [9].

(ii) An infective cause. In nuclear tissue removed under strict sterile conditions during surgery for lumbar herniated discs, 53% of patients were found to have present low virulent anaerobic microorganisms (*P. acnes* and *Corynebacterium propinquum*) [10]. This was in contrast to none of patients who had undergone surgery for other spinal disorders such as scoliosis [11]. *P. acnes* are commonly found in hair follicles in the skin and in the oral cavity. They frequently invade the circulatory system during tooth brushing where they do not present an immediate health risk due to the aerobic environment of the blood stream [12-14]. When an intervertebral disc is herniated nuclear material extrudes into the spinal canal. Within a short time, neocapillarisation begins in and around the extruded nucleus material [15-19] and inflammation occurs with an increased presence of macrophages [17-19]. As the avascular anaerobic disc provides an ideal environment for anaerobic bacteria it is plausible that these low virulent anaerobic bacteria may enter the disc and give rise to a slowly developing infection. Local inflammation in the adjacent bone (MC type 1) may be a secondary effect due to cytokine production or microbial metabolites (e.g. propionic acid) entering the vertebrae through normal disc nutrition. Studies have shown a dramatic (310%) increase in the frequency of MC type 1 following a lumbar disc herniation [20]. The anaerobic bacteria are located in the anaerobic disc, and the MC may result from this adjacent infection. P. acnes is known from the skin to trigger and adjacent inflammatory response [21]. *P. acnes* cannot multiply in the highly vasculated aerobic bone and are therefore not present where the MC occur as, shown by Wedderkopp et al [22].

A small cohort study has also demonstrated a very positive response to treatment with antibiotics in patients with MC after a lumbar disc herniation [23]. These findings lend credence to the theory that

MC that develop after a lumbar disc herniation may be caused by a bacterial infection in some patients.

Aims:

1. To investigate if herniated nucleus material from lumbar disc herniations is infected with bacteria.
2. To determine if patients with an anaerobic infected disc are more likely to develop MCs following a disc herniation as compared to patients with sterile discs.

METHODS

Study subjects

This study involved a cohort of patients undergoing primary surgery at a single spinal level for lumbar disc herniation. Patients were included if they were between 18 and 65 years of age and had an MRI-confirmed lumbar disc herniation, where the annular fibres were penetrated by visible nuclear tissue. Patients were excluded if they had received any antibiotic treatment within the previous two weeks. All patients had an MRI at baseline and 1-2 years after surgery.

Collection of biopsies

To avoid skin contamination, stringent antiseptic sterile protocols were followed, including, cleaning the skin in the operation field, preoperatively, for 2 minutes with 2% chlorhexidine in 70% isopropyl then allowing the solution to dry. The nucleus material was evacuated in 5 biopsies, each with a new set of sterile instruments. The five glass vials in which the nucleus material had been placed were immediately frozen at –80 degrees Celsius. One high dose 1.5 g. Cefuroxin (antibiotics) were administered intravenously after the biopsies were obtained in order to avoid inhibiting any bacteria present in the biopsies before the tissue was examined. The samples were transported to Aston University in the special thermal transport boxes used for organ transport between hospitals. They were covered in frozen carbon dioxide (-80°).

The follow- up MRI's was all to be performed in the same MRI-scanner. An open low field 0.2 T, MRI unit with a body spine surface coil. The patients were placed in the supine position. Five sequences of localized images were taken, two coronal and three sagittal.
Patients were included regardless if they had Modic changes at baseline. Both baseline and follow-up MRI were evaluated by the same consultant radiologist, who was an experienced research radiologist, blinded to the laboratory results. The type, size and volume were graded according to the Nordic Modic Protocol [24]. At the follow-up MRI the occurrence of new Modic changes type 1 or 2 at the level of the previous disc herniation was graded a positive finding.

Detection, culturing and identification of bacteria

Microbiological examination of tissue samples
Five tissue samples were collected from each patient. Under sterile conditions, extracted tissue was sectioned and finely ground using an individually packaged sterile, gamma irradiated scalpel (Swann Morton) and a sterile, gamma irradiated petri dish. Once the packaging was opened all scalpels were flamed before use as an extra precaution to ensure sterility. Tissue samples were then

spread onto and embedded into Columbia blood agar plates (Oxoid UK,) which were then incubated under aerobic and anaerobic conditions for 7 days at 37°C.

Microbial identification following anaerobic and aerobic culture growth

Following aerobic and anaerobic culture incubation resulting colonies were sub-cultured onto Columbia blood agar plates and incubated for 24 hours at 37°C in aerobic and anaerobic conditions. All colonies were investigated by Gram staining. Presumptive *P. acnes* colonies were identified following Analytical Profile Index (API) biochemical analysis using the Rapid ID 32A kit (bioMerieux) and by Polymerase Chain Reaction (PCR) amplification of 16S rDNA. Specific primers designed for amplification of *P. acnes* 16S rDNA were used to amplify a 600bp region: forward primer 5′- GGGTTGTAAACCGCTTTCGCCT -3′ and reverse primer 5′- GGCACACCCATCTCTGAGCAC -3′ [25]. Chromosomal DNA was extracted from single selected colonies by a rapid boil extraction method [26]. PCR was performed in a 25µl volume containing 19.8 µl of SDW, 2.5µl of 10x PCR buffer (10mM Tris HCl pH 8.3, 3.5mM $MgCl_2$, 25mM KCl), 0.2µl of each primer (25pmoles/µl), 0.2µl of dNTPs (10mM each nucleotide), 0.1µl of *Taq* DNA polymerase (1.25units/µl) and 2µl of template DNA. A positive control (with previously amplifiable *P. acnes* DNA) and a negative control (sterile water as template) were included. The following PCR conditions were used: initial 94°C denature for 4 min; 35 cycles of 94°C denature for 30 sec, 54°C annealing for 30 min, and 72°C extension for 1min; 72°C extension for 4 min; 4°C hold. A 2% agarose gel containing 1µg/ml of ethidium bromide was used to separate amplified fragments. Electrophoresis was performed in 1x TAE (40mM Tris, 1mM EDTA and 0.1% ($^{v}/_{v}$) glacial acetic acid buffer at 100 volts. Gram-positive cocci were further analysed using standard biochemical tests (oxidase and catalase) to identify presumptive staphylococci. In addition, latex agglutination for clumping factor/protein A was used to distinguish presumptive *S. aureus* and coagulase negative staphylococci.

Detection of bacterial DNA in tissue samples by PCR

To determine the presence of bacterial DNA in all tissue samples, including culture-negative samples, PCR amplification of bacterial 16S rDNA was undertaken. A sample of each tissue (approximately 50-100 mg wet weight) was aseptically transferred to a sterile 1.5ml microcentrifuge tube. Each tissue sample was suspended in 200µl of Tris-EDTA buffer, 100 µl proteinase K (10mg/ml) and 240µl sodium dodecyl sulphate (10% w/v) and incubated for 48 hours in a water bath at 45°C. Following incubation, DNA was extracted from the lysed tissue samples with phenol/chloroform. Detection of microbial 16S rDNA was performed by PCR using broad range universal bacterial primers to amplify a 466bp region: forward primer 5′- TCCTACGGGAGGCAGCAGT-3′ and reverse primer, 5′- GGACTACCAGGGTATCTAATCCTGTT-3′ [27]. Amplification was performed as described above for the *P. acnes* identification. A positive control (using previously amplifiable *P. acnes* DNA) and negative control (sterile water as template) was run for every PCR performed. All samples giving a positive result with the broad range primers were also subjected to PCR using the *P. acnes*-specific primers as described above for identification of *P. acnes* colonies.

Statistics

To determine the association between anaerobic culture and new Modic changes a Fisher's Exact Test and relative risk calculations were utilised. The Statistical programme used was SPSS 13.

Ethical considerations

The study was approved by The Scientific Ethics Region of Southern Denmark no: VF-20060085. All patients signed a written informed consent.

RESULTS

Sixty-seven patients were included in the study. In three patients, it was not possible to evacuate nuclear material during surgery, and another three patients did not have an MRI taken at 1 year follow-up due to the long distance to The Spine Centre. Of the remaining 61 patients who were included in the study, the mean age was 46.4 years (SD 9.7) and 27% were female. Of the 61 baseline MRI's, 50 were performed at the operating hospital. 11 came from 11 other hospital, and had a sufficient quality to evaluate the disc herniation and possible Modic changes. All follow-up MRI were performed at The Back Research Center. The mean time between the two MRI were 424 days (range 334 – 546)

All patients were immunocompetent. No patients had received a previous epidural steroid injection or undergone back surgery. None of the patients developed clinically evident post-operative discitis.

In total, cultures were positive in 28 (46%) of the 61 patients. Anaerobic cultures were positive in 26 (43%) patients, and of these 4 (7%) had two microorganisms present with all having, both one aerobic and one anaerobic microorganism present. No patients had more than two types of bacteria isolated. Two (3%) patients had only aerobic bacteria isolated. This distribution of the 61 who had two MRI is summarised in Table 1. The remaining three patients who did not have a follow-up MRI, one was infected with *P.Acnes* two were negative. All tissues demonstrating positive cultures of *P. acnes* produced single bands of the expected size following PCR amplification for both the general bacterial and *P. acnes*-specific primers. All culture negative tissues were also PCR negative.

	Isolated micro organisms	Herniated discs N=61		Of the herniated discs with positive microbiology n=28
Anaerobic				
	Propionibacterium acnes	24 (40%)		86%
	Coagulase-negative staphylococci	2 (3%)		7%
Aerobic				
	Gram positive cocci (1 single)	4 (6%) *		14% *
	Gram negative rod (1 single)	1 (1.5%)		3%
	Neisseria species	1 (1.5%) **		3% **
	Positive cultures	52% (46%)****		113% ***

Table 1.
The distribution of bacteria in the positive cultures.

*** This figure is 113% is due to the fact that 4 patients had 2 microorganisms isolated with one anaerobic and one aerobic culture.

**** This figure is 52% as opposed to the overall 46% because 4 patients had mixed microorganisms isolated and one patient had a positive anaerobic and one aerobic culture.

In the discs with a nucleus from which anaerobic bacteria were isolated, 80% developed new MCs in the vertebrae adjacent to the previous disc herniation. In contrast, 0% of those infected with aerobic bacteria developed new MCs and 44% did so in the negative cultures. Table 2 shows the numbers of patients developing new MCs, stratified by infection type.

The association between anaerobic culture and new Modic changes is shown as a contingency table in Table 2. A Fisher's Exact Test shows this association to be highly statistically significant ($p=0.0038$), with an odds ratio of 5.60 (95% CI 1.51 to 21.95). If the contingency table is reconstructed using only those patients whose disc material was infected the association between anaerobic infection and new MCs is a relative risk of 'infinity' compared with those with aerobic infection, as no MCs developed in those with aerobic infection.

	New MCs at the site of the disc herniation	No new MCs	Totals
Positive anaerobic culture	20	5	25
Pure aerobic culture	0	2	2
Negative culture	15	19	34
Totals	35	26	61

Table 2. Contingency table for the association between anaerobic culture and new Modic changes.

DISCUSSION

In this study, 46 % of the 61 patients with a lumbar disc herniation were found to have microorganisms present in extruded nuclear tissue. The microorganism most frequently cultured was the anaerobic bacterium, *P. acnes.* The discs from which anaerobic bacteria were isolated were statistically significant ($p<0.0038$) more likely to develop MC, than those in which no bacteria or aerobic bacteria were detected. The findings of this study could be interpreted as a support the theory that the occurrence of Modic Changes (MCs) Type 1 in the vertebrae adjacent to the previously herniated disc might be due to oedema surrounding an infected disc.

This study confirms the original findings of Stirling et al [10,11] demonstrating that extruded nucleus material frequently has microorganisms present, a finding replicated in other studies. Corscia et al [28] evacuated extruded disc material in 30 lumbar disc herniations and reported that 71 % had microorganisms present, 36 % with a staphylococcus and 18 % with *P. acnes*. In 30 cervical disc herniations they found that 59 % had microorganisms present, 37 % with *P. acnes*. Agarwal et al [29] similarly cultured material excised from 52 patients treated with a single level microdiscecetomy for lumbar disc herniation. Ten (19%) of the patients had microorganisms

present and in 7 (70%) of those, *P. acnes* was the sole microorganism isolated. Fritzell et al [30] investigated 10 patients with a lumbar disc herniation without any clinical signs of an infection. Those biopsies were analysed with Polymerase Chain Reaction (PCR) and in 2 patients (20%) bacteria were identified, either *Bacillus cereus* or *Citrobacter braakii/freundii.* The authors expressed their surprise that a normally anaerobic tissue such as the disc had bacteria present in 20% of their patients.
Ben-Gamlin et al [31] expressed an alternative explanation of these findings. In their samples of evacuated nucleus material, they suspected that contamination was the reason for 2 (7%) of the 30 patients having coagulase-negative staphylococci. The operations were performed under stringent sterile conditions and the samples were cultured both anaerobically and aerobically, but all patients received large doses of antibiotics (Cefazolin) before the operation when the biopsy was later obtained. These high doses of antibiotics to which P. acnes is sensitive may partly explain the low isolation rate noted in this study.
Six studies have demonstrated the presence of bacteria in nucleus tissue evacuated from patients with lumbar disc herniation. In these studies the rates of isolation ranged from 7% to 53%, which may reflect the extended length of incubation P. acnes requires for growth and the need for rapid processing following excision both of which can influence successful isolation. Different culture conditions will also affect the growth of *P. acnes*. In addition, further work by our group has demonstrated in a pilot study that patients with Modic type 1 changes in the adjacent disc following a lumbar disc herniation respond to long term antibiotic treatment, which could be interpreted as a possibility that the presence of these organisms is resulting in an infection. [23].

The current study confirms the findings of other studies [10,11,28-30] where the microorganism most frequently found in the extruded nucleus material is *P. acnes*, accounting for approximately 80 % of the positive cases. The predominance of *P. acnes* might reflect the unusual environment in the disc where the lack of vascularity results in a very low oxygen tension and a low pH which provides ideal conditions for low virulent anaerobic bacteria to grow. These bacteria are unlikely to survive so well in the highly vasculated aerobic bone and are therefore not present where the MC occur which was confirmed by Wedderkopp [22].

The key element of concern is whether the detected bacteria found in the nuclear material from the herniated discs of these patients are indicative of an infection or possibly due to intra-operative skin contamination. Every possible precaution to avoid contamination was undertaken, and the operation procedures were conducted under the strictest sterile conditions. If skin contamination was the cause of infection, a pattern of multiple skin bacteria cultures would be observed, usually include coagulase negative staphylococci. However, this was not the case in this study, where the majority were monocultures, a few were mixed cultures with 2 microorganisms, multi-cultures were absent. In addition, Stirling et al [11] showed in their study (using the same skin cleaning procedures as this study) that skin contamination was absent in their control patients. In 207 lumbar disc herniation operations they found 37 % of the nucleus material had bacteria present. This was in direct contrast to the 27 control patients (who underwent a similar spinal procedure but for scoliosis, trauma, or malignancy by the same surgical team using the same antiseptic procedures) from which no microorganisms were isolated. If the presence of microorganisms in the nuclear material was a result of skin contamination then some of these 27 control patients would have been expected to have demonstrated positive cultures.

Evacuations of herniated disc material from the patients were not caused by a suspicion of a clinical relevant spinal infection. This study was done for research purpose only, the cultures and the data analysis was performed with this aim.

A further question which needs addressing is why do some patients develop MC when no microorganisms are present in their herniated nuclear tissue? In the paper by Albert et al [9], at least two causal pathways for the development of new MCs are proposed. One theory is the infective pathway of the extruded nucleus material investigated in this study. But possibly the infection takes place later, after the nucleus materiel has been removed. After disc herniation surgery MRI shows contrast enhancement along the surgical tract in all patients 6 weeks and 6 months after surgery [32]. This enhancement is portraying a hypervascularisation, and it is possible that the bacteria might enter the disc at this later point. The other theory is the biomechanical pathway. All patients in this study had a lumbar disc herniation where there degeneration was observed, portraying loss of soft nuclear material, reduced disc height and hydrostatic pressure. These changes increase the shear forces on the endplates and micro fractures may occur. The observed MC that developed in the absence of infection is possible due to the result of a biomechanical impact, reflecting oedema secondary to micro fractures and subsequent inflammation, or a result of an inflammatory process from pro-inflammatory chemicals penetrating through the micro fractures from the nucleus pulposus as proposed by Adams et al [33,34].

Conclusions

This study confirms the findings of five previous studies which suggested that up to half of patients undergoing surgery for a first time disc herniation have infected extruded nucleus tissue, which is normally sterile. The predominant causative microorganism was the bacterium *P. acnes).* The findings of this study could be interpreted as a support the theory that the occurrence of Modic Changes Type 1 in the vertebrae adjacent to a previously herniated disc might be due to oedema surrounding an infected disc hence the discs that were infected with anaerobic bacteria were significantly more likely to develop MCs in the adjacent vertebrae than those in which no bacteria were found.

References

1. Jensen TS, Karppinen J, Sorensen JS, Niinimäki J, Leboeuf-Yde C (2008) Prevalence of vertebral endplate signal (Modic) changes and their association with non-specific low back pain - A systematic literature review" Eur Spine J.17:1407–22.
2. Albert HB, Manniche C. (2007) Modic changes following lumbar disc herniation. Eur Spine16:977-82.
3. Airaksinen O, Brox JI, Cedraschi C, et al (2006) European Guidelines: COST B13 Working Group on Guidelines for Chronic Low Back Pain. Eur Spine J. 15 Suppl 2:S192-300
4. Modic MT, Steinberg PM, Ross JS, et al (1988) Degenerative disk disease: assessment of changes in vertebral body marrow with MR imaging. Radiology 166:193-9.
5. Wang Y, Videman T, Niemeläinen R, Battié MC (2011) Quantitative measures of modic changes in lumbar spine magnetic resonance imaging: intra- and inter-rater reliability. Spine 36:1236-43.
6. Peterson CK, Gatterman B, Carter JC, Humphreys BK, Weibel A (2007) Inter- and intraexaminer reliability in identifying and classifying degenerative marrow (Modic) changes on lumbar spine magnetic resonance scans. J Manipulative Physiol Ther. 30:85-90.
7. Jensen TS, Sorensen JS, Kjaer P (2007) Intra- and interobserver reproducibility of vertebral endplate signal (Modic) changes in the lumbar spine: the Nordic Modic Consensus Group classification. Acta Radiol. 48:748-54.
8. Solgaard Sorensen J, Kjaer P, Jensen ST, Andersen P (2006) Low-field magnetic resonance imaging of the lumbar spine: reliability of qualitative evaluation of disc and muscle parameters. Acta Radiol. 47:947-53.
9. Albert HB, Kjaer P, Jensen TS, Sorensen JS, Bendix T, Manniche C (2008) Modic changes, possible causes and relation to low back pain. Med Hypotheses 70:361-8.
10. Stirling A, Worthington T, Rafiq M et al. (2001) Association between sciatica and Propionebacterium acnes. Lancet 357:2024-2025.
11. Stirling AJ, Jiggins M (2002) Association between Sciatica and Skin Commensals. International Society for the Study of the Lumbar Spine. Cleveland, USA.
12. Bhanji S, Williams B, Sheller B, Elwood T, Mancl L (2002) Transient bacteremia induced by tooth brushing a comparison of the Sonicare toothbrush with a conventional toothbrush. Pediatr Dent 24:295-9.
13. Roberts GJ, Holzel HS, Sury MR (1997) Dental bacteremia in children. Pediatr Cardiol 18:24-7.
14. Farrar MD, Ingham E (2004) Acne: Inflammation. Clinics in Dermatology 22:380-4.
15. Doita M, Kanatani T, Harada T, Mizuno K (1996) Immunohistologic study of the ruptured intervertebral disc of the lumbar spine. Spine 21:235-41.
16. Hirabayashi S, Kumano K, Tsuiki T, Eguchi M, Ikeda S (1990) A dorsally displaced free fragment of lumbar disc herniation and its interesting histologic findings. A case report. Spine15:1231-3.
17. Ito T, Yamada M, Ikuta F et al. (1996) Histologic evidence of absorption of sequestration-type herniated disc. Spine 21:230-4.
18. Lindblom K, Hultquist G (1950) Absorption of protruded disc tissue. J Bone Joint Surg 32-A:557-60.
19. Gronblad M, Virri J, Tolonen J et al (1994) A controlled immunohistochemical study of inflammatory cells in disc herniation tissue. Spine 19:2744-51.
20. Albert HB, Manniche C (2007) Modic changes following lumbar disc herniation. Eur Spine J. 16:977-82.

21. Lomholt HB, Kilian M (2008) Er acne vulgaris en infektion med den »forkerte« Propionibacterium acnes? Ugeskr Læger 170:1234-1237. [Danish]
22. Wedderkopp N, Thomsen K, Manniche C, Kolmos HJ, Secher Jensen T, Leboeuf Yde C (2009) No evidence for presence of bacteria in modic type I changes. Acta Radiol. 50:65-70.
23. Albert HB, Manniche C, Sorensen JS, Deleuran BW (2008) Antibiotic treatment in patients with low-back pain associated with Modic changes Type 1 (bone oedema): a pilot study. Br J Sports Med. 42:969-73. Epub 2008 Aug 21
24. Jensen TS, Sorensen JS, Kjaer P. Intra- and interobserver reproducibility of vertebral endplate signal (modic) changes in the lumbar spine: the Nordic Modic Consensus Group classification. Acta Radiol. 2007;48:748-54.
25. Sfanos, K. S and Isaacs, W. B (2008) An evaluation of PCR primer sets used for detectionof Propionibacterium acnes in prostate tissue samples. The Prostate 68:1492-95.
26. Caddick, JM, Hilton AC, Rollason J, Lambert PA, Worthington T, Elliott TS. (2005) Molecular analysis of methicillin-resistant Staphylococcus aureus reveals an absence of plasmid DNA in multidrug-resistant isolates. FEMS Immunol Med Microbiol 44:297-302.
27. Nadkarni MA, Martin FE, Jacques NA, Hunter N (2002). Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148: 257–266.
28. Corsia MF, Wack M, Denys G. (2003).Low virulence Bacterial infections of intervertebral discs and the resultant spinal disease processes. Abstract from Scoliosis Research Society (SRS) annual meeting
29. Agarwal VJ, Golish R, Kondrashov D, Alamin TF (2010) Results of bacterial culture from surgically excised intervertebral disc in 52 patients undergoing primary lumbar disc microdiscectomy at a single level. The Spine Journal 10 IS-149S
30. Fritzell P, Bergström T, Welinder-Olsson C (2004) Detection of bacterial DNA in painful degenerated spinal discs in patients without signs of clinical infection. Eur Spine J. 13:702-6.
31. Ben-Galim P, Rand N, Giladi M, Schwartz D et al (2006) Ashkenazi E, Millgram M, Dekel S, Floman Y. Association between sciatica and microbial infection: true infection or culture contamination? Spine 31:2507-9.
32. Van Goethem JW, Van de Kelft E, Biltjes IG et al (1996) MRI after successful lumbar discectomy. Neuroradiology. 1996;38 Suppl 1:S90-6.
33. Adams MA, McNally DS, Dolan P. (1996) 'Stress' distributions inside intervertebral discs. The effects of age and degeneration. J Bone Joint Surg Br;78:965-72.
34. Adams MA, Freeman BJ, Morrison HP, Nelson IW, Dolan P (2000) Mechanical initiation of intervertebral discs degeneration. Spine 25:1625-36.