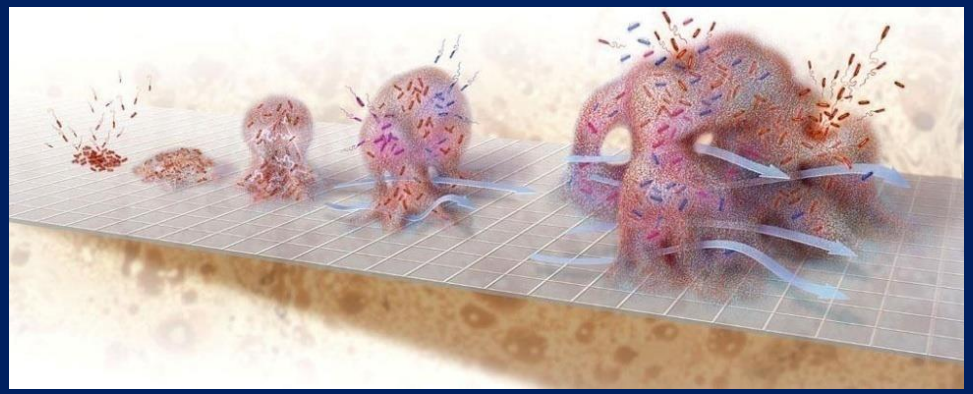
Prosthetic Joint Infections

Review of diagnosis including the role of molecular techniques



Dr Prema Singh

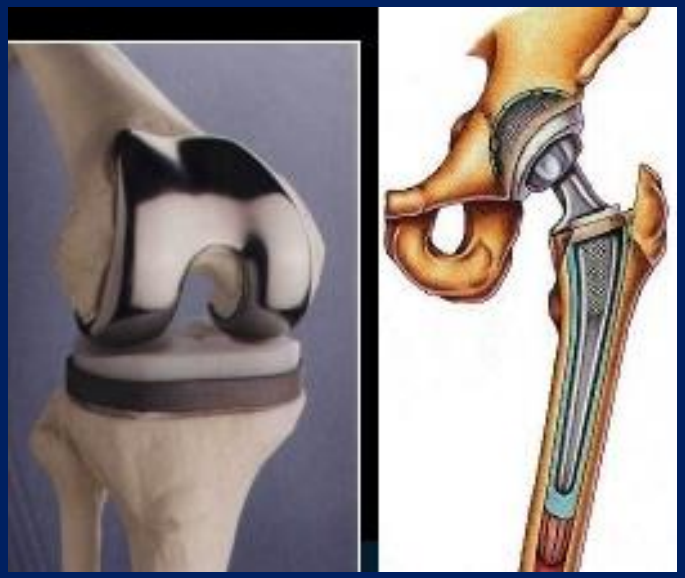
Consultant Microbiologist

Watford General Hospital

Structure of talk

- Introduction
- Case scenario
- Conventional diagnostic methods
- Non conventional methods
- Molecular techniques
- Future outlook

Prosthetic Joint Infections



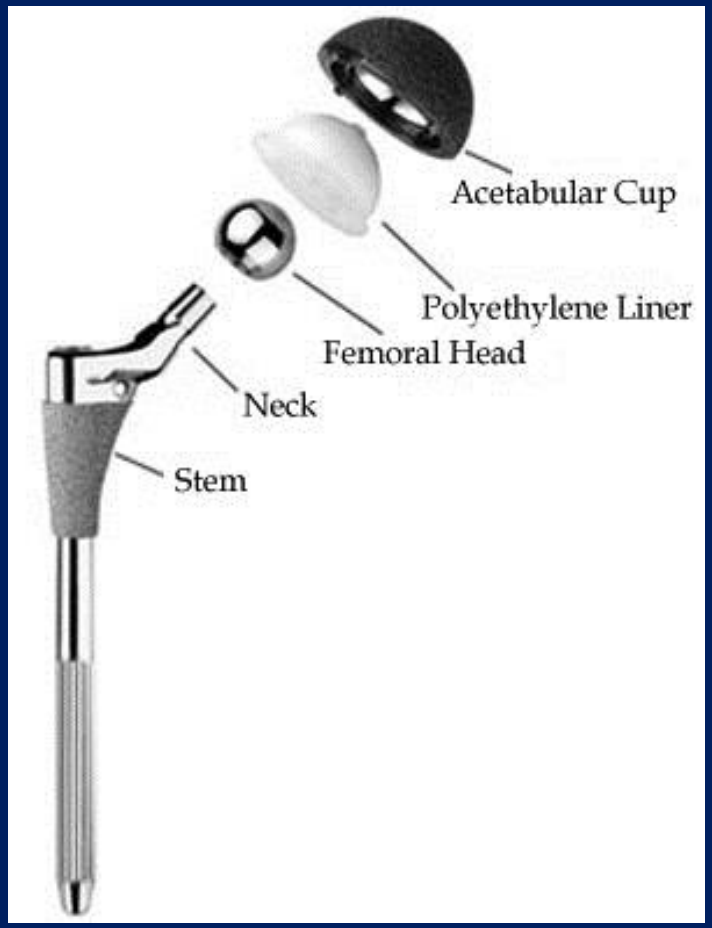
Infection rates

- Hip replacement = 1.2%
- Knee replacement = 1.7%

- Total no. Hip & Knee replacements/year = 180,000 (*National Joint Registry)*

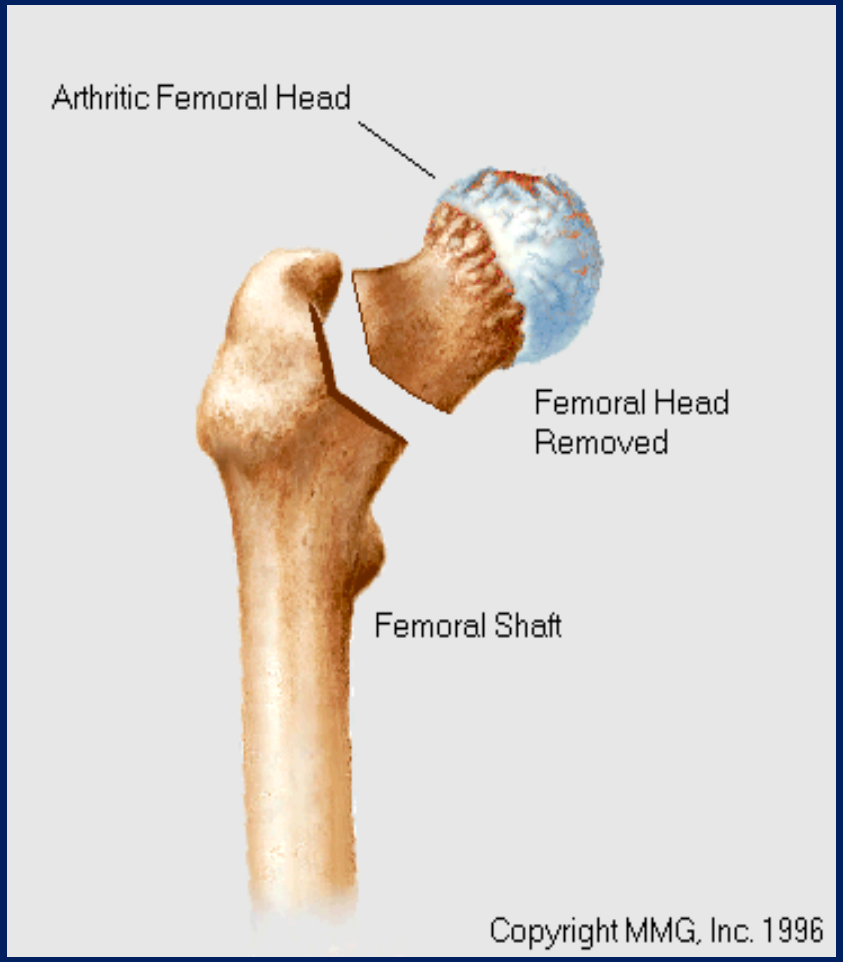
- *Report of the Mandatory Surveillance of Surgical Site Infection (SSI) in Orthopaedic Surgery - 2009-2013*

Total Hip Replacement Implants



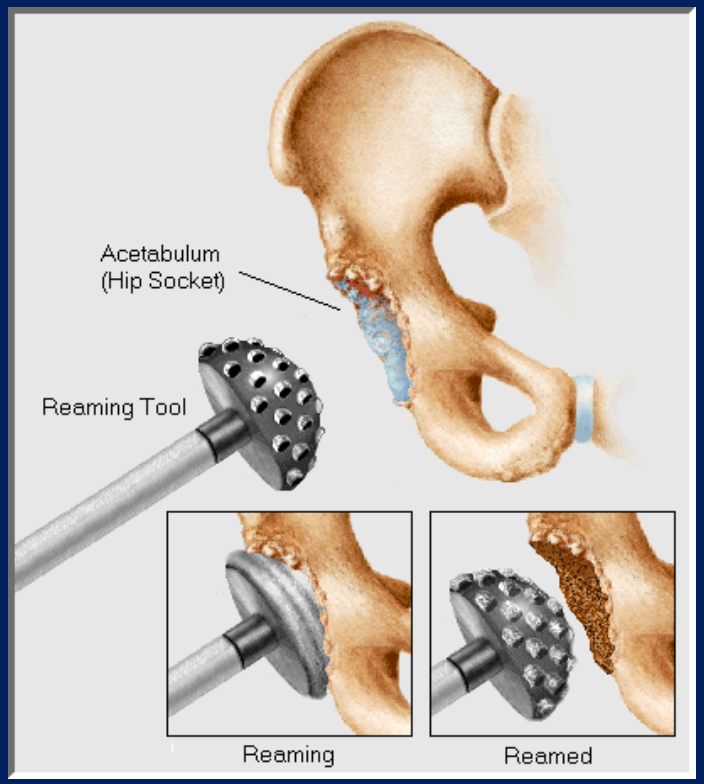
Technique: Total Hip Replacement

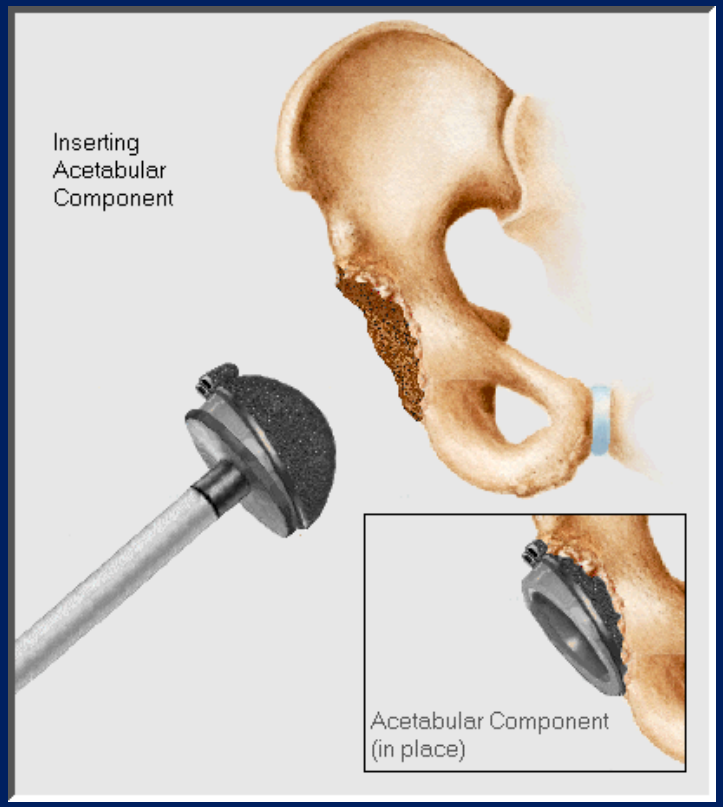
- Femoral neck resection



Technique: Total Hip Replacement

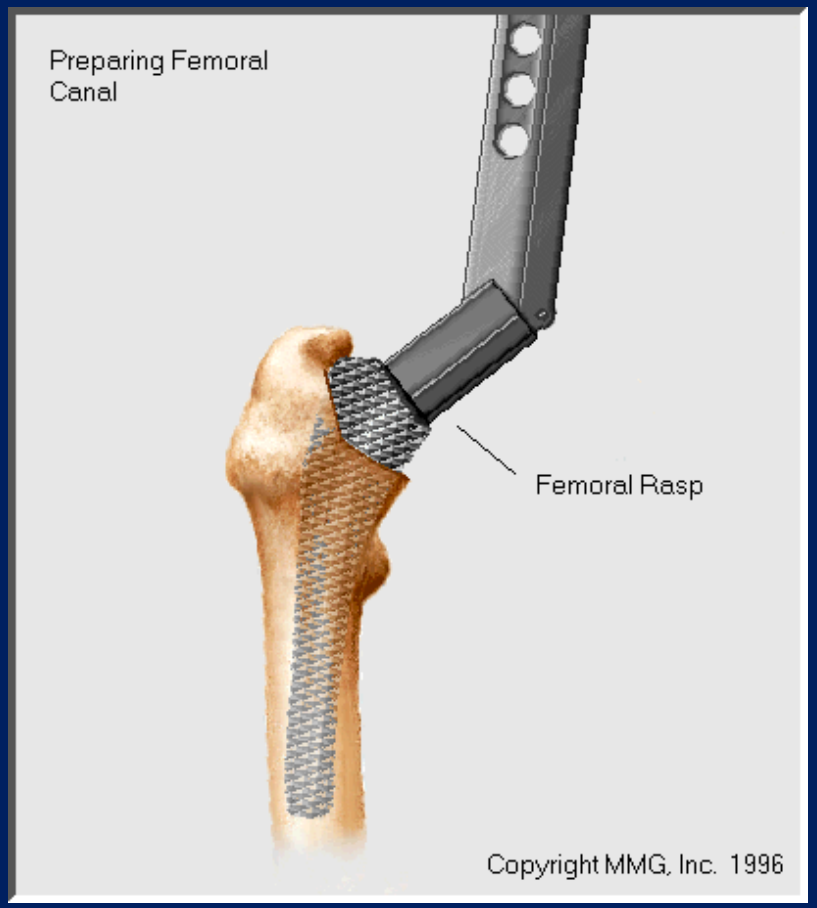
- Acetabular reaming
  - Insertion of acetabular component



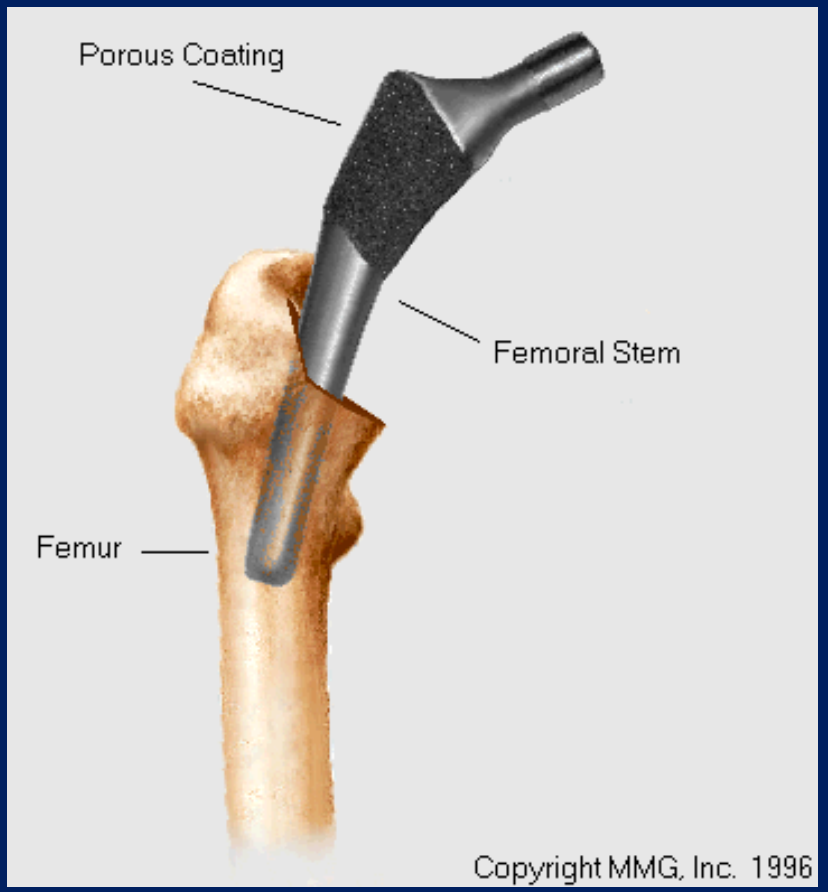


Technique: Total Hip Replacement

- Reaming of femoral component

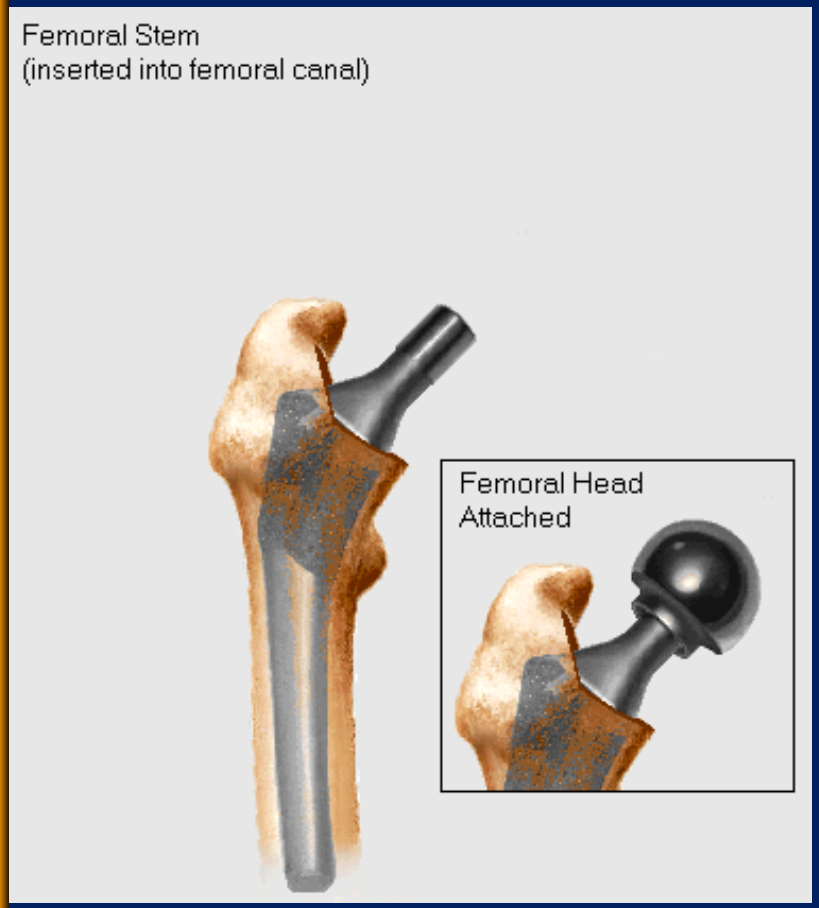


- Insertion of femoral component

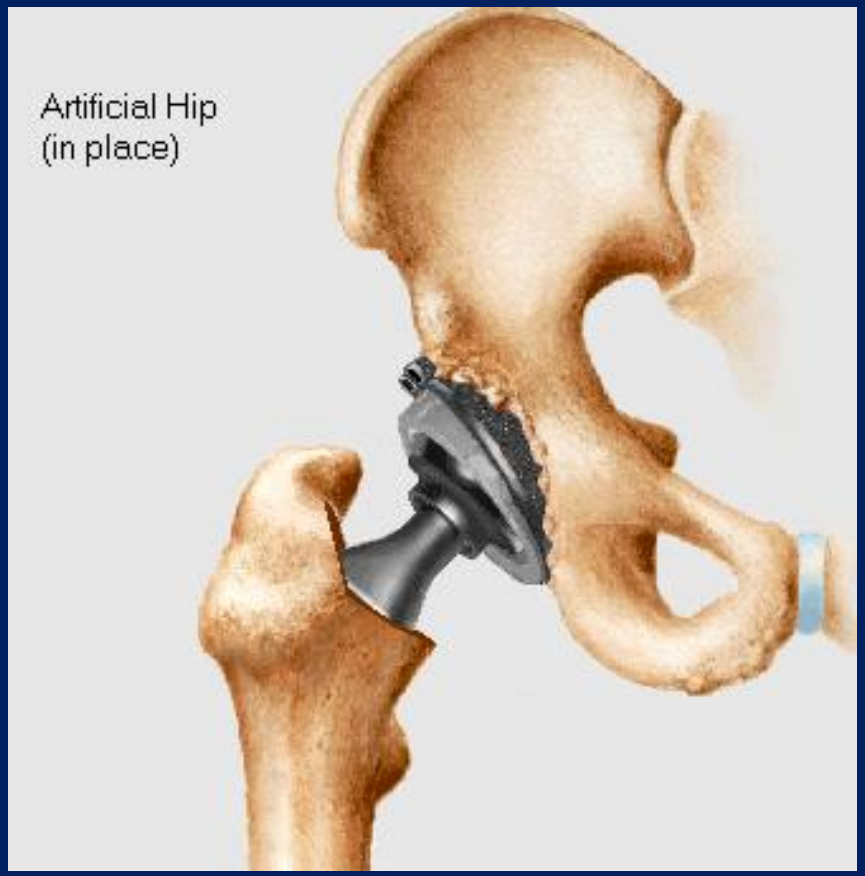


Technique: Total Hip Replacement

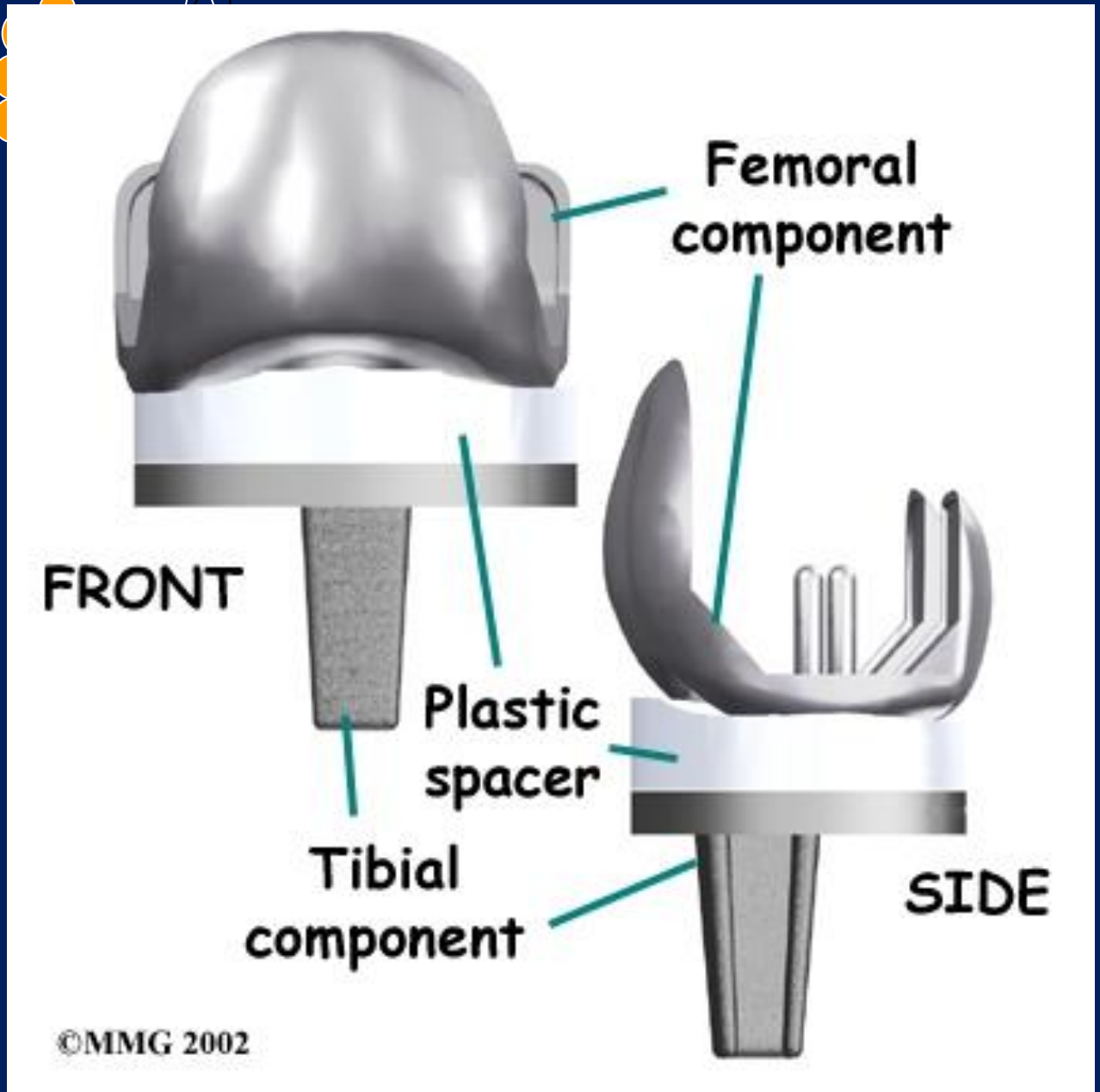
- Femoral head impaction



- Final implant

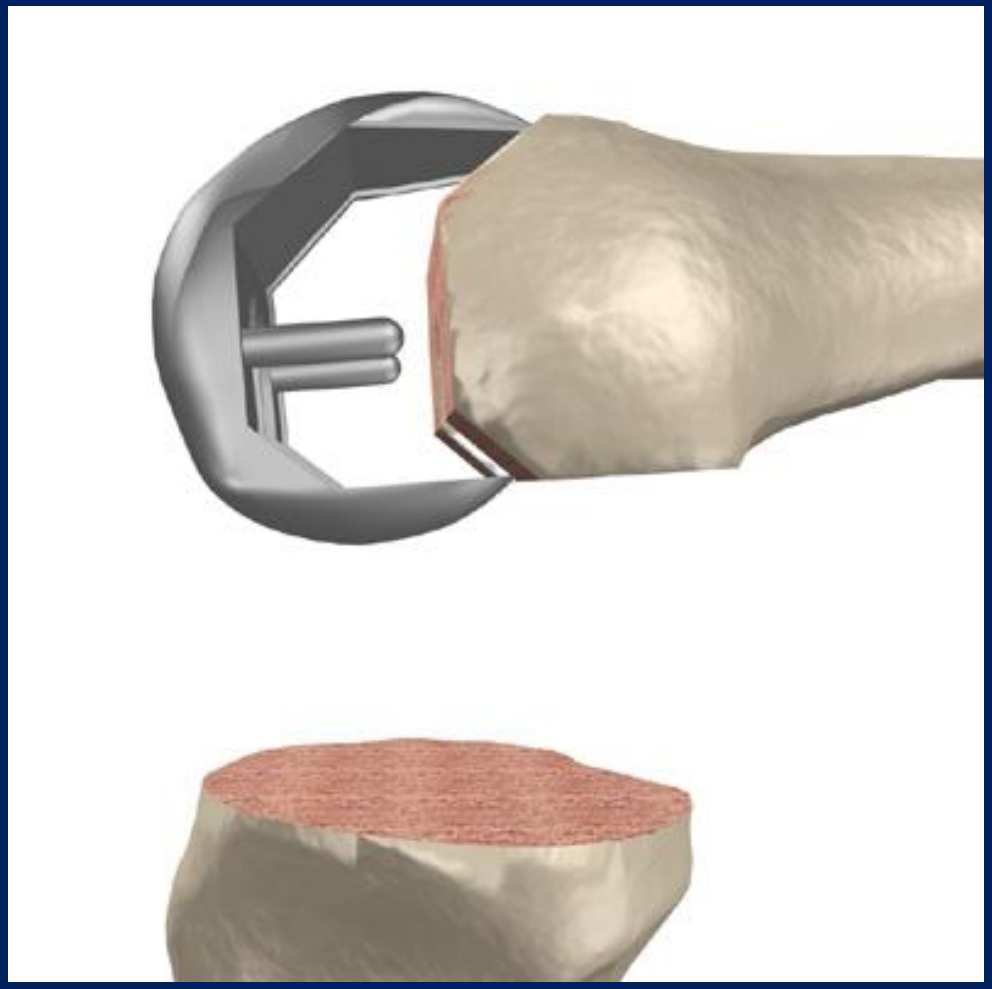


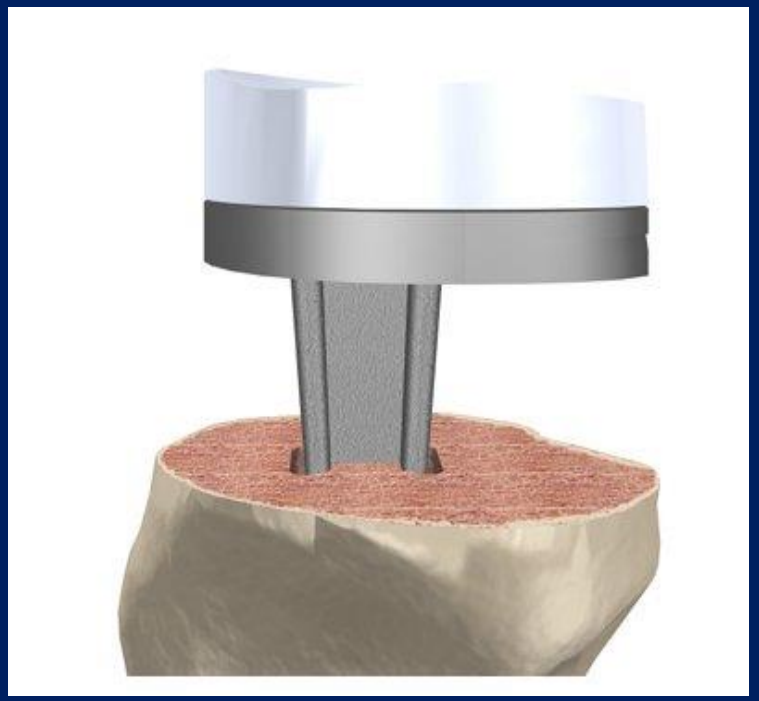
Knee Replacement—Implants



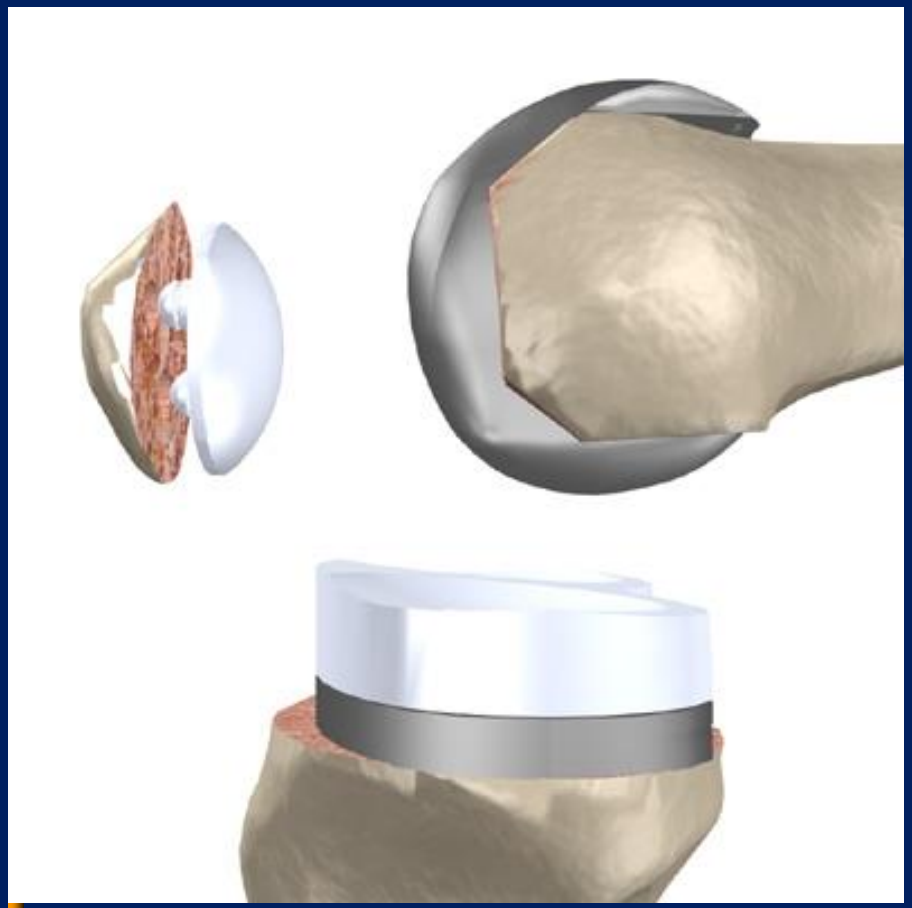
Patellar component

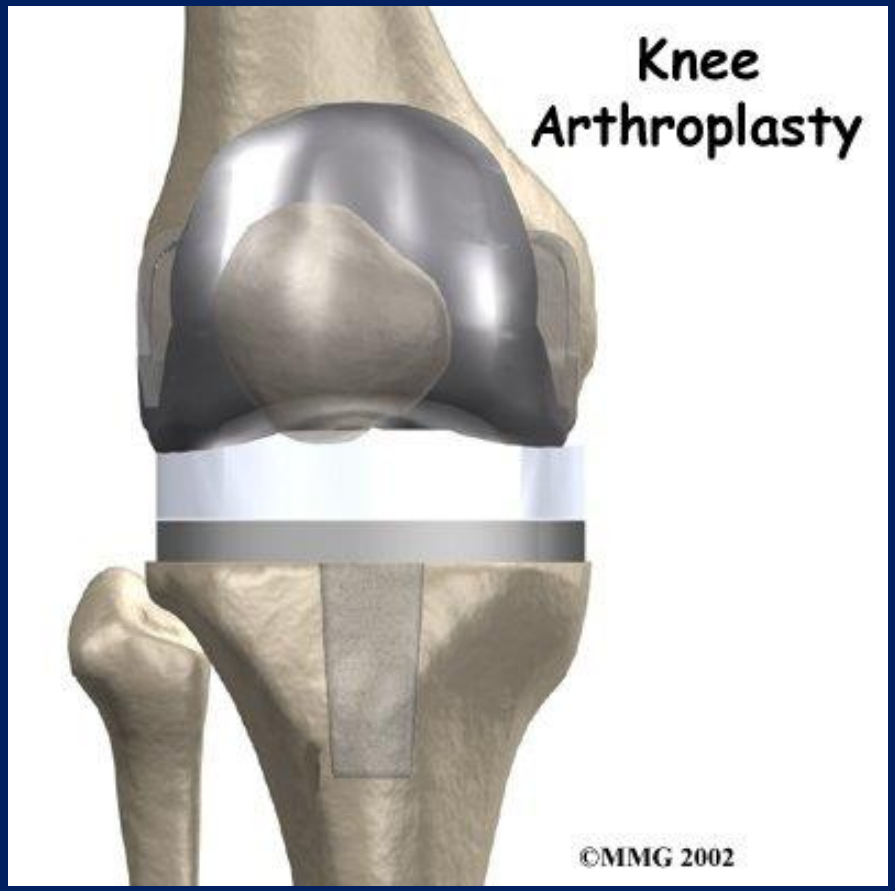
Knee Replacement—Implants



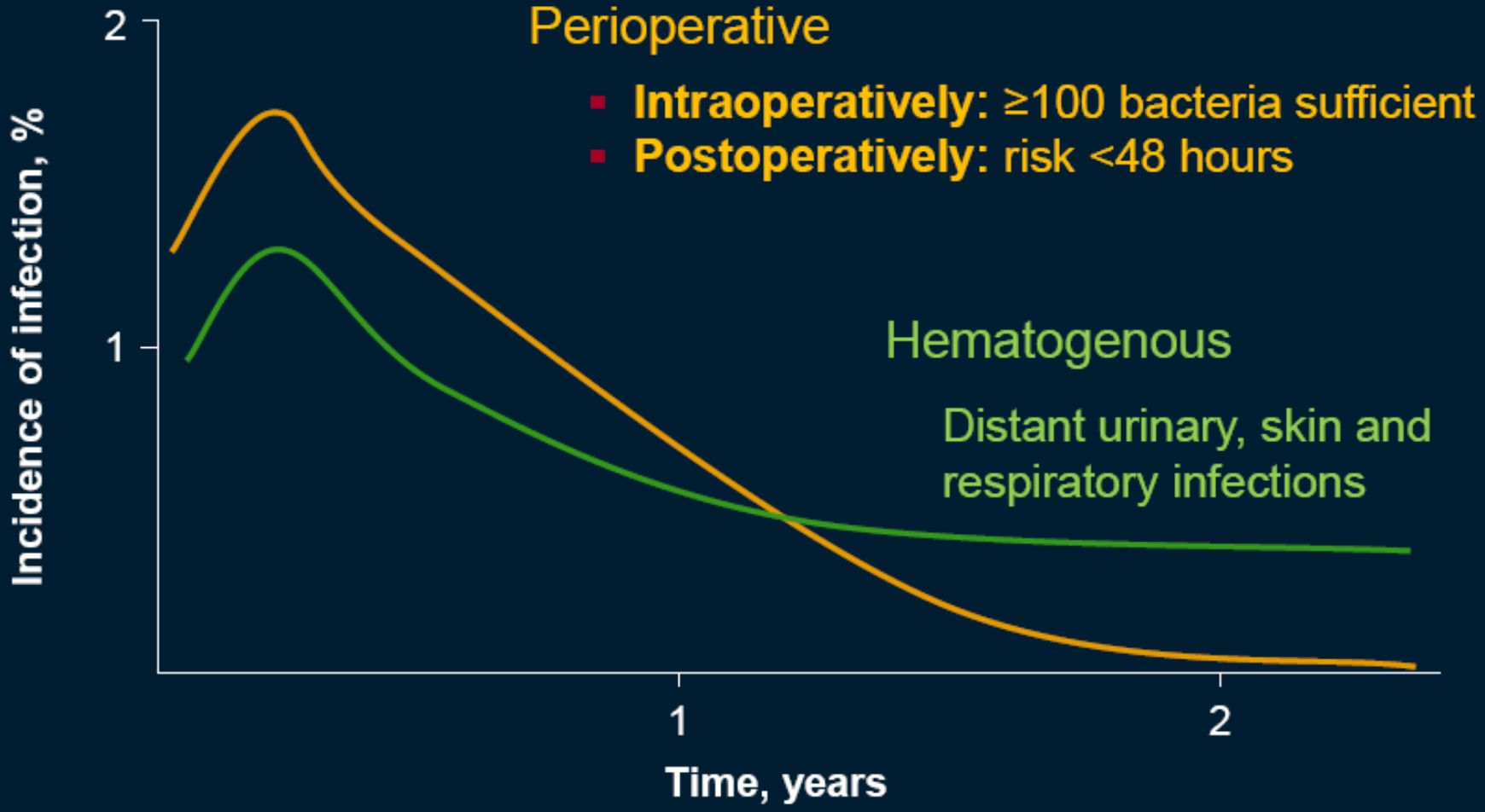


Knee Replacement—Implants





Route of implant infection



Case Study 1

- A 70 year old gentleman had been referred for a total hip replacement due to severe osteoarthritis of his left hip

- He underwent a successful operation, had an uneventful recovery and was discharged 5 days later.

Case study 1

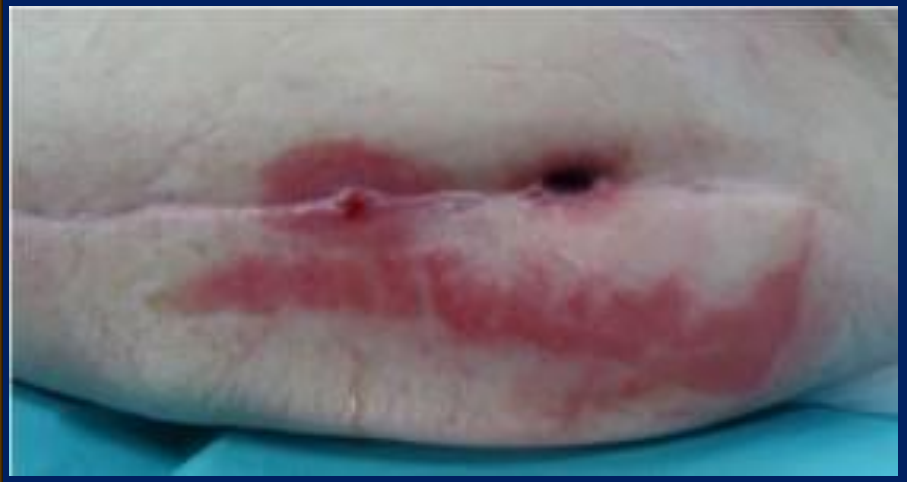
- A year later he presents back to the orthopaedic team with one month history of pain over his left hip with a discharging wound and a sinus. He has also been having difficulty in mobilising.

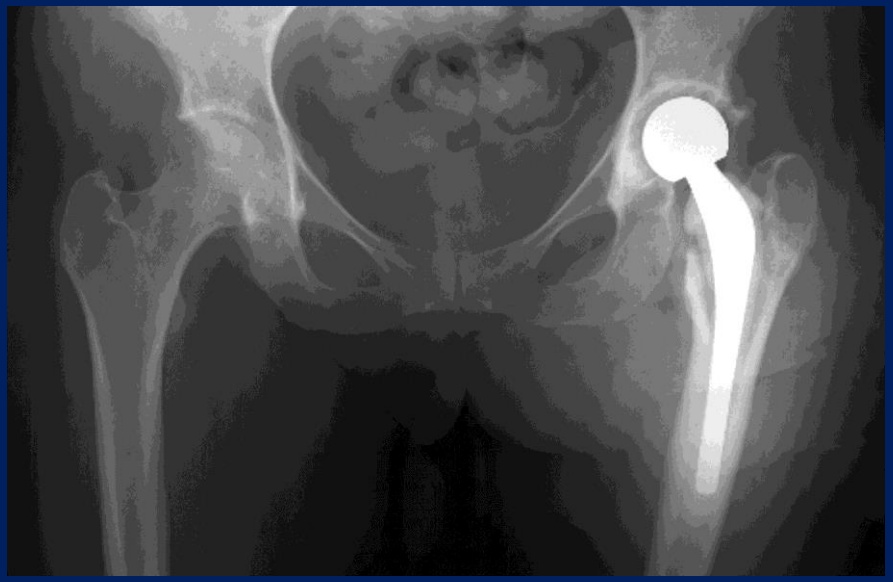
- He has a raised WCC of 20 and a CRP of 200. He has an X ray of his hip which shows loosening.

- He is reviewed at the fortnightly MDT meeting.

Local multidisciplinary team

Sinus & X ray





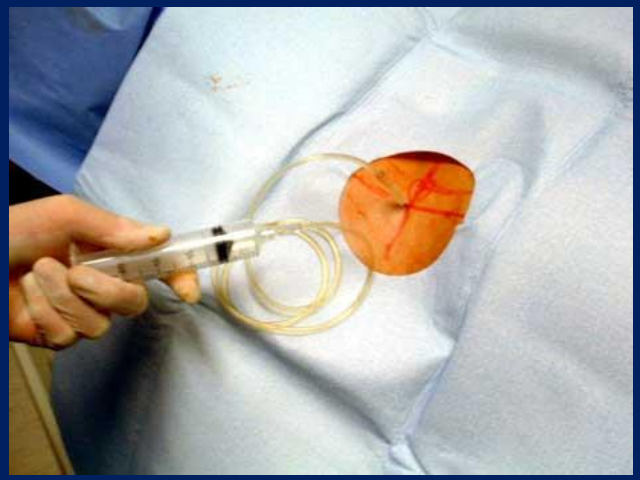
What are the features which suggest an infection?

- Presence of a discharging wound & sinus
- WCC =20
- CRP=200
- X Ray picture
- All of the above

Which one of the following investigations most accurately detects infection ?

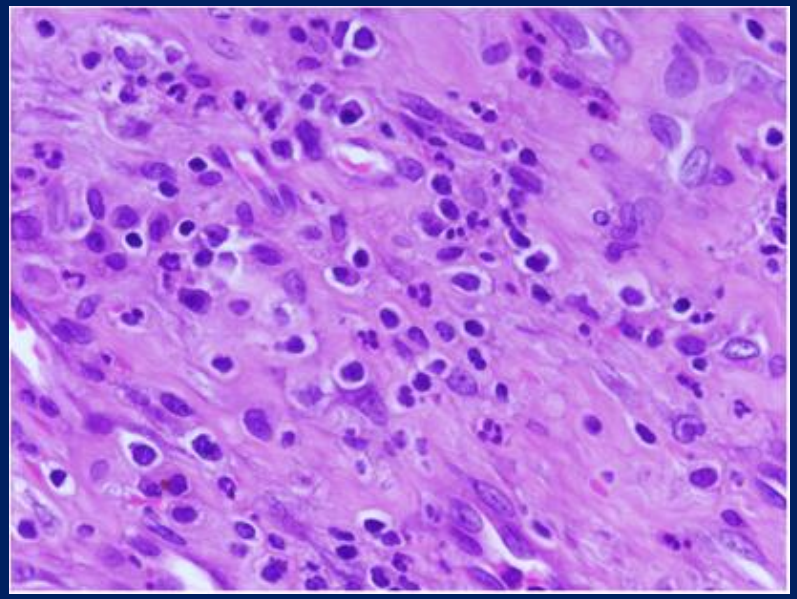
- WCC
- CRP
- Procalcitonin
- Imaging
- Histopathology
- Synovial fluid WCC count & culture
- Tissue culture

Joint aspiration



- Synovial fluid leucocyte count and differential
- Knee (≥1.7 x 10^9/l leucocytes, ≥65% neutrophils)
- Hip (≥4.2 x 10^9/l leucocytes, ≥80% neutrophils)

Histopathology



- Acute inflammation in periprosthetic tissue histopathology

  defined variably as ≥1 to ≥10 neutrophils/high power field

Definition of PJI

- Presence of a sinus tract
- Acute inflammation on histopathology
- Presence of purulence around the prosthesis
- 2 or more intraoperative cultures

  or

  combination of preop aspiration & intraoper. cultures that yield same organism

- *IDSA guidelines Dec 2012*

Intra-operative tissue culture

- At least 3 and optimally 5 or 6 periprosthetic intraoperative tissue samples should be submitted for aerobic and anaerobic culture at the time of the surgical debridement or prosthesis removal to maximise the chance of obtaining a microbiological diagnosis.
- No swabs or sinus tract cultures
-

Atkins BL et al J Clin Microbiol 1998;36:2932-9

Multiple samples & containers & separate instruments

BACTEC for Diagnosis of PJI

Recent prospective study in Oxford

Sensitivity = 87% vs 83% (RCM)

Specificity = 97% vs 100% (RCM)

>96% of culture positive detected within first 3 days of culture

99.9% culture positive within 5 days

Clinically significant samples = 203

Positive (both bottles) = 166 (62%)

Positive (only aerobic) = 42 (20%)

Positive (only anaerobic) = 49 (18%)

Both BACTEC bottles – Overall determination of culture positive in >20%.

Prolonged microbiological culture > 2 weeks may not be necessary.

Poster 0031 (Oxford Bone Infection Conference – 2012)
Minassian A et al, BMC Infectious Diseases 2014, 14; 233

Blood Tests

1. WBC
2. CRP
3. ESR
4. Procalcitonin
5. Interleukin

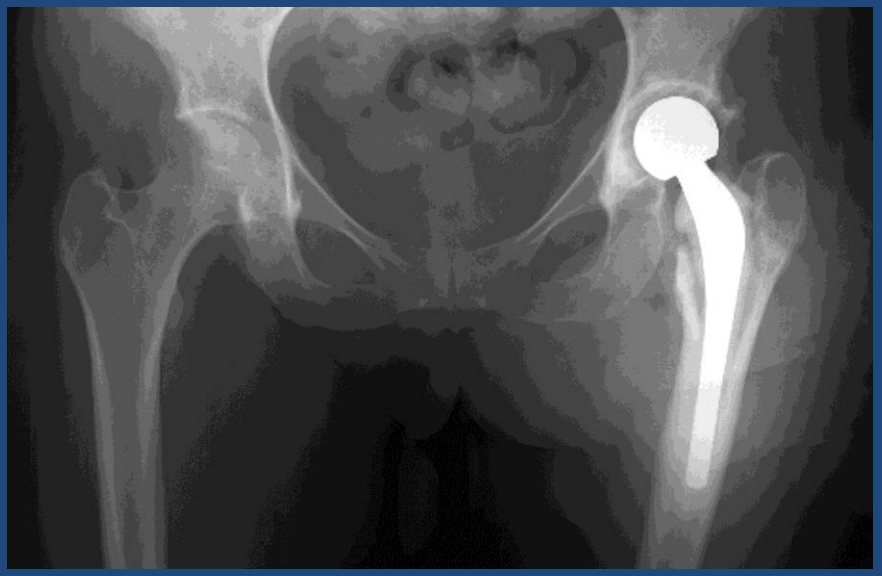
Imaging Diagnostics

- Conventional x-ray
- Ultrasound
- Computed tomography (CT)
- Magnetic resonance imaging (MRI)
- Scintigraphy
- Single photon emission computed tomography (SPECT/CT)
- PET / CT

Conventional X-Ray

Drawbacks

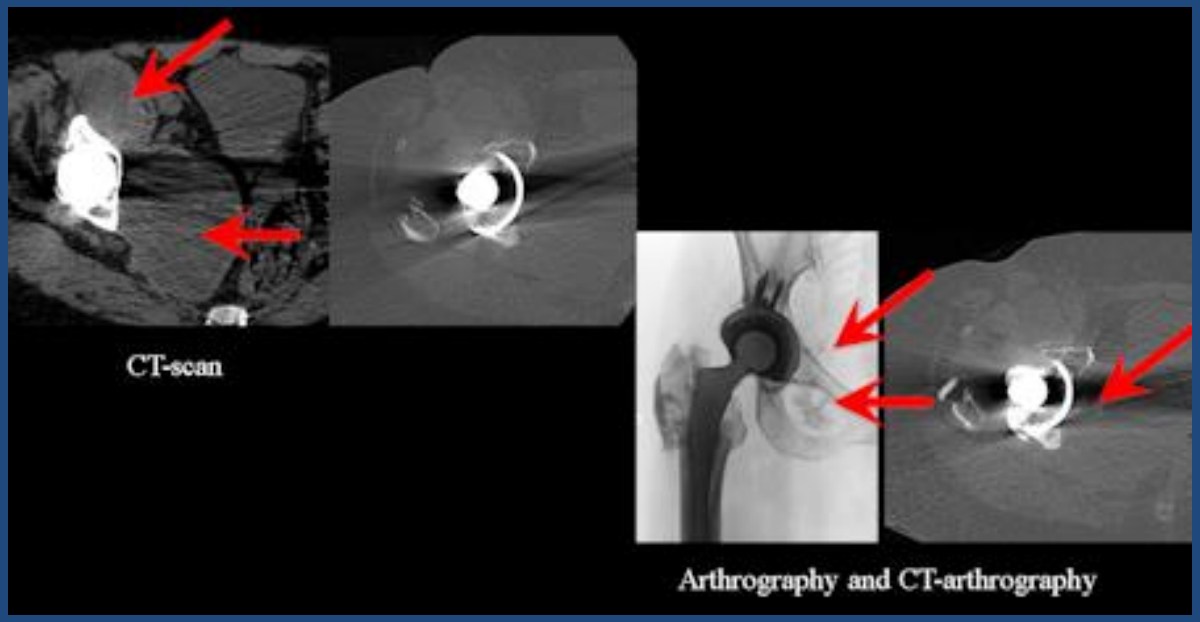
- Changes less specific for infections
- Poor imaging of soft tissue
- Sequestra, fistulas, abscesses partially detectable.



Ultrasound

- Detects effusions
- Synovial thickening
- Useful for aspiration

CT with Contrast



- Poor imaging of soft tissue
- Metal artefacts
- Radiation exposure

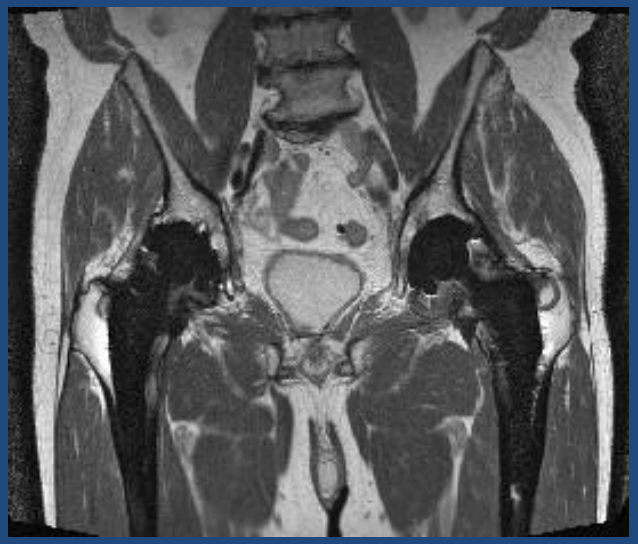
M R I

Advantages

- Shows acute inflammations with high sensitivity or specificity
- Shows complication, e.g. abscesses and fistulas

Drawbacks

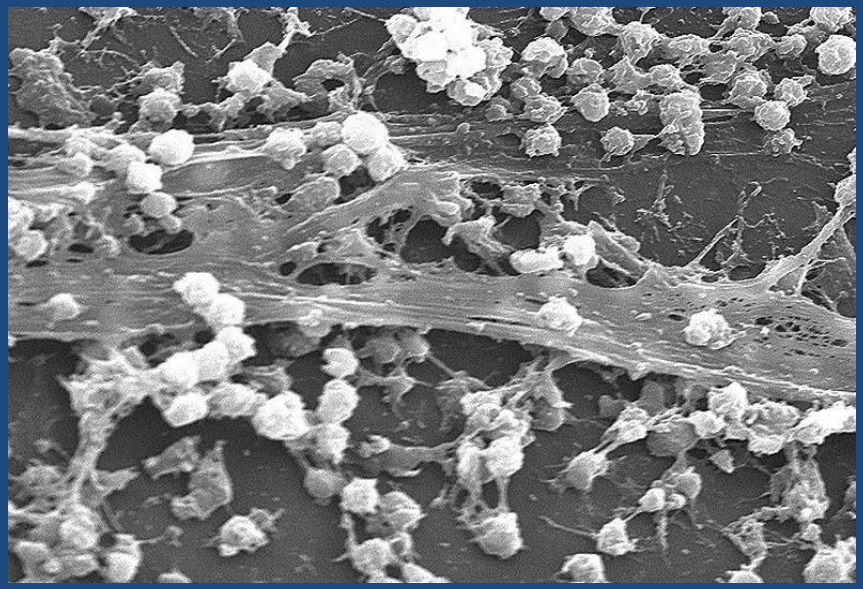
- Specificity is reduced with chronic infections
- Bone oedema is over-estimated
- Susceptibility artefacts



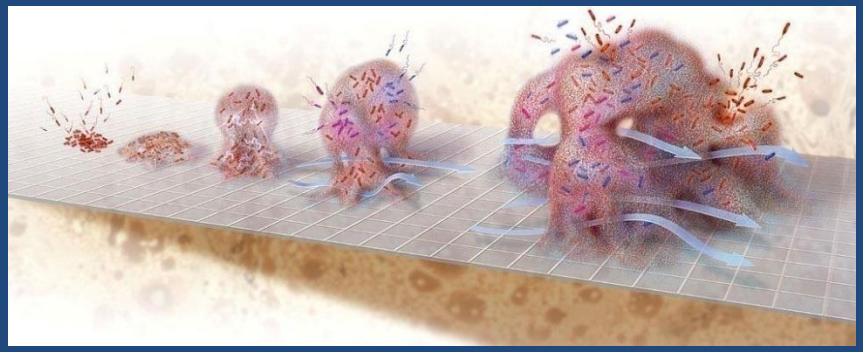
Frequency of microorganisms causing prosthetic joint infection

Microorganism	Frequency (%)
Coagulase-negative staphylococci	30-43
Staphylococcus aureus	12-23
Streptococci	9-10
Enterococci	3-7
Gram-negative bacilli	10-17
Anaerobes	2-4
Candida spp.	1-3
Polymicrobial	10-20
Unknown (culture false-negative)	10-30

Biofilm



Formation of biofilm on implant surface.



Bacteria in biofilm resist antibiotics and defence system.

Principle of BactoSonic®

The implants are placed in the air-tight transport containers and sonicated in the specially designed ultrasonic device BactoSonic®. Compared to other ultrasonic baths, BactoSonic® works with a very low ultrasound intensity. The biofilm is removed without killing the bacteria, a quantitative assessment is possible.

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Sonication of Removed Hip and Knee Prostheses for Diagnosis of Infection

Andrej Trampuz, M.D., Kerryl E. Piper, M.S., Melissa J. Jacobson, A.S., Arlen D. Hanssen, M.D., Krishnan K. Unni, M.D., Douglas R. Osmon, M.D., Jayawant N. Mandrekar, Ph.D., Franklin R. Cockerill, M.D., James M. Steckelberg, M.D., James F. Greenleaf, Ph.D., and Robin Patel, M.D.

N ENGL J MED 357;7 WWW.NEJM.ORG AUGUST 16, 2007

Sonication study

- Number of patients = 331
- Number of Total Knee prostheses= 207
- Number of Total hip prostheses = 124
- Number of aseptic failure = 252
- Number of prosthetic joint infection= 79

Sonication study

- Sensitivity of prosthetic tissue culture = 60.8%
- Sensitivity of sonicate fluid culture = 78.5 %
- 14 cases of PJI detected by sonicate fluid cultures but not by prosthetic tissue culture.
- In patients receiving antibiotics within 14 days before surgery:
- Sensitivity of prosthetic tissue culture = 45.0%
- Sensitivity of sonicate fluid culture = 75.0%

IDSA guidelines

Intraoperative Diagnosis of PJI

"At least 3 and optionally 5 or 6 periprosthetic intraoperative tissue samples or the explanted prosthesis itself should be submitted for aerobic and anaerobic culture at the time of the surgical debridement or prosthesis removal to maximise the chance of obtaining a microbiological diagnosis (B-II)"

Scope of delivery – ready-to-use

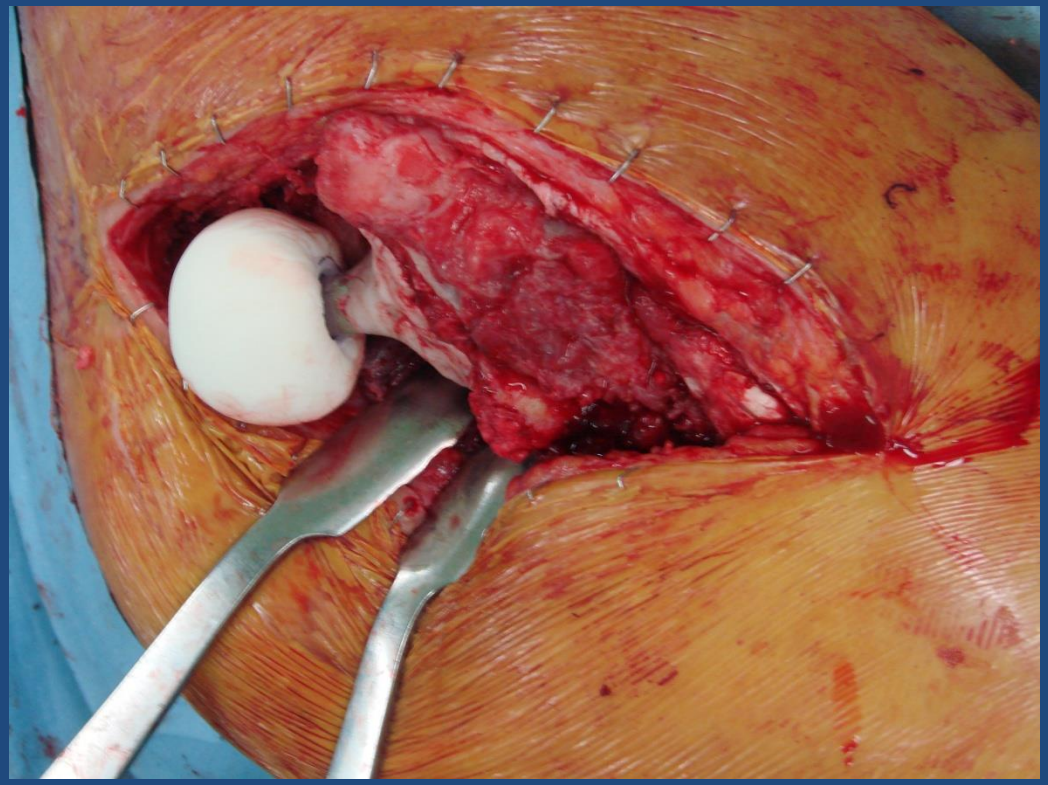
The BactoSonic® includes an ultrasonic bath BS 14, different sizes of implant containers, corresponding holders, other accessories and standard operating procedure of the sonication.

Endoprostheses not included.

Local Setting

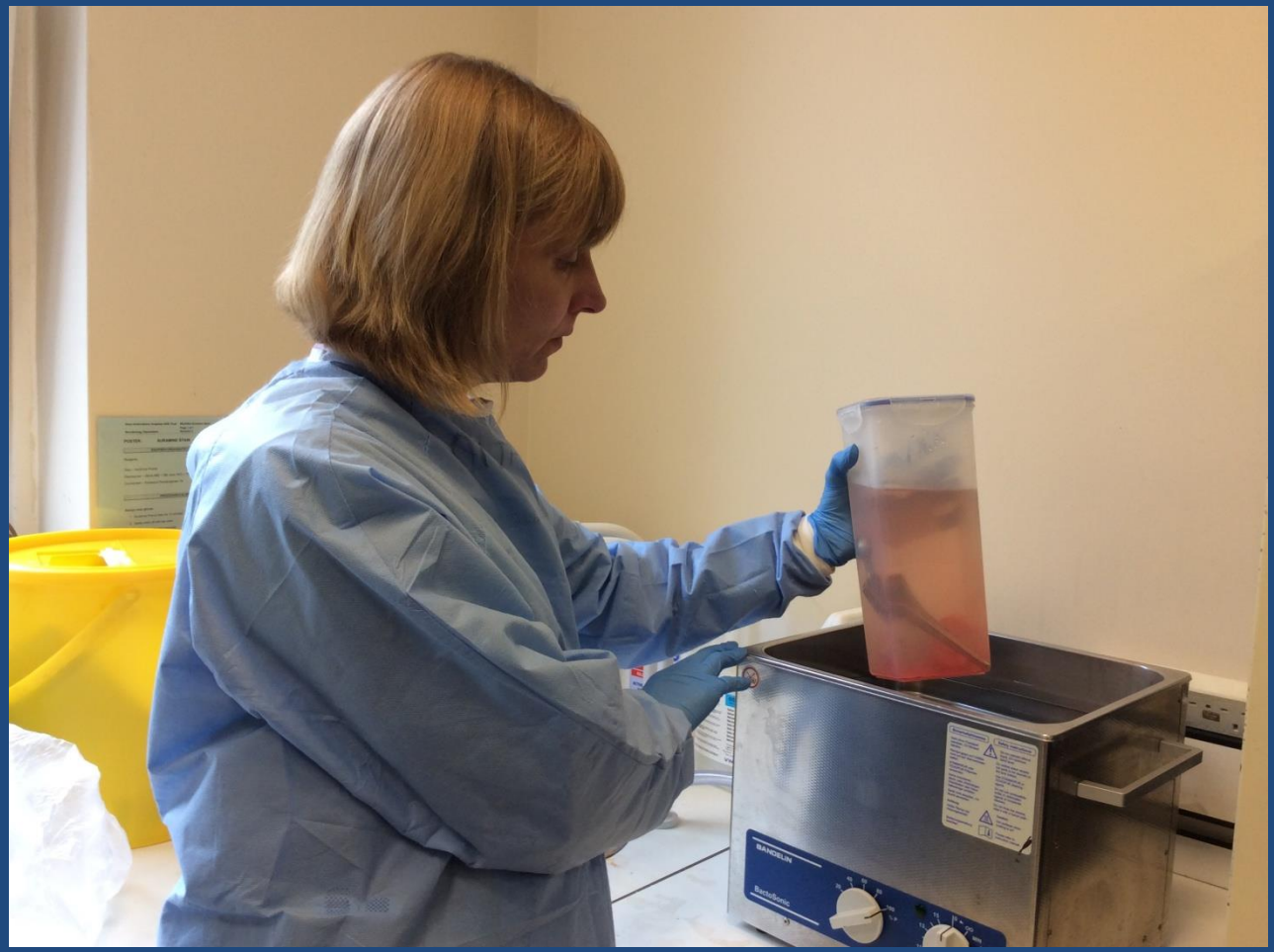
- Number of hip/knee replacements annually = 1150
- Number of Primary Joint replacements = 950
- Number of revisions = 150
- Number of unicompartmental/patello femoral = 50
- Percentage of revisions (septic loosening) = 10-15%

Implant sent in sterile container



Implant Processing

- Add sufficient Ringer's to the implant within the transport container to cover at least 90% of the implant. Ensure the container lid is closed and airtight.
- Hold the container in a wad of paper towel and vortex for 30 seconds or shake rigorously by hand for 30 seconds.
- Place the vortexed/shaken container in the BactoSonic ultrasound bath using the appropriate carrier insert.
- Sonicate at 100% power (40 kHz) for 1 minute.


BANDELIN

Implant Processing - continued

- Hold the container in a wad of paper towel, vortex or shake by hand again for 30 seconds.
- Open the containers and aliquot 15ml of the sonicate fluid into each of 2 labelled universal containers.
- One 15ml aliquot is used for culture. The second 15ml aliquot is stored at -20^{o}C.

Culture

- Inoculate 100µl of sonicate fluid to each culture plate (blood, chocolate, MacConkey, FHB).
- Spread across the entire surface using a sterile plate spreader.
- Inoculate 3ml into each culture broth (BHI, RCM).
- Incubate and examine culture media as described in the table.

Media	Incubation	Period	Read
Blood agar	35-37°C in 5% CO_2	48hrs	Daily
Chocolate agar	35-37°C in 5% CO_2	48hrs	Daily
MacConkey agar	35-37°C in air	48hrs	Daily
FHB (+MZ disc)	35-37°C in AnO_2	6 days	48 hrs then at 6 days
BHI broth	35-37°C	6 days	Daily
RCM broth	35-37°C	6 days	Daily

*Sub-culture terminally to BA, MacConkey and FHB or if growth is evident and incubate sub-cultures for up to 48 hours.

Sonicate fluid culture on agar plate

Interpretation

- The number of colony forming units (CFU) of each distinct isolate is determined.
- Identification to the species level.
- Appropriate susceptibility testing on significant isolates.

CFU per plate*	Broth (either/both)	Identification and susceptibility testing required?
>5	+	Yes
1 – 4	+	Discuss with Consultant Microbiologist and perform if requested
0	+	Discuss with Consultant Microbiologist and perform if requested
0	–	No

*If the CFU count differs between plates, interprete using whichever plate yields the highest count.

Reporting and Evaluation of Results

- Any growth MUST be brought to the attention of the Consultant Microbiologist.
- Results are reported qualitatively and quantitatively.
- For each distinct isolate, multiply CFU per plate by 10.
- Report as CFU/ml.
  6 colonies ⟶ Report as 60 CFU/ml.

Reporting and Evaluation of Results

- continued

- Counts of >50 CFU/ml are usually significant. Significance of 10-40 CFU/ml determined in the Clinical context.
- If broth culture only yields growth, report isolates with code “on enrichment only”.
- Quantitative results cannot be obtained from broth culture.

Reporting and Evaluation of Results

- continued

- Isolates from broth culture only are not usually clinically significant.
- Probably due to contamination during collection, transportation or processing.
- Exceptions are with anaerobics or in patients receiving antibiotics.
- Additional investigations such as periprosthetic tissue cultures, joint aspirate cultures, histopathology or intraoperative – findings need to be considered and assessed.

Advantages of sonication

- Higher sensitivity
- High accuracy- bacteria dislodged but not killed
- Difficult to detect organisms
- Mixed infections
- Increased yield following previous antibiotics
- Rapid result
- Quantitative assessment

Sonication studies with implants

- Shoulder prosthesis (**Piper KE et al. JCM 2009**)
- Orthopedic devices (**Gorisek N, Bombek M. In preparation**)
- E. coli small-colony variants (**Sendi P et al. JCM 2010**)
- Breast implants (**Del Pozo JL et al. JCM 2009**)
- Breast implants (**Rieger UM et al. Aesth Plast Surg 2009**)
- Bone grafts and substitutes (**Clauss M et al. Acta Biomat 2010**)
- Electrophysiologic cardiac devices (**Rohacek M, Weisser M et al. Circulation 2010**)
- Ureteric catheters (**Bonkat G et al. W J Urol 2010**)
- Multiplex PCR (**Achermann Y et al. JCM 2010**)
- Fast-track microbial detection (microcalorimetry) and identification (MALDI-TOF) (**Steinrücken J, Clauss M, Borens O**)

Problems/Issues Encountered

- Secure initial funding
- Training of orthopaedic colleagues
- Training of biomedical scientists
- Availability and cost of containers
- Sterilisation of containers
- Leaking specimen containers

Progress So Far

- Methodology well established
- 100 implants/mobile parts processed so far
- Staphylococcus spp (S.aureus and CNS)=55%
- Gram negative= 17%
- *A multidisciplinary team approach to the management of infected Joint replacements: A fifteen month review in a large general hospital in the EoE (Abstract 2546-EFORT-May 2015 Prague)*

Molecular analysis

Molecular Analysis

Different techniques have been used on sonicate fluids.

1. Real time multiplex PCR (Septifast, Light cycler).
2. 16S ribosomal RNA-PCR.
3. Real time probe PCR assays.

Multiplex PCR of Sonication Fluid

37 PJI cases

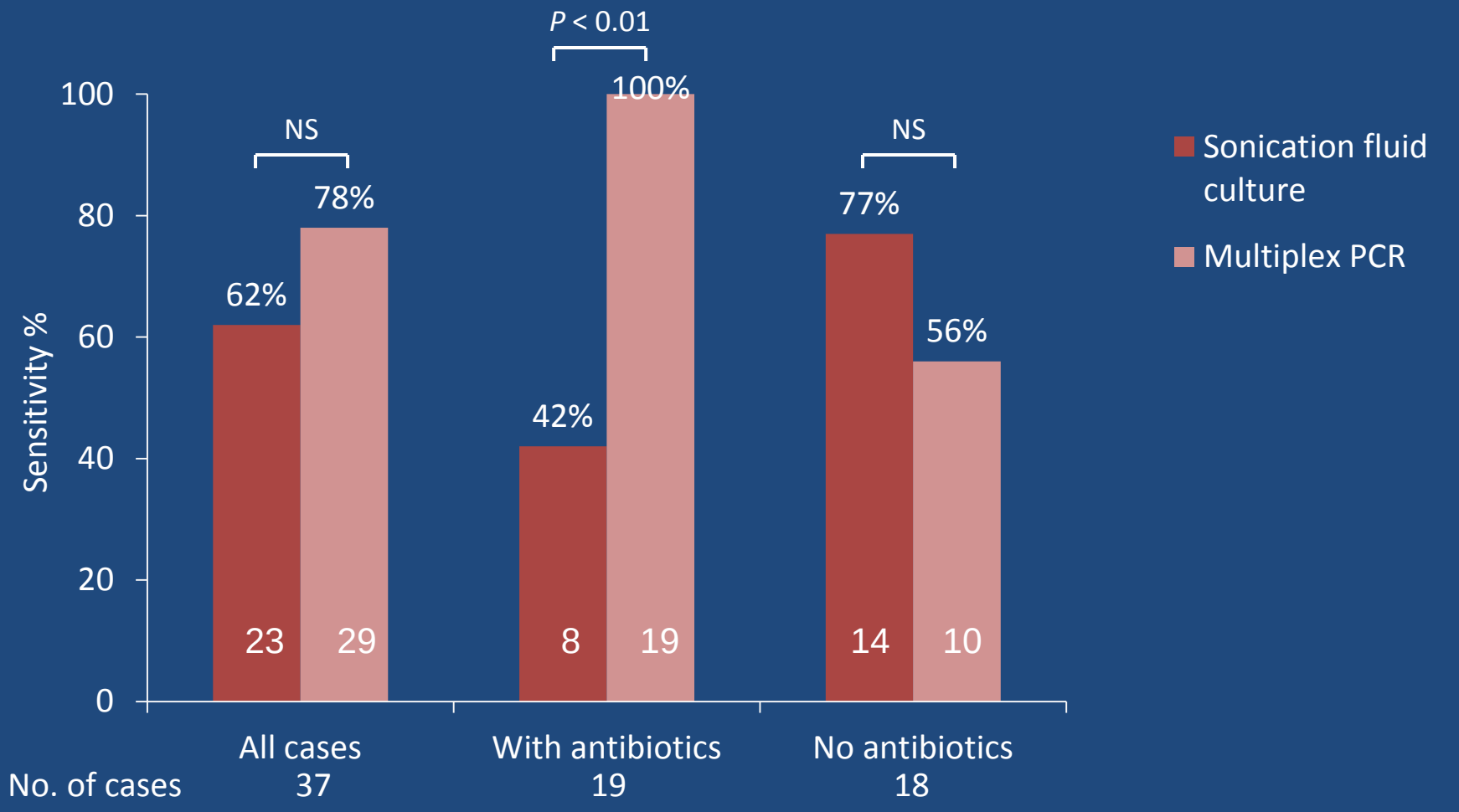
Hip Prosthesis	=	17
Knee Prosthesis	=	14
Shoulder Prosthesis	=	4
Elbow Prosthesis	=	1
Ankle Prosthesis	=	1

Y. Achermann et al – J.Clin.Microbiol.2010, 48(4):1208

Comparison of periprosthetic tissue culture, sonication fluid culture, and multiplex PCR of sonication fluid in 37 cases of PJI

Infection type and microorganism	No. of episodes	No .of episodes with positive result by diagnostic test		
		Perisprosthetic tissue culture	Soncation fluid culture	Multiplex PCR of sonication fluid
Single microorganism	31	22	20	26
Staphylococcus aureus	9	5	5	9
Coagulase-negative staphylococci	11	9	8	11
Steptococcus mitis	1	1	1	1
Steptococcus agalactiae	1	1	1	1
Steptococcus dysgalactiae	1	1	0	1
Steptococcus gallolyticus	1	0	0	1
Steptococcus pneumoniae	1	0	0	1
Propionibacterium acnes	5	4	4	0
Candida albicans	1	1	1	1
Polymicrobial infection*	6	2	2	3
Total no. of episodes (%)	37 (100)	24 (65)	23 (62)	29 (78)

* Included coagulase-negative staphylococci. *Klebsiella pneumoniae/oxytoca* and *Enterococcus faecalis* (n = 1). *P.acnes* and coagulase-negative staphylococci (n = 1), *S.aureus* and coagulase-negative staphylococci (n = 2), *Corynebacterium* species and coagulase-negative staphylococci (n = 1), and *P.acnes* and *S.aureus (n = 1).*



Sensitivity of culture and multiplex PCR of sonication fluid. The sensitivity values are shown overall (all cases) and stratified according to patients who had received antimicrobial therapy previously (n = 19) and patients who had not received antimicrobial therapy (n = 18). Eight pathogens (7 *Propionibacterium acnes* and 1 *Corynebacterium* species) were missed by multiplex PCR due to lack of specific primers for these species. The value at the bottom of each bar indicates the number of PJI cases in that group and the percentage above each bar indicates the sensitivity. NS, not significant.

16S Ribosomal RNA

No. of patients = 26

No. of episodes = 29

Criteria for PJI fulfilled = 12

Sensitivity of bacterial culture = 58%

Sensitivity of PCR = 50%

F.H. De Man et al – Infection 2009 37(3) 92-4

Results of bacterial culture and broad-range PCR in 12 episodes of confirmed PJI			
Episode	**Sample origin**	**Bacterial culture – Pathogen**	**16S rRNA-PCR – Pathogen**
1	Synovial fluid	*Streptococcus pyogenes*	*Streptococcus pyogenes*
2*	Periprosthetic tissue	*Propionibacterium* sp.	*Streptococcus infantis*
3	Synovial fluid	*Staphylococcus aureus*	Negative
4	Periprosthetic tissue	No growth	Negative
5	Periprosthetic tissue	No growth	Negative
6	Synovial fluid	No growth	*Streptococcus bovis*
7	Periprosthetic tissue	*Staphylococcus aureus* *Enterococcus faecalis*	*Staphylococcus aureus*
8	Periprosthetic tissue	No growth	Negative
9	Periprosthetic tissue	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
10	Periprosthetic tissue	*Pseudomonas aeruginosa* *Enterococcus faecalis*	*Pseudomonas aeruginosa*
11	Periprosthetic tissue	*Staphylococcus epidermidis*	*Staphylococcus epidermidis*
12	Periprosthetic tissue	No growth	Negative

* Identification of the pathogen in this episode was inconclusive, since in both diagnostic methods the ratio of number of positive: total number of specimens was low. However, based on the previous history (duration of symptoms 19 months) and the small fragment for amplification isolated from the biopsy, *Propionibacterium* sp. was interpreted as possible pathogen, and *Streptococcus infantis* as contamination.

Genus/Group Specific PCR Assay

No. of patients = 144

Sensitivity of tissue culture = 70.1%
Sensitivity of sonicate fluid culture = 72.9%
Sensivitity of PCR = 77.1%

Specificity of tissue culture = 97.9%
Specificity of sonicate fluid culture = 98.3%
Specificity of PCR = 97.9%

Cazanave et al – J.Clin.Microbiol. 2013

Microbiological findings in 24 cases with prosthetic joint infection, according to the type of diagnostic specimen

	Periprosthetic tissue culture[a]	Sonication fluid culture[b]	Multiplex PCR or sonication fluid
No. of all detected pathogens[a]	18	19	31
Type of organism			
Gram-positive cocci	15 (83%)	16 (84%	25 (81%)
Coagulase-negative staphylococci	6	7	12
S. aureus	7	7	11
Enterococcus spp.	2	2	2
Gram-negative bacilli	2 (11%)	3 (16%)	6 (19%)
Escherichia coli	1	1	1
Enterobacter spp.	0	1	3
Klebsiella spp.	0	0	1
Proteus spp.	1	1	1
Other organisms	1 (6%)	0	0
P. acnes	1	0	0
Type of infection			
Monomicrobial	14 (58%)	12 (50%)	16 (67%)
Polymicrobial	3 (13%)	4 (17%)	7 (29%)
Negative culture	7 (29%)	8 (33%)	1 (4%)

Note: The percentages were rounded and the sum may not equal 100%.

[a] Periprosthetic tissue cultures were considered positive if ≥1 tissue specimen were positive. For low virulent organisms, growth in ≥2 tissue specimens or an additional clinical criterion for PJI was required.

[b] Sonication fluid cultures were considered positive, if ≥50 CFU/ml grew in sonication fluid.(*Portillo et al*)

Innovative techniques in progress

- RT-PCR
- FISH (Fluorescence in situ hybridisation)
- Whole Genomic sequencing

Commonly used definition criteria for prosthetic joint infection

Definition criteria	Sensitivity	Specificity
Cutaneous sinus tract communicating with the prosthesis	20-30%	~100%
Acute inflammation in periprosthetic tissue histopathology[a]	95-98%	98-99%
Synovial fluid leukocyte count and differential[b]		
Knee (≥ 1.7 x 10^9/l leukocytes, ≥65% neutrophils)	96%	98%
Hip (≥4.2 x 10^9/l leukocytes, ≥80% neutrophils)	95%	98%
Visible purulence (wound secretion, pus around prosthesis)	20-30%	~100%
Microbial growth		
Synovial fluid	60-80%	97%
Periprosthetic tissue[c]	70-85%	92%
Sonication fluid (≥50 CFU/ml)	85-95%	95%

[a] Defined variably as ≥1 to ≥10 neutrophils/high-power field.

[b] Early postoperative and inflammatory joint diseases excluded.

[c] For low-virulent microorganisms (e.g. coagulase-negative staphylococci, *Bacillus* spp., *Propionibacterium* spp.), growth of the same organism in ≥2 tissue

Future outlook

- Sonication constitutes an additional tool for the diagnosis of PJI
- With modified primer sets multiplex PCR has the potential for further improvement of the diagnosis of PJI
- More studies are required to identify not only a validated PCR based technique but also the right diagnostic strategy and patient population in which its use can be of most benefit.

Acknowledgements

- *Local MDT*
- Tim Waters
- Paul Whittingham-Jones
- Neil Davies
- Theresa Maunganidze
- Jane Goldman
- Vanessa Wong
- SpRs & fellows

- *Swiss Group*
- Andrej Trampuz
- Olivier Borens
- Werner Zimmerli

- *Oxford Group (OBIC)*
- Bridget Atkins
- Tony Berendt
- Andrew Woodhouse
- Philip Bejon

Thank you