

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MEDICINE

Clinical and Experimental Sciences

Prosthetic Joint Infections, Biomarkers and Antibiotic Stewardship

by

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Thesis for the degree of MD

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For Zephan and Naz

ABSTRACT

Infection, microbiology, diagnostics, antibiotic stewardship and infection prevention/control are interweaving matters, one benefit from the other and all advance each other.

The complex nature of medical sciences and the advances in medical, surgical, cancer care and technologies currently available, particularly in the western world, means population live longer. However in certain cases, these advances result in increased susceptibility to infection that needs novel diagnostics as well as the provision of excellent antimicrobial and infection prevention programmes to treat, prevent spread as well as limit or prevent the rise of antimicrobial resistance.

A good example is joint replacement (Arthroplasty), the majority of which are successful. Not only do they provide pain relief, restore function and independence, but also improve the patients' quality of life. Prosthetic (or periprosthetic) joint infection (PJI) or arthroplasty associated infections (AAI) which is defined as infection involving the joint prosthesis and adjacent tissue are rare, although the effect on patients and health economy can be detrimental in the era of rising antimicrobial resistance.

An accurate diagnosis of PJI remains a challenging clinical problem. Despite aggressive investigations, the distinction between PJI and other causes of joint failure, such as aseptic loosening, can frequently be convoluted even among experts in the field. Chapters one and two of this thesis will review diagnostic tests in PJI including the original application of novel biomarkers in the synovial fluid as well as the application of specific polymerase chain reaction (PCR) technology. Chapter three aims to highlight the issues of antimicrobial resistance through original research into novel strains of methicillin resistant *Staphylococcus aureus* (MRSA) and a review on hidden resistances associated with *S. aureus*, an organism that is associated with bone and joint infections including PJI. Finally in chapter four, my aim was to provide further insights through application of biomarkers into antibiotic decision making process and stewardship programmes and specific novel application in antibiotic delivery in PJI.

This thesis is the result of research and publication over the past ten years. It is mainly intended for infection specialists, antimicrobial pharmacists and orthopaedic surgeons, aiming to provide professionals with insight to these novel applications and technologies, encourage collaborative work among these multidisciplinary teams, and generating more research questions and future studies.

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Academic Thesis: Declaration Of Authorship

I, Kordo Saeed, declare that this thesis and the work presented in it are my own and have been generated by me as the result of my own original research.

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I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;
2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;
3. Where I have consulted the published work of others, this is always clearly attributed;
4. Where I have quoted from the work of others, the source is always given. With the exception of such quotations, this thesis is entirely my own work;
5. I have acknowledged all main sources of help;
6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;
7. Parts of this work have been published as: List of references, 1,4,36,69,72,160,191,193,307, 329 as below:

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Definitions and Abbreviations

AAI	Arthroplasty Associated infection
AL	Aseptic loosening
AR	Antimicrobial Resistance
AUC	Area under the curve
BCBs	Blood culture bottles
CDAD	<i>Clostridium difficile</i> associated diarrhoea
CF	Cystic fibrosis
CM	Cooked meat
CoNS	coagulase-negative staphylococci (CoNS),
CRP	C-reactive protein
CV	Coefficient of variation
DAIR	debridement, antibiotic and implant retention
EBJIS	European Bone and Joint Infection Society conference
EIA	Enzyme immunoassay
ELISA	enzyme linked immunosorbant assay
EMRSA	Epidemic strains MRSA
ESR	erythrocyte sedimentation rate
FISH	Fluorescence <i>in situ</i> hybridization
HAI	Healthcare-associated infection
HIPEC	Hyperthermic intraperitoneal chemotherapy
HIV	human immunodeficiency virus
HPA	Health Protection Agency (Currently Public Health England)

hVISA	Heteroresistant vancomycin- intermediate <i>S. aureus</i>
ICU	Intensive care unit
IDSA	Infectious diseases society of America
Ig	Immunoglobulin
IL	Interleukin
MALDI-TOF MS	Matrix-assisted laser desorption ionization time-of-flight mass spectrometry
MAU	Medical admission unit
MIC	Minimum inhibitory concentration
MRCNS	Methicillin resistant coagulase negative staphylococci
MRI	Magnetic resonance imaging
MRSA	Methicillin (meticillin)-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin (meticillin)-sensitive <i>Staphylococcus aureus</i>
NPV	Negative predictive value
NPWT	Negative Pressure Wound Therapy
NPWTiaI	Negative Pressure Wound Therapy and Intra-Articular Antibiotics Instillation
OS-MRSA	Oxacillin-susceptible methicillin-resistant <i>Staphylococcus aureus</i>
PAP	Population analysis profile
PBP	Penicillin-binding protein
PCR	Polymerase chain reaction
PCR-ESI/MS	PCR-electrospray ionization mass spectrometry
PCT	Procalcitonin
PFGE	Pulsed- field gel electrophoresis

PHE	Public Health England
PJI	Prosthetic Joint Infection
PM	Peritoneal malignancy
POD	Post operative day
PPV	positive predictive value
PVL	Panton-Valentine Leukocidin
ROC	Receiver Operating Characteristic
RT-PCR	real-time polymerase chain reaction
SCC	staphylococcal cassette chromosome
sce-LTA	short-chain exocellular lipoteichoic acid
SCVs	Small colony variants
sICAM-1	Soluble intercellular adhesion molecule 1
SIRS	systemic inflammatory response syndrome
SSTIs	Skin and soft tissue infections
TKA	total knee arthroplasty
TNF- α	tumour necrosis factor alpha
VISA	vancomycin-intermediate <i>S. aureus</i>
VRSA	vancomycin-resistant <i>S. aureus</i>
VSSA	vancomycin-sensitive <i>S. aureus</i>
WBC	White blood cell
WCC	white cell counts

Chapter 1 **Diagnostics in Prosthetic Joint Infections**

Note: This overview has already been peer reviewed and published (1).

Globally, the incidence of arthroplasty is continuing to rise. In the United States alone, there were around 1.05 million total hip and knee arthroplasties performed in 2010 (2). The numbers are projected to reach 4.05 million by 2030 (3). Despite low complication rates, the rise in the number of joint replacement procedures performed worldwide could result in an increasing actual number of complications; the most severe and challenging of these include prosthetic (or periprosthetic) joint infection (PJI) or arthroplasty associated infections (AAI) which is defined as infection involving the joint prosthesis and adjacent tissue (4,5).

Prosthetic joint infection (PJI) may be present clinically without meeting criteria from the Proceedings of the International Consensus on Periprosthetic Joint Infection (5). Diagnostic techniques have received increasing consideration from multidisciplinary teams involved in managing PJI. To date, there is no diagnostic test with absolute accuracy for the diagnosis of PJI. Intraoperative tissue/fluid/prosthetic samples for culture have historically been used as the gold standard in most hospitals.

In general diagnostic tests can be grouped into pre-operative and intra-/postoperative diagnostics, some of which are excessively overused and others are underutilised for a number of reasons, including conflicting results in the literature, lack of availability and cost. The purpose of this chapter is to provide an overview of a range of diagnostic techniques that have been established in the diagnosis of PJI and some novel techniques that could become key diagnostics in the future (Figure 1.1).

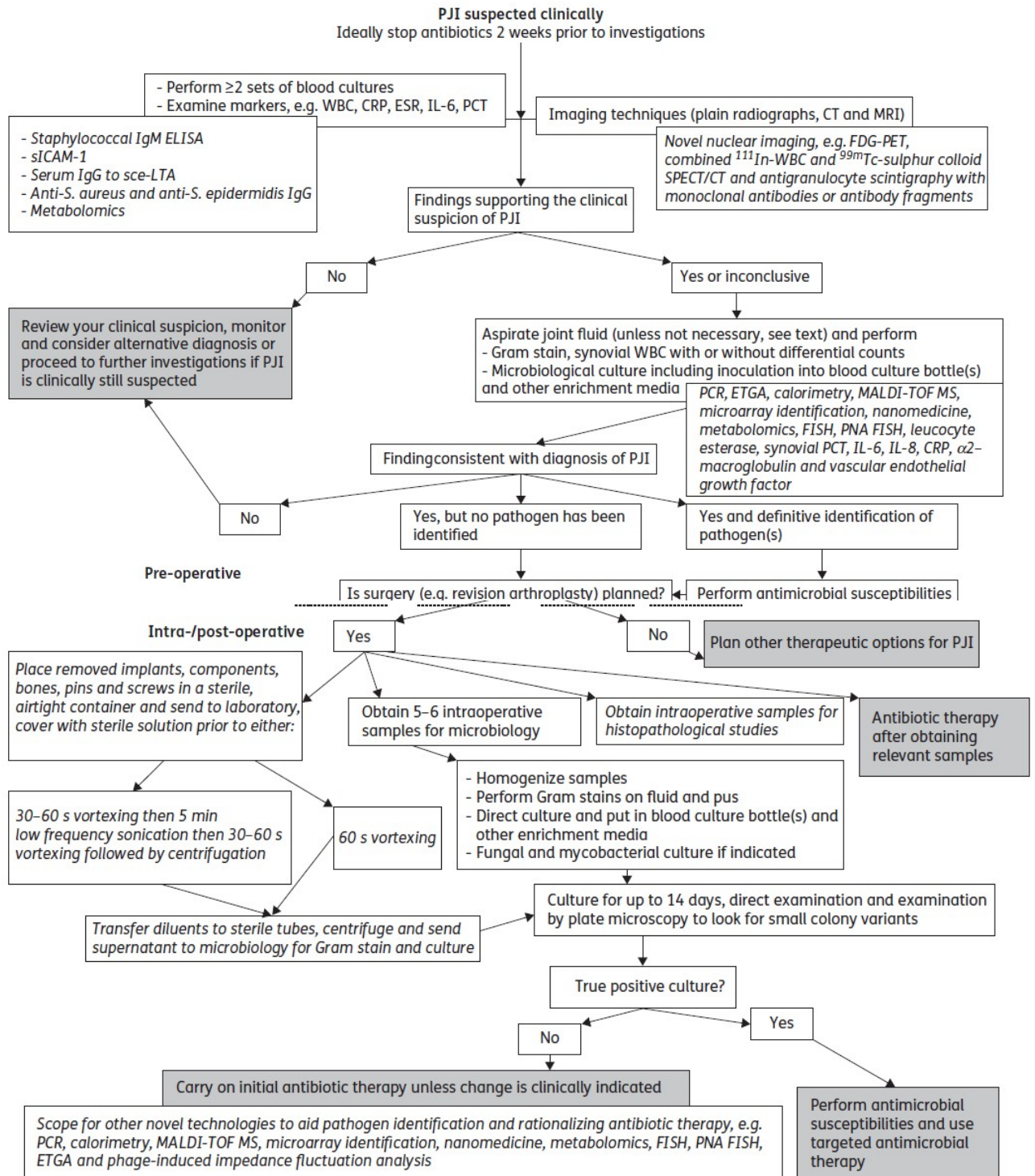


Figure 1.1 Diagnostic algorithm for PJI, including some novel science, tests and technologies (shown in italics) that may not be available in many centres or require further investigations.

PJI, prosthetic joint infection; WBC, white blood cell; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; PCT, procalcitonin; sICAM-1, soluble intercellular adhesion molecule 1; sce-LTA, short-chain exocellular lipoteichoic acid; MRI, magnetic resonance imaging; CT, computed tomography; FDG-PET, 18F-fluoro-2-deoxyglucose positron emission tomography; SPECT, single-photon emission computed tomography; PCR, polymerase chain reaction; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry; FISH, fluorescence in situ hybridization; PNA, peptide nucleic acid probe; ETGA, enzymatic template generation and amplification.

From Saeed K. J Antimicrob Chemother. 2014;69(SUPPL1)

1.1 Pre-Operative diagnostics

1.1.1 Biochemical, haematological, serological and microbiology studies

White blood cell (WBC) counts, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels tend to be first-line screening tests for evaluating patients with suspected PJI, probably due to their relatively low cost and widespread availability. However, they are not essential when making a diagnosis of PJI, particularly in clinically apparent infections. Indeed, increased WBC, ESR and CRP levels are neither particularly sensitive nor specific for PJI (6–11).

False-negative or low values could occur in the context of suppressive antimicrobial therapy, low-virulence pathogens, chronic infections and/or infections with a fistula, which are all common occurrences in PJI (12,13). Equally, they may be elevated due to concomitant inflammatory conditions or after primary uncomplicated arthroplasty (14). Furthermore, the diagnostic effectiveness of CRP and ESR can also be different according to the type of prosthesis or surgery. Piper et al (15) found CRP and ESR values were higher in knee arthroplasty and spine implant patients than in hip arthroplasty patients with infection and showed the lowest sensitivity for diagnosis of shoulder arthroplasty infection. Some advocate measuring baseline levels followed by serial measurements to assess trends, while others suggest that corroborating ESR and CRP values may provide the best positive (PPV) and negative predictive values (NPV) for the diagnosis of PJI (13,16–19). However, in a study of > 3500 CRP measurements in > 250 PJI patients, CRP values were neither sensitive nor specific as indicators for infection following second-stage revision surgery or for debridement, antibiotic and implant retention (DAIR) procedures. More importantly, the authors concluded that routine CRP monitoring should not be recommended in the prosthetic joint setting as results can lead to inappropriate management decisions and increased cost (20).

Other biomarkers have been evaluated or studied in the diagnosis of PJI (Table 1.1) (21–26). Studies have shown that the serum concentration of interleukin 6 (IL-6) is significantly higher in patients with septic loosening compared with aseptic loosening: cut-off levels of 8 or 9 pg/mL provided sensitivities of 40% and 80% and specificities of 81% and 77%, respectively (22,25) with the latter cut-off providing a PPV of 65%, an NPV of 50% and 78% accuracy for the diagnosis of PJI (22). Some

have suggested that elevated IL-6 levels of 12 pg/mL combined with 'high' CRP levels provide a good screening test to identify patients with PJI (21,26). However, the normal range of serum IL-6 varies, which may reflect a considerable variation in cut-off ranges in different studies (21,22,25,27–29). Furthermore, like CRP, IL-6 is not a specific marker for bacterial infection and its concentration in the peripheral blood increases after trauma, chronic inflammatory conditions and arthroplasty (6,30–32).

Since the early 1990s, there has been much interest in procalcitonin (PCT). Although studies have often given conflicting results regarding the superiority of PCT, it is largely believed that PCT is a more accurate indicator of bacterial infection than the biomarkers mentioned above (33–36). Unlike other surgical procedures, it appears that serum PCT levels are not significantly elevated following arthroplasty (23). In one study, serum PCT was evaluated within 10 days after orthopaedic surgery and was useful in differentiating infectious from non-infectious causes of fever (37). However, in other studies, despite striking specificity, the sensitivity of serum PCT has not been found to be of value in the diagnosis of PJI (Table 1.1) and hence, at present, serum PCT cannot be considered a superior marker to identify patients with PJI (21,22,24,25).

The association of tumour necrosis factor α (TNF- α) with other cytokines was previously reported in knee synovial tissue from patients with rheumatoid arthritis (38); however, there is insufficient information on the value of TNF- α as a diagnostic marker for infection or PJI (21). Soluble intercellular adhesion molecule 1 (sICAM-1) is a member of the immunoglobulin superfamily. Expression of the gene encoding sICAM-1 can be induced by cytokines and/or bacteria (39,40). Worthington et al (22) found that median sICAM-1 concentrations in the serum of patients with septic loosening (330 ng/mL) were significantly higher than in those with aseptic loosening (180 ng/mL) (Table 1.1). Drago et al (24) considered sICAM-1 to be a good marker for distinguishing cases of PJI from comparison groups consisting of patients without infection or those with previous infections that had been cleared.

As the majority of cases of PJI are due to coagulase-negative staphylococci (CoNS), serum IgG to short-chain exocellular lipoteichoic acid (sce-LTA) (previously termed lipid S) produced by CoNS (22,41) could represent a valuable diagnostic marker in patients with device-related infections including PJI due to CoNS (11,22,42,43). A staphylococcal IgM ELISA has been adapted for the diagnosis of delayed PJI. The test detects serum IgM antibodies to staphylococcal biofilm

polysaccharide antigens and researchers found a significant difference in levels between delayed PJI cases, non-infected implants and cases without prosthesis and infection. Using a cut-off value of 0.35 ELISA units, the test showed sensitivity of 90% and specificity of 95% (44). Other serological tests have also been adapted for use, with Luminex technology able to detect anti-*Staphylococcus aureus* and anti-*Staphylococcus epidermidis* IgG in 2 h (45). Studies evaluating these biomarkers in PJI are limited. The majority are small studies with different designs and inclusion and exclusion criteria. They fail to provide information regarding assay reproducibility and other factors that can affect the kinetics of some of these markers, e.g. presence of comorbidities and treatment with antibiotics, steroids and other immunomodulators at the time of testing. Hence, to a diagnostician, the role of these biomarkers in the diagnosis of PJI still remains to be fully defined. Undertaking tests with higher sensitivity followed by tests with higher specificity may prove to be of more value in assessing patients where there is a clinical suspicion of PJI (21,23,26,46,47). However, larger prospective and ‘real-life’ studies are needed to define performance, cut-offs, diagnostic utility, clinical reliability and cost-effectiveness of individual as well as combinations of biomarkers, not only in the diagnosis of PJI but also in the evaluation of response to therapy after DAIR or persistent infection in the period between explantation and reimplantation during two stage revisions.

Biomarkers	Cut-off	Sensitivity (%)	Specificity (%)	PPV	NPV	Comments
TNF- α (21)	≥ 40 ng/mL	43	94	75	85	testing needs to be done within 1 h and is time-consuming
Serum PCT (21,25)	≥ 0.3 ng/mL	<30-33	98-100	87	80	Routine test that can be performed on routine immunoassay platforms; however, it lacks sensitivity, particularly in localised infections
IL-6(21,22,25)	8–12 pg/mL	40-95	80-87	65-74	50-98	Routine test; however, its normal range varies in adults, which may reflect a considerable variation of cut-off ranges in different studies
sICAM-1 (22)	250 ng/mL	94	74	65	65	Insufficient information regarding clinical utilization in routine diagnostic laboratories
Serum IgG to sce-LTA (22)	3 out of 4 cases with CoNS PJI showed elevated levels					Specific only for coagulase-negative staphylococci; insufficient information regarding clinical utilization in routine diagnostic laboratories
Anti-staphylococcal IgM (44)	≥ 0.35 unit	90	95	90	95	May increase due to staphylococcal infection elsewhere in the body

Table 1.1 Biomarkers other than CRP, ESR and WBC that have been used or studied in the diagnosis of PJI

1.1.2 MRSA screening, blood cultures and swabs

Methicillin-resistant *S. aureus* (MRSA) screening, especially prior to orthopaedic implantation, is normal practice in UK hospitals. Documented concomitant or past MRSA carriage acts as a surrogate marker for clinicians to treat empirically with an MRSA-active antibiotic (48,49). However, a number of reports suggest current or past colonization with MRSA does not necessitate empirical antibiotic coverage for MRSA in PJI (50,51).

Blood cultures should be performed to exclude concomitant bacteraemia in suspected cases of PJI or if the patient is febrile and/or if there are concerns of metastatic infection. However, they often remain negative due to prior empirical antimicrobial therapy (52). Ideally, two or more sets or repeat samples should be taken prior to commencing antibiotic therapy to ascertain significance, particularly in cases of positive cultures yielding skin flora (53). Superficial and sinus tract swabs are not helpful, as the organisms cultured do not predict those causing deep infection (54).

1.1.3 Imaging studies

Plain radiographs are widely used in the initial evaluation of painful arthroplasties, despite the lack of sensitivity and specificity. They are most helpful in the diagnosis of PJI when studied serially over time, after implantation, and may guide further diagnostics. Ultrasonography could also be helpful in detecting joint effusions and guide arthrocentesis. Magnetic resonance imaging (MRI) and CT are not considered to be first-line imaging modalities for evaluating PJI. They can help detect sinus tracts, soft tissue abscesses, bone erosion and periprosthetic lucency. Detecting periostitis has 100% sensitivity but only 16% specificity for PJI. Associated joint distension and soft tissue fluid collections around arthroplasties increase this specificity to 87% (55). However, MRI should only be performed in patients with implants that are safe for this technique.

A recently published comprehensive review evaluated the diagnostic accuracy and clinical value of more advanced imaging techniques (Table 1.2) (56,57). The roles of these techniques in assessing patients for PJI are expanding, particularly for the detection of prosthetic hip infections. In practice,

however, these advanced imaging techniques still play only a limited role in the diagnosis of PJI as they can be resource- and time-consuming compared with other tests.

1.1.4 Pre-operative arthrocentesis

Pre-operative arthrocentesis is a valuable procedure for the investigation of PJI or failed arthroplasties (58–66). It must be performed aseptically and ideally in all patients with suspected PJI unless this is contraindicated (e.g. uncontrolled coagulopathy) or when the diagnosis of PJI is obvious prior to planned surgery (13,19,61–63). In revision total knee arthroplasty (TKA), it was demonstrated that synovial fluid WBC counts < 1100/mL containing < 64% neutrophils resulted in 99.6% NPV for excluding PJI (67). In contrast, a synovial fluid WBC count of > 1700/mL or >.65% neutrophils had sensitivities for knee PJI of 94% and 97%, respectively, and specificities of 88% and 98%, respectively, in patients without underlying inflammatory joint diseases and who were > 6 months from TKA implantation (65). WBC counts of > 27800/mL and 89% neutrophils have also been predictive of early TKA infections (66). With regard to hip arthroplasty, in a study of .200 patients with painful hip arthroplasties, a synovial fluid WBC count > 4200/mL was 84% sensitive and 93% specific, and 80% neutrophils was 84% sensitive and 82% specific to detect all those with hip PJI (59).

Imaging techniques	Sensitivity (%)	Specificity (%)	Comments
Radionuclide imaging and ¹⁸ F-fluoro-2-deoxyglucose positron emission tomography (56)	91-100	9-97	specificity varies depending on diagnostic criteria used
Combined ¹¹¹ In-WBC and ^{99m} Tc-sulphur colloid single-photon emission computed tomography/CT (56)	96-100	91-97	reported diagnostic accuracy of 95%–97%; appear to be promising tools in diagnosis of PJI
Antigranulocyte scintigraphy with monoclonal antibodies or antibody fragments (57)	83	80	routine test; however, its normal range varies in adults, which may reflect a considerable variation of cut-off ranges in different studies

Table 1.2 Diagnostic and clinical value of more advanced imaging techniques

Saeed K. Diagnostics in prosthetic joint infections. J Antimicrob Chemother. 2014;69(SUPPL1)

In general, the leucocyte differential varies between septic and aseptic loosening of prosthetic joints, with a predominance of neutrophils in PJI compared with lymphocytosis in inflammatory conditions (64). However, the interpretation of synovial WBC is complex when there is bleeding into the joint or in concomitant inflammatory arthritis; like other investigations, the synovial WBC count and its ability to predict or confirm PJI must be interpreted in light of clinical findings, the aspirated joint, the type of prosthesis and the time from implantation.

In a novel application, synovial aspirates were tested for the presence of leucocyte esterase enzyme using a simple colorimetric strip test. In 30 cases of PJI of the knee, when the leucocyte esterase reading was considered positive by the investigators, the test was 80.6% sensitive and 41.7%–100% specific with a PPV of 41.7%–100% and an NPV of 92.3% (68). Although analysis of the colorimetric strip test is subjective, these results are encouraging, especially as a point-of-care test in the diagnosis of PJI. Furthermore, in our institution, we have studied synovial PCT levels in pre-defined cases of joint infections and control groups including cases of PJI and aseptic loosening. We observed higher synovial PCT levels in infection cases including PJI compared with aseptic loosening and other inflammatory arthritis (69) as detailed below.

1.1.4.1 Measuring synovial fluid procalcitonin levels in distinguishing cases of septic arthritis, including prosthetic joints, from other causes of arthritis and aseptic loosening

Note: this original work has been peer reviewed and published (69)

1.1.4.1.1 Background

Clinical features of septic (pyogenic or bacterial) arthritis, including PJI, can mimic those of non-septic arthritis (e.g. degenerative, crystal and other inflammatory arthritis). In addition, manifestations of aseptic loosening can also resemble those of PJI. Studies have shown that the cause of arthritis remains unknown in about 16–36 % of patients (70). Joint aspiration for cytology

examination and bacterial culture, in combination with clinical, radiological and biochemical findings, remain the standard tests for the diagnosis, but none of these have a satisfactory efficacy, sensitivity or specificity (70,71). Although a true bacteriological culture can be regarded as a “gold standard” diagnostic test, in reality this approach is time consuming, especially when these infections require urgent antimicrobial treatment; in addition, a negative bacteriological culture does not always exclude an infective process, especially in those who has been on empirical antimicrobials prior to sampling. PCR assay have been used to detect causative bacterial agents, but sensitivities and specificities vary among the different assays and are not routinely available in many UK hospitals (72).

Strategic biomarkers are needed to assist in a rapid diagnosis and differentiation of these cases. The utility of serum PCT in detecting bacterial infection has been recognized in multiple studies, and different serum cut-off levels have been suggested for various clinical conditions (36,73–78). Serum PCT has been previously evaluated as a biomarker for differentiating septic from non- septic arthritis with conflicting results. The variability in these results may be explained by both the presence or absence of systemic inflammatory response syndrome, the assays used and/or the whole study design, with some of these studies excluding certain groups of patients making their results less representative of real-life situations (9,10,25,79–82). At a cut-off value of ≥ 0.3 ug/L, however, serum PCT has been found to be very specific (98 %), although its sensitivity is $< 35\%$, particularly in PJIs (9,10,25,79–82). The objectives of this study were to establish if PCT measurement from synovial fluid (usually validated as a serum assay) is reproducible and to evaluate the usefulness of measuring PCT levels directly from synovial fluid for differentiating septic arthritis, including PJI, from other forms of arthritis and aseptic loosening. We have also produced Receiver Operating Characteristic (ROC) analysis and calculated the sensitivity, specificity and predictive values for various synovial fluid PCT cut-off levels in predefined cases of septic and non-septic arthritis, including where an implant is present. To our knowledge this is the first report presenting such data.

1.1.4.1.2 Methods

This non-interventional, unblinded comparative study was performed at the Royal Hampshire County Hospital (Hampshire Hospitals NHS Foundation Trust), Winchester, UK. The study received ethical approval from the Health Research Authority NRES committee South West, Exeter, UK REC

No. 12/SW/0070. Using a standard quantitative PCT enzyme immunoassay kit (Brahms Diagnostica, Berlin, Germany) on a mini VIDAS analyser (BioMérieux, France), synovial PCT level was measured retrospectively in 76 adult patients. The patients were categorized into two groups, with those in group A ($n = 26$) diagnosed with septic arthritis (eight cases of prosthesis-related infections and 18 cases of native joint arthritis) and those in group B ($n = 50$) with non-septic arthritis (including 6 cases of aseptic prosthetic loosening). Case selection, definition, inclusion and allocation to each group were based on clinical, radiological, microbiological culture and biochemical results, the patients' final diagnosis, including intraoperative findings in implant-related cases and response to treatment. Patients without a diagnosis suitable for placement in either group A or B or patients who had conflicting clinical and diagnostic findings were excluded from the study, including those who possibly or probably had a clinical infection, but for whom no confirmatory positive bacteriological cultures were available.

Between 2009 and 2012, synovial samples were taken from these patients aseptically as part of their routine investigations by appropriate clinical staff at different hospital source points. On arrival in the laboratory, the samples were processed for routine diagnostic tests, and the surplus from each sample was divided into aliquots and frozen at $-20\text{ }^{\circ}\text{C}$ for subsequent use in the PCT investigations. At the time of synovial PCT testing, the frozen samples were thawed and tested neat within 2 hours; however, highly viscous joint fluid samples were tested neat and also diluted 1:4 (100 μL of joint fluid sample with 300 μL of serum-free reagent; ref. 66581, BioMérieux).

Given that synovial fluid is not the usual medium for which the PCT assay was designed, the reproducibility of synovial PCT measurements was also assessed on synovial PCT samples containing lower (<0.05 and $1\text{ }\mu\text{g/L}$) and higher ($4.6\text{ }\mu\text{g/L}$) PCT concentrations using a previously validated formula (83). Statistical analysis was performed using SPSS ver. 20 software (SPSS, Chicago, IL) to compare the mean synovial fluid PCT scores of the two groups. We also performed a ROC analysis and determined the sensitivity, specificity, PPV and NPV for various synovial fluid PCT concentrations.

1.1.4.1.3 Results

A total of 76 patients (26 in group A and 50 in group B) were enrolled in the study (Table 1.3). There was a statistically significant difference in the mean synovial fluid PCT values between the two

groups (Figure 1.2). The ROC analysis and the sensitivity, specificity and PPV and NPV for various synovial PCT values are given in (Figure 1.3). Within the batch of synovial PCT samples containing different concentrations of PCT, the coefficient of variation (CV) was 0, 4.1 and 3.3 % at cut off points of <0.05, 1, and 4.6 ug/L, respectively.

Patient characteristics	Group A ^a	Group B
Median age, years (range)	78.7 (43–88)	66.5 (30–90)
Diagnosis (number of cases)	Prosthetic joint infection (PJI) (8) ^b Native joint septic arthritis (18)	Aseptic loosening (6) ^b Crystal arthropathy (20) Osteoarthritis, rheumatoid arthritis, psoriatic arthritis and other inflammatory and non-specific arthritis (24)
Total number of cases (male: female)	26 (15:11)	50 (33:17)

Table 1.3 Age, male to female ratio and diagnosis in each study group

^a Microorganisms detected were *Staphylococcus aureus* (12 cases), other *Staphylococci* spp. (4 cases), b-haemolytic *Streptococci* (3 cases), *pneumococci* (1 case), *enterococci* (1 case) and *Escherichia coli*, *Proteus* spp. and other coliforms (remaining 5 cases)

^b Mixture of knee and hip arthroplasties. Median synovial procalcitonin (PCT) level in the PJI group was 3.12 vs. 0.05 ug/L (aseptic loosening group). Only two cases of PJI had concomitant positive blood cultures. The highest synovial PCT levels were seen in *S. aureus* infections regardless of the presence of concomitant positive blood cultures. Note this is just an observation and the numbers are too small to extract statistical power

From Saeed K, et al. Infection. 2013;41(4).

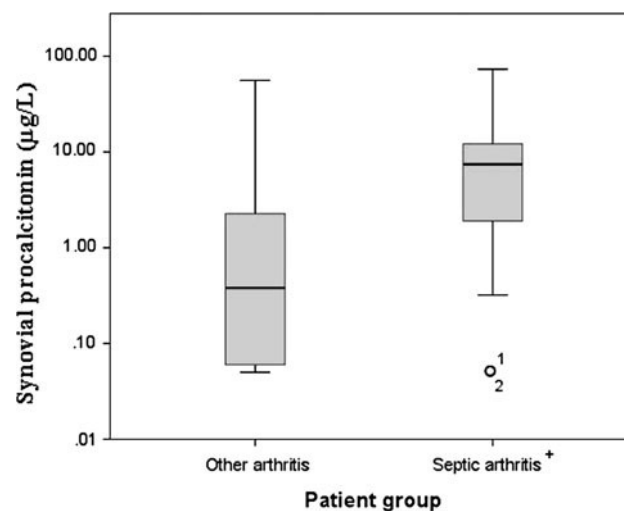


Figure 1.2 Synovial concentration of procalcitonin (PCT) according to the diagnosis of septic vs. non-septic (other) arthritis.

Under the assumption of no equal variance, the independent-samples *t* test suggested that there was a significant difference in the mean values for non-septic arthritis [in ug/L; median 0.415, range 0.05–56.06, mean 2.61; standard deviation (SD) 8.22] and septic arthritis (in ug/L; median 7.435, range 0.05–73.33, mean 10.37, SD 14.95; $p = 0.020$).

1, 2: Patients with confirmed septic arthritis and low synovial PCT; both had been on appropriate antibiotics for some time prior to sampling although this treatment may not be the reason for the low PCT level. *Plus symbol* All cases had a positively significant microbiological culture in the synovial fluid; there was no apparent relation between the nature of the organism and the synovial PCT value. PCR was not used due to lack of availability in-house and funding. Blood cultures were taken in all these cases in this group (group A), with only 6 of the 26 cases with a concomitant (same isolate) positive blood culture

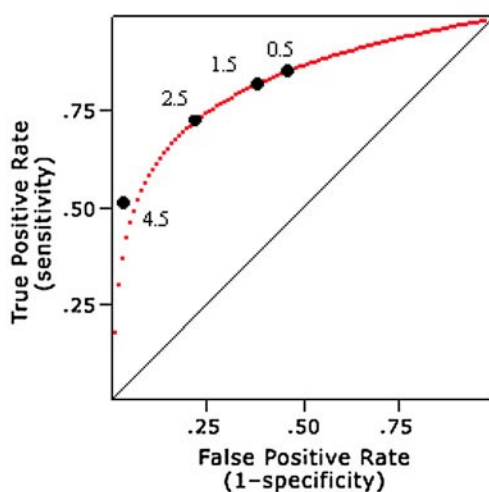
From Saeed K, et al. Infection. 2013;41(4).

1.1.4.1.4 Discussion

Our results suggest that synovial PCT may be a valuable biomarker in supporting clinicians in differentiating septic from non-septic arthritis as in our study cohort group A patients (diagnosed with septic arthritis) had significantly higher mean synovial PCT values than group B patients (diagnosed with non-septic arthritis) (Figure 1.2). However, as with any diagnostic test, synovial fluid PCT values have to be interpreted in the context of the clinical setting. Based on our cohort a synovial fluid PCT cut-off value of < 0.5 ug/L is likely to exclude the diagnosis of septic arthritis with a NPV of 0.90 (95 % confidence interval 0.73–0.97), but a poor PPV (Figure 1.3). Two patients in group A had synovial PCT values of < 0.5 ug/L despite having a genuine infection: one had a low grade PJI due to CoNS, and the other had a native joint infection due to *Escherichia coli*; both patients received the appropriate antibiotics prior to synovial fluid aspiration (Figure 1.2; Table 1.3). However, there were seven other patients in group A which received antibiotics prior to sampling, and their synovial PCT levels remained elevated. Therefore, prior antibiotic therapy in these two cases does not entirely explain the low synovial PCT values, although the low grade PJI infection with CoNS may be a factor. In contrast, higher synovial PCT levels among group B patients were observed in cases of crystal arthritis, particularly where there was a concomitant malignancy; two of the highest values in this group were found in patients with a history of renal cell carcinoma and carcinoid tumour. In the absence of pre-existing inflammatory conditions or malignancy, a value of ≥ 0.5 ug/L may also be supportive for the diagnosis of an infection process, and overall synovial fluid PCT values of > 4.5 ug/L provided a PPV 0.82 and NPV of 0.79 for the diagnosis of septic arthritis (Figure 1.1). This cut-off value may be particularly valuable in cases of crystal arthritis with simultaneous septic arthritis, but further studies are needed to confirm this hypothesis.

Although previous reports have shown that serum PCT measurements lack sensitivity in the diagnosis of PJI, with some authors attributing this to the absence of a systemic inflammatory response in most PJI (9,10,25,82), our observations is that (apart from the one patient infected with CoNS discussed above) cases of PJI, regardless of the presence of concomitant positive blood cultures, were associated with higher synovial PCT values than cases of aseptic loosening (Table 1.3). We therefore believe that synovial PCT measurements warrant further evaluation in cases of early and delayed PJIs in larger centres and with appropriate controls.

ROC Curve for $y = 0.18\ln(x) + 1$
 Area under curve = 0.824



Cut-off µg/L	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)	PPV (95% Confidence Interval)	NPV (95% Confidence Interval)
0.5	0.88 (0.69-0.97)	0.57 (0.42-0.71)	0.52 (0.37-0.67)	0.90 (0.73-0.97)
1.5	0.85 (0.64-0.95)	0.62 (0.55-0.81)	0.59 (0.42-0.75)	0.89 (0.74-0.96)
2.5	0.69 (0.49-0.85)	0.78 (0.63-0.88)	0.62 (0.42-0.79)	0.83 (0.68-0.92)
4.5	0.54 (0.34-0.73)	0.94 (0.82-0.98)	0.82 (0.56-0.95)	0.79 (0.66-0.88)

Figure 1.3 Area under the receiver operating characteristic (ROC) curve and the ability of synovial PCT to distinguish septic arthritis from non-septic arthritis [sensitivity, specificity, positive and negative predictive values (95 % confidence interval)] at different cut-off level

From Saeed K, et al. Infection. 2013;41(4).

From the limited number of tests that we have conducted, it appears that our PCT assay performs relatively well and is reproducible (CV range from 0 to 4.1 % at various cut-offs). The test takes up to 30 min to perform in the laboratory and may have the potential to become a point of care test performed by those who obtain the synovial fluid. Some of the limitations of this study include a relatively small number of patients and the absence of other biomarker assays performed concomitantly, including serum PCT, to compare to values reported in other studies and to synovial PCT values in this study. Additionally, the retrospective aspect of our study was a major limitation in terms of comparing synovial PCT values to full clinical details, criteria of systemic inflammatory response and the severity of arthritis. Nevertheless, our results provide some evidence that synovial fluid PCT may become a useful tool in the evaluation and management of patients with painful and swollen joints, including those with orthopaedic implants, and that it may supplement other clinical, radiological and laboratory findings in differentiating septic from other arthritis. Differentiating

septic from non-septic arthritis with a simple biochemical test is a major challenge. Based on our findings, we believe that synovial PCT measurements would be more informative than serum PCT measurements in differentiating infection from non-infection cases of arthritis, particularly when the nature of the infection is more localized and in PJIs. Very high synovial fluid PCT levels (e.g. >4.5 ug/L) may support a diagnosis of an infective process with subsequent initiation or continuation of antibiotic therapy; at the same time, the high NPV of this measurement with lower synovial PCT levels (e.g. <0.5 ug/L) could aid clinicians to exclude a diagnosis of septic arthritis, directing them towards alternative diagnoses and prompting cessation of antibiotic therapy once started. This in turn could lead to a reduction in unnecessary antibiotic use, a valuable aspect of antimicrobial stewardship in the era of rising antimicrobial resistance, as well as a change in the management of implant related infections. Before synovial PCT becomes a routine diagnostic test, larger, prospective studies are needed to further validate these findings, define optimal synovial cut-off levels in both native and prosthetic joints and perform a cost-effectiveness analysis.

1.1.4.1.5 Further work

The above work has led to further works specifically related to synovial PCT and PJI. This work is ongoing and so far synovial PCT remains a promising tool in the diagnosis of PJI. Preliminary results of this work has been presented as an Abstract (code P16) at the European Bone and Joint Infection Society conference (EBJIS) in Utrecht/ The Netherlands in September 2014 (Appendix A Synovial fluid Procalcitonin and the diagnosis of potential implant related infections)

1.2 Intra-/post-operative diagnostics

1.2.1 Histopathological studies

Histopathological examination of intraoperative samples could provide additional information to gross surgical appearance and aid in the diagnosis of PJI and acute inflammation (84,85), although such examination is unlikely to identify causative organisms. A further consideration is that the presence of particular pathogens and previous antibiotic therapy could modify the nature of the inflammatory response and alter the results (86). A neutrophil count of 5–10 cells per high power field, at a magnification of $\times 400$, has a sensitivity of 50%–93% and specificity of 77%–100% for predicting PJI and has been used to decide between the need for revision versus resection arthroplasty when other pre-operative evaluation has failed to confirm PJI (13,19,87–92). Unfortunately, not all centres have specialist histopathologists to carry out this type of analysis, which is crucial in making these decisions.

1.2.2 Microbiology studies

Samples for microbiological investigation can include pus, synovial fluid, soft tissue, bone and prosthetic components. Cultures of sinus tract exudates are often positive due to skin flora and intraoperative swab cultures have low sensitivity and should be avoided (93). Withholding or stopping antimicrobial therapy (if possible for ≥ 2 weeks) prior to collecting the specimens increases the yield of recovered organisms (13,94).

The importance of at least five separate biopsy samples for bacterial culture, taken in proximity to hip prostheses for the optimal diagnosis of PJI, was first propounded in the 1980s (95) and later confirmed by other investigators (13,53,91,94). Combination of synovial fluid and periprosthetic tissue may provide the best sensitivity, specificity and accuracy (96). Each specimen should be obtained with a separate set of sterile instruments and placed into a separate sterile container. At

this stage, a frozen section, if available, may also be performed (97). Samples should be transferred as soon as possible to the laboratory for culture, but if delays are inevitable, they can be kept either at 4°C or at room temperature. Maintaining anaerobic conditions during transportation and, if needed, using amies transport medium may yield better viability (98).

Various preparatory methods have been applied in the processing of tissue samples prior to culture on assorted media, e.g. partitioning bigger samples into smaller pieces with surgical knives (98,99) homogenization using Ballotini beads or grinding with a mortar and pestle or a Seward stomacher (100). Gram staining of tissue samples can be performed but has low sensitivity.

Inoculating synovial fluid into paediatric blood culture bottles (BCBs) was reported to detect more pathogens than direct culture methods (101). A prospective study evaluated four different culture media used in the diagnosis of PJI; BACTEC[®] BCBs and cooked meat (CM) broth were significantly more sensitive than direct plates and fastidious anaerobic broths for various intraoperative samples, with no significant difference in sensitivity between BACTEC[®] and CM (102). Equally, others demonstrated higher sensitivity and specificity for synovial fluid inoculation into aerobic and anaerobic BCBs (93,103). However, despite the above reports, this practice has not been featured in more recent guidelines (104). Most studies recommend 5 days of incubation for aerobic cultures and 7 days for anaerobic cultures (94,105,106), but prolonged incubation for up to 13–14 days may help with pathogen isolation, particularly *Propionibacterium* spp. and small colony forms, e.g. in *Escherichia coli* (104,107,108). Special culture techniques for fungi and mycobacteria are necessary if clinically suspected. Isolation of identical organism(s) from two to three or more independent specimens is highly predictive of infection (sensitivity: 65%; specificity: 99.6%) (53,95,107). The prevalence of small colony forms in PJI is unclear. These organisms may be present in areas near the prosthesis with low concentrations of antibiotic diffusing from the cement. Hence, the UK Standards for Microbiology Investigations recommends examination of culture plates with a plate microscope to detect small colony forms of staphylococci (109).

False-negative culture results in PJI may be due to sampling error, prior antibiotic therapy, low quantity of microorganisms, fastidious organisms, use of inappropriate culture media and delays in processing samples. Furthermore, organisms may be concentrated in the periprosthetic tissue and in biofilm-related implant infections and, for these reasons, conventional culture methods developed

for planktonic bacteria are not reliable and have led to the erroneous diagnosis of 'aseptic loosening or failure' in what were genuine infections (110). Hence, obtaining samples from the prosthesis could improve the diagnosis of PJI (94,111,112). The explanted prosthesis, joint components, bones, pins and screws can be placed in a sterile, airtight container and covered with Ringer's solution or saline prior to submission to the laboratory for sonication and subsequent centrifugation and subculture of sonication fluid. Studies have demonstrated that the diagnostic sensitivity and specificity of Gram staining of sonication fluid are ~45% and ~100%, respectively (94). Subculture of sonication fluid has shown a sensitivity of 79% compared with 60% for conventional cultures. Additionally, the sensitivities of periprosthetic tissue and sonication fluid culture in patients receiving antimicrobial therapy within 14 days before surgery also differed significantly (45% and 75%, respectively) (94). The specificities of sonication fluid culture, tissue culture and synovial fluid culture are ~99%, ~99% and ~98%, respectively (27,94,105,113–115).

Optimizing sonication parameters such as duration, temperature and centrifugations are critical for better microbial yield (101,112). Vortexing samples for 30–60 seconds before and after 5 min of low-frequency sonication also leads to better pathogen recovery (94,116,117). However, sonication is not widely available in routine diagnostic centres; moreover, it may damage bacteria, especially Gram negatives and anaerobes, and there is a risk of contamination during the procedure (94,118). A recent prospective study compared the efficacy of vortexing alone for 1 min versus vortexing plus sonication for biofilm disruption in the diagnosis of PJI. Among 135 removed prostheses, 35 were diagnosed with infection and 100 with aseptic failure. Using a cut-off of ≥ 50 cfu/mL, vortexing plus sonication showed higher sensitivity than vortexing alone (60% versus 40%) while the specificity was 99% for both methods. At this cut-off, the sensitivities of sonication and vortexing fluid culture were reduced to 39% and 30%, respectively, in patients who previously received antibiotics. However, at a lower cut-off of ≥ 1 cfu/mL, vortexing alone and the combined method were nearly identical in both sensitivity (69% versus 71%) and specificity (92% versus 93%). Therefore, the authors advocated using the lower cut-off (119). Vortexing is not technically challenging, does not appear to be harmful to bacteria and, for laboratories that cannot perform sonication, vortexing a resected device without sonication is probably a reasonable alternative.

1.3 Novel technologies, tests and potential future diagnostics

A summary of novel technologies are listed below:

- Microcalorimetry: Measures heat intensity produced by dividing microorganisms in relation to their replication and metabolism in real time (120). *In vitro* analysis has shown it can be a suitable tool for accurate, non-invasive and real-time microbiological assay of biofilms (121). A recent review cited unpublished data indicating that microcalorimetric analysis of synovial fluid from patients with acute arthritis discriminated between septic and non-septic arthritis within 8 h (45).
- Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS): MALDI-TOF MS has transformed pathogen identification (122). A recent study demonstrated the ability of MALDI-TOF MS to recognize and identify staphylococci in patients with PJI (123). Another group used Ibis T5000 biosensor technology combined with broad-range PCRs and MS to detect infected cases of PJI that were miscategorised as aseptic loosening or failure (124).
- Bead mill processing: Involves agitation of tissue samples with glass beads to release intracellular pathogens from biofilms. In a retrospective study this technique detected organisms in 84% of patients who were undergoing revision arthroplasty (125), a better yield than conventional culture.
- Fluorescence *in situ* hybridization (FISH) and peptide nucleic acid probe: FISH can identify and enumerate specific microbial groups in <1 h in positive blood culture bottles (126). Combining FISH with confocal laser scanning microscopy has created 2D and 3D images of biofilms that can help to assess the effectiveness of antimicrobial agents and which offer great potential for the diagnosis of PJI (127,128).

- Synovial biomarkers: in addition to synovial PCT (69), the use of synovial biomarkers (IL-6, IL-8, CRP, α 2-macroglobulin and vascular endothelial growth factor) as rapid and inexpensive diagnostic tools in PJI is the subject of current research (129,130).
- Enzymatic template generation and amplification: A novel and rapid technology for the detection of viable organisms in blood culture bottles. The technique determines the level of microbial DNA polymerase within clinical samples in <3 h with sensitivity and specificity of 90.6% and 99.0%, respectively (Dr M. Dryden, Hampshire Hospitals NHS Foundation Trust, personal communication).

Synovial fluid or intraoperative tissue samples after sonication or bead mill homogenization could potentially be investigated by these techniques. Other potentially useful technologies under development include microarray (131), phage-induced impedance fluctuation analysis (132), nanomedicine (133) and metabolomics (134). These may potentially be applied in the routine management of PJI in the future, not only to identify pathogens, virulence factors and antimicrobial susceptibilities, but also to provide information on disease process and progression as well as response to therapy. PCR amplification and sequencing analysis of 16S rRNA, specific PCRs and reverse transcription PCR are attractive tools especially in culture-negative infections or in the presence of fastidious microorganisms. These techniques will be discussed in more detail in Chapter 2.

Chapter 2 Application of Specific PCR in the diagnosis of PJI and a review of this technology and its impact on the PJI management

This chapter included an original published work related to specific Staphylococcal PCR followed by literature review evaluated the impact of PCR in the management of PJI that was also peer reviewed and subsequently published.

2.1 Specific staphylococcal polymerase chain reaction can be a complementary tool for identifying causative organisms and guiding antibiotic management in orthopaedic infections

Note: this original work has been peer reviewed and published (72).

Staphylococcus species are one of the most common organisms isolated from bone and joint infections including PJIs in adults. Rates up to 39% for *S. aureus* and 37.5% for coagulase-negative staphylococci have been reported. European data support that *S. aureus* remains the most common cause of septic arthritis (135–137). These infections tend to be complex, their microscopy and culture-based microbiological identification can be challenging and in many cases inconclusive (138). Delayed treatment can have devastating effects on patients' outcomes. Therefore, treatment with broad-spectrum antibiotics active against staphylococci usually is started in the community, often for a prolonged period before sampling in surgery, particularly in infections of native joints. Consequently, this can prevent growth of bacteria on standard culture media even after thorough surgical

debridement, obtaining multiple tissue samples and extended 5-14 days culture period based on previously published results (107,139,140).

Improved and more rapid microbiological diagnostic techniques such as real-time polymerase chain reaction (RT-PCR) are becoming cornerstones in the identification of causative agents for many infections. PCR involves a process of selective enzymatic amplification and detection of known target DNA or RNA sequence present in a suspected organism. Amplified DNA is later analysed for bacterial identification. In this article we demonstrate how useful an assay specific for methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA) and methicillin resistant coagulase negative staphylococci (MRCNS) RT-PCR can be in the diagnosis and antimicrobial management of bone, joint and prosthetic joint infections. Our aims were:

- i) to perform a RT-PCR method to identify the presence of two genes (*mecA* and *femB* genes) in bone and joint infection or prosthetic joint infection specimens and
- ii) to discuss positive and negative results with clinicians and advise on modification of antibiotic therapy.

2.1.1 Materials and Methods

2.1.1.1 Nucleic Acids Extraction from Specimens

Tissue samples were lysed in MagNA Pure DNA tissue lysis buffer (Roche[®], Roche Diagnostics Limited, West Sussex UK) and extracted using a MagNA Pure LC by Roche[®] using the MagNA Pure LC DNA isolation kit III (bacteria and fungi, Roche[®]) using the manufacturer's protocol.

2.1.1.2 Duplex RT-PCR for *mecA* and *femB*

At the Molecular Diagnostics Unit of the Health Protection Agency [HPA , currently Public Health England (PHE)] Southampton regional laboratory, 19 primary samples, which failed to grow on culture after 5 days of incubation, were examined by using duplex 5' endonuclease (TaqMan[®], Roche Diagnostics Limited, West Sussex, UK) RT- PCR (141–143), for the presence of *mecA* (this lies within the staphylococcal cassette chromosome mec (SCCmec) mobile cassette, encodes methicillin resistance) and *femB* (factor essential for methicillin binding, and is targeted at a *S. aureus* specific sequence genes; Table 2.1). Samples included six bone and tissue specimens, six prosthetic joint fluid aspirates, and seven native joint fluid aspirates taken from adult patients with suspected bone, joint or prosthetic infection who had been on various anti-staphylococcal antibiotics before surgery (Table 2.2).

Primers were used at a working concentration of 300 nM each, while TaqMan[®] probes were used at a working concentration of 200 nM each per reaction, with Light- Cycler 480 Probes master (Roche, Mannheim, Germany) universal master mix. Five uL of DNA template was added to a final volume of 20 uL per reaction. After extraction, RT-PCR was carried out using the Rotor- geneQ (6000) (Qiagen, Crawley, UK) (141). Cycle conditions were 95 °C for 5 minutes, then 50 cycles of 15 seconds at 95 °C and 60 seconds at 60 °C with signal acquisition at the end of each 60 °C step. Interpretation of RT-PCR results was as follows:

positive *femB* and *mecA*= MRSA;

positive *femB* and negative *mecA*= MSSA;

negative *femB* and positive *mecA*= MRCNS;

negative *femB* and *mecA*= staphylococcal DNA not detected.

Discriminate detection of the two targets in the duplex assay was possible by use of two different fluorophors: 6-carboxyfluorescein (FAM: emits a fluorescent signal

at 525 nm) for detection of *mecA*, and 6-carboxy-2',4',7',4',7'-hexachlorofluorescein (HEX: emits a fluorescent signal at 560 nm) for detection of *femB* (Table 2.1).

Primer name*	Sequence (shown 5' to 3')
femBF1	GACATTTGATAGTCAACGTAACGTAATATT
femBR1	GCTCTTCAGTTTCACGATATAAATCTAAGA
mecAF	CATTGATCGCAACGTTCAATTT
mecAR	TGGTCTTTCTGCATTCTGGA
Probe name*	Sequence (shown 5' to 3')
<i>femB</i> VICP1†	TCATCACGTTCAAGGAATCTGACTTTAACACCATAGT
<i>mecA</i> P†	TGGAAGTTAGATTGGGATCATAGCGTCAT

* Primers and probes obtained from eurofins MGW Operon, Anzingerstr 7a, 85560 Ebersberg, Germany.
 † The *mecA* probe was labeled with a 5' FAM fluorophor and a 3' TAMRA quencher. The *femB* probe was labelled with a 5' HEX fluorophor and a 3' TAMRA quencher.

Table 2.1 Primer and probe sequences

From Saeed K, et al. Curr Orthop Pract. 2010;21(6).

2.1.2 RESULTS

Nineteen suspected cases of bone, joint or PJI from patients who were on anti-staphylococcal antibiotics with intra-operative samples that failed to grow on conventional culture were identified. These were tested using the RT-PCR duplex for *mecA* and *femB*. The results were communicated to the orthopaedic consultants (Table 2.2); the surgeon was not obliged to take our advice on the basis of these results, especially with regard to discontinuing antibiotics. Ten samples (57.8%) gave positive results RT-PCR, four for MSSA, two for MRSA and four for MRCNS. The remainder were negative for any staphylococcal DNA. The decision in these patients was left to the orthopaedic team on the basis of clinical, intraoperative findings and inflammatory biomarkers such as unexplained loosening with diagnostic analyses, such as measurement of white blood cell (WBC) count and erythrocyte sedimentation rate, C- reactive protein assays and radiographic examinations.

No	Specimen type	PCR ID	Already on empirical antibiotics	Comments
1	Metal work joint fluid	DNAND*	Vancomycin and rifampicin	Decision by consultant orthopaedics on the basis of intraoperative, clinical and biochemical findings
2	Metal work joint fluid	DNAND	=	=
3	Tissue knee	DNAND	Cefuroxime and fusidic acid	=
4	Tissue knee	DNAND	=	=
5	Tissue knee	DNAND	=	=
6	Metal work joint fluid	DNAND	Vancomycin	=
7	Elbow aspirate	DNAND	=	=
8	Aspirate from hand	DNAND	=	=
9	Femoral membrane tissue	DNAND	=	=
10	Metal work joint fluid	MRCNS†	Vancomycin and rifampicin	Confirmation that patient on the right antibiotics, continue same treatment
11	Metal work joint tissue	MRCNS	=	=
12	Tissue knee	MRCNS	=	=
13	Tissue knee	MRCNS	=	=
14	Shoulder aspirate	MRSA‡	Vancomycin and fusidic acid	=
15	Synovial fluid elbow	MRSA	Flucloxacillin	Antibiotic changed to vancomycin and fusidic acid eventually patient became positive for MRSA in the nose and continued treatment with oral doxycycline and fusidic acid.
16	Joint fluid knee	MSSA§	Cefotaxime	Antibiotic changed to flucloxacillin
17	Shoulder aspirate	MSSA	Flucloxacillin	Confirmation that patient on the right antibiotics, continue same treatment
18	Shoulder aspirate	MSSA	=	=
19	Prosthetic hip aspirate	MSSA	Vancomycin	Antibiotics changed to flucloxacillin

*Staphylococcal DNA not detected. = Same as above.
†DNA of Methicillin resistant coagulase negative Staphylococci detected.
‡DNA of Methicillin resistant Staphylococcus aureus detected.
§DNA of methicillin sensitive Staphylococcus aureus detected.

Table 2.2 Samples with negative cultures after prolonged incubation and results of PCR and effect on patients' management

From Saeed K, et al. Curr Orthop Pract. 2010;21(6).

2.1.3 DISCUSSION

Ten of 19 samples gave positive results using RT-PCR (Table 2.2). In seven patients [patients 10, 11, 12, 13, 14, 17 and 18] identification of a causative organism was of great reassurance for the surgical team. These individuals were on effective and adequate antibiotic therapy. In three patients the results of the RT-PCR led not only to identification of a causative organism, but also a complete change of antibiotics. In two patients [16, 19], RT-PCR identified that infection was caused by MSSA, hence the surgical team and microbiologists agreed to narrow the antibiotic spectrum by changing therapy from cefotaxime and vancomycin, the former of which is an agent heavily associated with *Clostridium difficile* associated diarrhoea (CDAD) (144,145), to flucloxacillin which is not only the drug of choice in treatment of infection associated with MSSA but also is cheaper with fewer adverse side effects. In the remaining patient [patient 15], the RT-PCR resulted in identification of MRSA in samples taken from this patient. This led to a change in antimicrobial therapy from flucloxacillin to vancomycin and fusidic acid. Interestingly, this

patient was not known to be a carrier of MRSA. However, after we analysed this result the patient was re-screened and found to be colonized with MRSA in the nose.

In the remaining nine samples (42.2%), the RT-PCR results were negative and in concordance with conventional cultures. This may be attributed to either true negatives, prolonged antibiotic therapy before sampling, infection caused by organisms other than *S. aureus* or infection caused by methicillin sensitive coagulase negative staphylococci (MSCNS), which are not detected using this RT-PCR duplex. These strains are extremely rare in Southampton University Hospital Trust. On the basis of these results, the final decision for antibiotic continuation was made by the orthopaedic surgeons based on the clinical, intra-operative and biochemical biomarker findings.

Ideally in bone, joint and prosthetic joint infections, deep tissue and pus samples should be submitted before antibiotic therapy in hemodynamically stable patients. This does not often occur in clinical practice and sometimes patients are treated with antibiotics in the community before attending secondary or tertiary care. Failure to treat an infected bone or joint could have devastating consequences for the patient. Therefore, broad-spectrum antibiotics often are prescribed for prolonged periods before sampling. As a result, conventional microbiology culture is not always useful in identifying causative organisms even after 5 days incubation. Bacterial culture in combination with clinical findings, biochemical biomarkers, and histology remains the gold standard for the diagnosis of these infections (13,146,147). However, histological diagnosis requires expertise and significant funding, and therefore is not available in many hospitals in the UK. In addition, culture may not be precise for diagnosis in a considerable number of patients (148–150). False-negative cultures, possibly caused by the presence of a low-grade infection, are a significant problem because they may result in the delay of adequate antimicrobial therapy or prolonged therapy with expensive broad spectrum antibiotics with potential adverse effects. Such effects include colonization and infection with multi-drug resistant organisms, CDAD and an increasing cost of treating the patient.

Several studies on the use of PCR-targeted at the 16S ribosomal RNA gene, present in all bacteria, have been published (138,151–155). However, this is carried out by sequencing, a technology that is not available in most UK hospitals, usually is expensive and secondary confirmatory tests often are required to distinguish speciation e.g. MRSA, MSSA or MRCNS. RT-PCR has been used before in the diagnosis of *kingella kingae* infections (156). Our group also developed a multiplex PCR for the diagnosis of bone and joint infections in children (157), but we are not aware of any other report of a specific staphylococcal RT-PCR using *mecA* and *femB* in bone and joint infections. Specific duplex staphylococcal PCR has been reported to be a rapid method of detection of *S. aureus*, including MRSA, with sensitivity and specificity of 100% from screening swabs (143); our duplex has similar sensitivity and specificity from direct colonies of *Staphylococcus* spp. (unpublished data). We have shown that by using a specific duplex staphylococcal RT-PCR of *mecA* and *femB* genes, it is possible to identify *Staphylococcus* spp. including MRSA, directly from orthopaedic samples. Despite rising antibacterial resistance, the pharmaceutical pipeline in developing new antibiotics is drying up, therefore identification of causative organisms and tailored antibiotic therapy are of the utmost importance (158,159). Our duplex RT-PCR affected the management of just under 60% of these patients. As demonstrated, it provided an additional tool to clinicians predominantly regarding antimicrobial management. A positive result not only identifies the causative agent in suspected infection, but also aids in the modification of antibiotic treatment or confirmation of the correct initial choice. Equally significant, a negative result may reassure clinicians that infection is unlikely and hence unnecessary antibiotics can be stopped sooner rather than later.

In conclusion, modern medicine challenges us to seek new techniques to improve the care of our patients. We have shown that specific staphylococci RT-PCR can be a useful diagnostic tool in the identification of different staphylococci species in orthopaedic infections, especially when conventional cultures fail to grow. These techniques could have the potential in supporting

clinicians in choosing the most appropriate antibiotics, which should lead to reduction in morbidity and total cost of care.

2.2 The impact of PCR in the management of prosthetic joint infections

Note: this section has been peer reviewed and published as a review of literature (160).

Advances in molecular diagnostics has enabled laboratories to use techniques, such as PCR for the identification of microorganisms, including bacteria that are fastidious or slow growing, or in circumstances in which previous antimicrobial use may lead to false negative cultures from various clinical samples and settings. Synovial tissue and fluids, particularly in the case of low-grade infection and/or PJI, are kind of specimens for which poor outcome of conventional or traditional culture is known. In recent years, PCR for the diagnosis of PJI has received much attention. Below is a review on the impact of one the most common molecular-based techniques on the management of PJI.

2.2.1 Impact of PCR on the diagnosis & microbial identification in PJI

The use of specific PCR and non-specific broad-range PCR methods, from tissue samples, synovial fluid samples and sonication fluid samples, to diagnose PJI has been the subject of several studies.

In most studies, it is clear that PCR has shown considerably higher sensitivity compared with conventional tissue culture for detecting pathogens in the diagnosis of PJI (72,106,124,138,151,153,161–166) and most recent studies (published in the 3 years prior to the review) are summarized in (Table 2.3). One of the earlier prospective studies compared PCR and culture techniques from synovial fluid, which included 115 cases of potential PJI. The synovial fluid PCR had a higher sensitivity (71%) than conventional culture (43%). PCR specificity was 97% higher than culture 94%. The PCR accuracy, positive predictive value, negative predictive value, and likelihood ratio for positive results as follows: 88, 93, 87, and 23.6%, respectively. However, there was discordance between PCR and culture results in terms of the

microbe detected in 17% of the samples that were positive by both techniques (161). In addition, PCR has been useful in detecting organisms, even with recent antibiotic use (106,167). In one of these studies (106), among 19 cases receiving antibiotics, multiplex PCR was positive in all 19 (100%), whereas sonication cultures grew the organism in eight (42%) out of those cases. Earlier studies with PCR on sonication fluid may have suffered from previously observed gross contamination with some older sonication protocols that used bags to process prostheses. Over time, improved detection rates have been witnessed with PCR of sonication fluid from explanted material with and/or without traditional tissue culture in PJI (Table 2.3) (106,163,165,167,168).

A meta-analysis estimated the pooled sensitivity, specificity, and area under the curve for the detection of PJI using PCR were 86, 91, and 94%, respectively. For PCR, the sensitivity and specificity of the tissue samples were 95 and 81%, the sensitivity and specificity of the synovial fluid samples were 84 and 89%, and those of the sonicated prostheses fluid samples were 81 and 96%, respectively. Use of multiple reference standards had the lowest sensitivity and the highest specificity at 77 and 96%, respectively. Compared with non-quantitative PCR, quantitative PCR had a higher specificity of 94%. The sensitivity and specificity of the fresh samples was 89 and 91%, and that of the frozen samples was 81 and 90%, respectively (169). When 16S rRNA is used on intraoperative periprosthetic samples, the presence of the same microorganism in two out of five samples results in sensitivity of 94% and specificity of 100%, and the presence of only one positive sample results in specificity of 96.3% and positive predictive value of 91.7% (170). A study reported on the application of a multiplex PCR panel on periprosthetic tissues from 95 subjects undergoing resection knee arthroplasty, all of whom had had tissue cultures performed. A subset had synovial fluid culture and/or device vortexing and sonication with culture, and a smaller subset had sonicated fluid PCR. Of 64 subjects with PJI included, 10 and 44 had positive tissue PCR and cultures, respectively, one of whom had a positive tissue PCR with negative tissue cultures. The overall sensitivity of tissue PCR (16%) was significantly lower than that of tissue (69%), synovial fluid (72%), and sonicate fluid (77%) culture as well as sonicate fluid PCR (78%) (171). However, there is a question on how PJIs were defined in this study, which is acknowledged by the authors and it is possible that some PJI cases were misclassified as aseptic failure or vice versa.

A prospective study demonstrated the reliability of routine 16S rRNA gene PCR assays through the use of multicentre quality control. However, despite a sensitivity of >70% the authors were reluctant

to recommend the systematic use of the 16S rRNA gene PCR assay for optimal detection of micro-organisms causing mono-microbial or polymicrobial PJIs (172), instead the authors advocated the use of other techniques in addition to cultures, such as multiplex PCR or pathogen-specific PCR assays, for potentially culture negative infections (173,174).

Other PCR-based technology, such as PCR-electrospray ionization mass spectrometry (PCR-ESI/MS) has been used for the detection of broad-range bacteria and yeast in blood culture bottles and clinical samples (175–179). A group used an advanced version of this technology on sonicate fluid from 431 subjects with explanted knee or hip prostheses, with 152 PJI and reported sensitivities for detecting PJI of 77.6% for PCR-ESI/ MS versus 69.7% for culture, this difference was even more marked among the patients who had received antimicrobials before surgery. However, the observed specificities were 93.5 and 99.3%, respectively (180). Despite the increased sensitivity of PCR-ESI/MS compared with culture, in this study, there was 34 PCR-ESI/MS-negative PJI cases; 29 had negative sonicate fluid cultures. Using an older version of the same technique (the Ibis T5000 Biosensor; Ibis Biosciences, Carlsbad, CA) on synovial fluid specimens, Jacovides *et al* (162) reported organism detection in four culture-negative PJI cases as well as in 88% of cases with presumed non-infectious failure, organisms identified in these cases included many potential contaminants including: *Aureobasidium* spp, *Treponema* spp. and others, which raises questions regarding specificity and results interpretations.

Species-specific RT-PCR is becoming more common place in the laboratory environment and as such can be adopted by local laboratories adapted from published methods. Levy *et al.* carried out a study on the usefulness of 16S PCR and compared this to a panel of species-specific targets which included *S. aureus*, *S. epidermidis*, and *Pseudomonas aeruginosa* among others. They have concluded that the use of targeted RT-PCR against common organisms is a useful tool in the treatment on PJI, but it should be used ideally with culture as PCR is unable to give information on antibiotic susceptibility (181).

Achermann *et al.* (106) also discusses the merit of species- specific PCR on the detection of PJI. They performed real-time multiplex PCR test (SeptiFast; Roche Diagnostics, Basel, Switzerland). They had taken PJ implants and after sonication and nucleic acid extraction, carried out PCR using the SeptiFast kit. From a total of 37 samples, they identified a causative organism in 24. The SeptiFast kit contained an array of Gram positive and negative bacterial targets as well as fungal pathogens associated with PJI. A further study from 2003 by Veretass *et al* highlighted the usefulness of PCR for

the diagnosis of PJI associated with *Mycobacterium tuberculosis*. A nested PCR was established for the IS6110 insertion element of the *M. tuberculosis* complex which was used to identify the presence of the organism in six patients who all had bone and joint infection associated with the bacterium in as little as three days. This is in stark contrast to the 6 weeks culture time on solid culture media, which only has a 50–75% positivity rate. All six patients were afebrile, who did not have pulmonary TB and were not immunocompromised (182).

Single PCR-positive result has been shown to have a good PPV for PJI, whereas multiple positive culture results for the same organism are important to achieve a similar specificity from conventional culture (5). Using broad-range PCR and direct sequencing of 16S rDNA amplicons undoubtedly ensure that the bacteria amplified are potentially of clinical relevance or significance (164,170,183,184). However, the above studies are generally reporting irresolute sensitivities for PCR for example. Fihman *et al.* (183) utilized nucleic acid extraction with the semi-automated NucliSens miniMAG instrument coupled to 16S rDNA sequencing in 76 samples including 15 PJI. The authors demonstrated overall PCR sensitivity of 73.3% (only 53.8% for PJI and 88.2% in native joint infections), despite overall PCR specificity was 95.2%, whereas culture specificity was only 85.7%. Another study, involving only 26 patients with 29 episodes of PJI, compared 16S rRNA PCR and culture on synovial fluid and/or periprosthetic tissue, found lower sensitivity for PCR (50%) than that of culture (58%), but the specificity was higher for PCR 94% compared with 71% for culture (184). In addition, the specificity of PCR techniques for the diagnosis of PJI has also been inconsistent ranging from 0 and 100% (161,162,164–167,185). Consequently, one can conclude that existing data on the performance of PCR for PJI diagnosis are conflicting. Despite modification of the techniques, false positive results are an issue in PCR technology (163), these pseudo-identifications generate more challenges when clinicians and scientists are trying to interpret the presence of DNA from organisms such as coagulase-negative staphylococci or diphtheroids, which may represent true pathogens in the presence of prosthetics or contaminants from the skin during surgery or sampling. Similarly, this is particularly true if one sample is being tested with PCR as opposed to multiple intraoperative samples. Furthermore, the variability of reported sensitivities/specificities are the net result of the differences in technology, techniques, type of infections, expertise and the lack of standardized methods, for example, different pre-treatment procedures applied to samples before DNA extraction and different DNA extraction practices. Strategies such as testing multiple samples just like traditional culture methods, bead-mill processing of samples, vortexing and/ or sonication, aseptically, before performing PCR in a timely manner in an approved environment to overcome

these issues are required additionally PCR optimization and standardization need to be agreed among national review groups and/or researchers to produce consistent and comparable results.

Methodology	Sensitivity	Specificity	Samples	Comments	Ref.
Performing both culture and 16S rRNA gene PCR followed by sequencing	94% for PCR compared with 96% for culture	100% for PCR compared with culture 82%	122 patients	Examined the impact of obtaining multiple (five) specimens of synovial fluid or tissue and sensitivity and specificity are based on detection of the same microorganism in two samples or more	(170)
Modified commercially available 16S rRNA genes PCR assay on sonicate fluid. PCR was followed by hybridization to a nitrocellulose strip with probes specific for different species, allowing species identification without an additional sequencing step and providing the potential for detection of a polymicrobial infection	The sensitivity of PCR was 84%, slightly better than the observed 77% sensitivity for sonicate fluid culture. The sensitivities for patients receiving antibiotics preoperatively were 80 and 70% for PCR and sonicate fluid culture, respectively	The specificity of PCR was markedly lower (68%) than that of sonicate fluid culture (89%)	Among the 75 patients with prosthetic joints included in the study, there were 31 with clinically defined PJI		(163)
Compared sonicate fluid 16S rRNA gene real-time PCR and sequencing to culture of synovial fluid, tissue, and sonicate fluid for the microbiologic diagnosis of PJI. PCR sequences generating mixed chromatograms were decatenated using RipSeq Mixed	Synovial fluid, tissue, and sonicate fluid culture and sonicate fluid PCR had similar sensitivities (64.7, 70.4, 72.6, and 70.4%, respectively)	Synovial fluid, tissue, and sonicate fluid culture and sonicate fluid PCR specificities (96.9, 98.7, 98.3, and 97.8%, respectively)	135 PJI and 231 subjects with aseptic failure	Combining sonicate fluid culture and PCR, the sensitivity was higher (78.5%) than those of individual tests, with similar specificity (97.0%). A possible reason for the higher specificity reported in this study may be due to investigators were using a defined crossing-point threshold for a positive PCR result, thereby decreasing the number of false-positive PCR results. The use of a specific software program to analyze mixed sequences and detect polymicrobial infection was also used by the authors	(164)
Compared multiplex PCR approach in sonicate fluid with sonicate fluid and periprosthetic tissue culture	PCR on sonicate fluid had a higher sensitivity (96%) than tissue (71%) or sonicate fluid (67%) culture	100% specificity for PCR	86 patients (24 with PJI)		(167)

PCR panel on periprosthetic tissues from	The overall sensitivity of tissue PCR (16%) was much lower than that of tissue (69%), synovial fluid (72%) and sonicate fluid (77%) culture as well as sonicate fluid PCR (78%)	The specificities of tissue and sonicate fluid PCR and sonicate fluid and synovial fluid culture were 97, 100, 100, and 96%, respectively	95 subjects undergoing resection knee arthroplasty	This study did not apply strict criteria for the diagnosis of PJI based on Infectious Diseases Society of America guidelines (IDSA) or workgroup of the Musculoskeletal Infection Society, i.e., cases could have been misclassified as PJI or vice versa	(171)
Five perioperative samples per patient were collected for culture and 16S rRNA gene PCR sequencing	73.3%	95.5%	264 suspected cases of PJI and 35 control cases	The diagnosis of PJI was based on clinical, bacteriological, and histological criteria, according to IDSA. A molecular diagnosis was modeled on the bacteriological criterion (≥1 positive sample for strict pathogens and ≥2 for commensal skin flora)	(172)
Compared sonicate fluid culture and multiplex PCR and periprosthetic tissue culture	Sensitivities of multiplex PCR of sonicate fluid, sonicate fluid culture, and periprosthetic tissue culture were 77, 73, and 70%, respectively. For the patients receiving antibiotics in 2 weeks before surgery, PCR was 88% sensitive compared with 70% sensitivity for tissue or sonicate fluid culture	Specificities of tissue culture, of sonicate fluid culture, and of PCR were 97.9, 98.3, and 97.9%, respectively	434 subjects with arthroplasties (144 with PJI)	Sonicate fluid PCR was more sensitive than tissue culture with almost identical specificity. In this study, there were five PJI patients with a negative PCR result who had growth of <i>S. aureus</i> from sonicate fluid and/or tissue cultures	(173)

<p>Samples were cultured for 15 days, and underwent conventional broad-range polymerase chain reaction (PCR) (16S rDNA and 18S rDNA) as well as real-time PCR assays</p>	<p>Not available</p>	<p>Not available</p>	<p>3840 bone and joint culture-negative samples collected from 2308, including 1154 (50%) PJI patients (knee, hip or shoulders)</p>	<p>Specimens from 741 patients (32.1%) tested positive by culture, including 38 in which bacteria grew only after 6 days of incubation. PCR was positive in 141 (9%) culture-negative specimens. The systematic use of 18S rRNA enabled identification of a <i>Candida</i> species in only three cases, which were concordant with the results of cultures obtained after 6 days. There were six other fungal cases that were identified by the 18S rRNA (two <i>Cladosporium</i> sp., two <i>Penicillium</i> sp., one <i>Trichosporon</i> sp., and one <i>Schizophyllum</i> sp.), all were considered to be contaminants</p>	<p>(174)</p>
<p>Compared PCR-electrospray ionization mass spectrometry to culture using sonicate fluid using Plex-ID BAC detection assay</p>	<p>Sensitivity of this PCR approach for detecting PJI was superior to that of culture (78 vs 70%)</p>	<p>Specificity was inferior for the PCR (94 vs 99%)</p>	<p>431 subjects with explanted knee or hip prostheses (152 with PJI)</p>	<p>Only the first prosthesis removal surgery was studied; subjects undergoing only polyethylene liner exchange were excluded</p>	<p>(180)</p>

Table 2.3 The application of molecular diagnosis in work up of PJI

From Saeed K, Ahmad-Saeed N. Expert Rev Mol Diagn. 2015;15(7):957–64.

2.2.2 Impact of PCR on antimicrobial management in PJI

While molecular techniques have shown some promise in identifying a number of genes associated with antibiotic resistance (162,168,186), they do not yet match the microbiological applicability of testing the antibiotic susceptibility of organisms grown by traditional or conventional culture methods. We have reported specific staphylococcal PCR resulted in bacteriological identification in 10 (58.7%) of 19 infected samples (mixed native joints and PJI) that failed to grow. However, only three out of 10 patients had their antibiotics modified according to the PCR result, including escalation to broader spectrum antibiotics to cover for MRSA, based on a positive PCR. The rest of the cases were already on antibiotics clinically deemed appropriate and were not changed based on the PCR result (72).

A study highlighted the usefulness of 16S rDNA gene PCR in 3840 culture-negative samples collected from 2308 cases. Conventional broad-range PCR (16S rDNA and 18S rDNA) as well as real-time PCR assays was applied. The results allowed them to make 141 diagnoses in culture-negative patients. Isolates in this study, identified by PCR, were classified into two groups: fastidious bacteria in 35 cases, mostly anaerobes in adult patients, and *K. kingae* in children; and non-fastidious bacteria in 106 cases, mostly *S. aureus* (174). The study reported that these patients could have benefited from a specific antibiotic treatment. Strict criteria for the diagnosis of periprosthetic joint infection, as previously proposed (187) was not applied in this study, which may have altered results. In addition, in the same study, the authors also used 18S rDNA fungal PCR, which resulted in only three positive PCR results, all of which were *Candida* species that were diagnosed by positive culture after 6 days incubation. In none of these cases did the 18S rDNA PCR results have any impact on the therapeutic antifungal strategy. Similarly, Alraddadi *et al.* reviewed 36 patients with bone and joint infections, of which 17 patients were admitted with suspected PJI. Twenty-nine patients had a negative result and seven had a positive 16S PCR result with a range of organisms, including (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Klebsiella oxytoca*, *Staphylococcus lugdunensis*, *Bacteroides* species, *Staphylococcus* species, and *Streptococcus* species). The authors report that antimicrobials were discontinued for 19 patients based on negative PCR results and, in the case of seven patients; antimicrobials were maintained or changed in a manner consistent with the 16S PCR result. Nine patients were continued on antibiotics despite negative PCR. Of the 17 patients admitted with probable PJI in this study, four had a positive PCR assay, which led to a change in antimicrobial therapy. One patient not suspected to have infection had a positive 16S PCR assay (for a coagulase-negative *Staphylococcus*

species). This result was considered to be of uncertain significance and antibiotics were continued (188). According to the authors, there were no relapses among patients with negative PCR assays in whom antibiotics were discontinued within 5 months follow-up.

Most reports on PCR in PJI conclude that the technique can be a valuable tool in the management of patients with suspected bone and joint infections, who subsequently have negative bacterial cultures. Studies that comment on antimicrobial management and patient outcome are limited and they tend to suggest that clinicians receiving PCR results usually made important therapeutic antimicrobial choices (start, modify, or change) based on the PCR results and occasionally stopping antibiotics based on a negative PCR result alone, even in patients clinically suspected to have infection (188). However, these studies tend to be small in size, with no actual data on longer follow-ups of these patients in the years after the intervention. Better designed, multicentre studies are required to assess the impact of PCR results on the antimicrobial management in PJI and long-term clinical outcome.

2.2.3 Impact of PCR on economic outcomes in PJI

In addition to potentially conflicting data on sensitivity and specificity, the visible costs associated with this technology and the lack of its availability in many clinical diagnostic centres have limited its wider application, and could be the main reasons why PCR is not considered a standard tool in the work-up of PJI with PCR results have not being mentioned as a diagnostic marker for PJI in the Musculoskeletal Infection Society workgroup definition for PJI (187) and neither in the recent IDSA guides (104) nor by other expert groups (189). Despite that molecular diagnostics and PCR have shown constant advances and cost reductions over the past few years. To the best of our knowledge, no published study had looked at the cost-effectiveness and the overall health economy impact of PCR in the diagnosis or exclusion of PJI. Formal studies are needed to look at costs of PCR in PJI and its impact on the cost of hospital stay for unnecessary operations and/or unnecessary antimicrobial therapy or the opportunity to stop or directed antimicrobial management or the effect on the surgical intervention for example one stage, two stage revision, or implant retentions. We believe the savings or clinical and/or financial damage limitations almost certainly outweigh the both visible and hidden costs of PCR.

A prompt diagnosis and recognition of the aetiological agent are crucial for the effective management of PJI. The goal of optimally replacing culture methods with more rapid and informative methods for diagnosing aetiological agents in PJI has advanced dramatically over the past 10–20 years. Koch's culture methods are still widely used for microbiological investigation of PJI, even in many modern diagnostic microbiology laboratories. However, it is increasingly apparent that traditional methods are not fit for purpose, especially where biofilm infections are concerned and there is urgent need to deliver rapid, accurate diagnostics that can impact on antimicrobial stewardship as well as positive patients' outcomes. This highlights the urgent need for a change in the way we investigate and diagnose these types of infections. It is perhaps time to have a more detailed assessment of new technologies and to standardize technology performance. Until such time, rapid surgical exploration and microbiological sampling remains the recommended 'gold standard' diagnostic technique to achieve the optimal outcome.

Chapter 3 Challenges of antibiotic resistance

Antimicrobial Resistance (AR) is a natural phenomenon; it is an evolutionary process enabling microbes to survive exposure to antimicrobial substances. Looking for and detecting antimicrobial resistance has been a corner stone in diagnostic and research laboratories and through this strategies have been developed to tackle the issue of resistance which will be discussed in chapter four.

One of the major challenges that clinicians, scientists face is the evolution of microbes and the development what is known as “cryptic resistance” or hidden resistant mechanisms. In this chapter I would like to concentrate on Oxacillin-susceptible methicillin-resistant *Staphylococcus aureus* (OS-MRSA), a relatively novel hidden resistant mechanism and review some of other cryptic resistances in *Staphylococcus aureus* with potential implications for antibiotic management and infection prevention practices related to these in relation to of Skin and soft tissue infections (190–193).

3.1 Oxacillin-susceptible methicillin-resistant *Staphylococcus aureus* (OS-MRSA), a hidden resistant mechanism among clinically significant isolates in the Wessex region/UK

Note: This original work has been peer reviewed and published (193).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is defined genetically [presence of the *mecA* or *mecC* genes encoding a modified penicillin-binding protein 2a (PBP2a) with reduced affinity for β -lactams and a *S. aureus*—specific sequence such as *femB*] or phenotypically [oxacillin minimum inhibitory concentration (MIC) >2 mg/L]. However, oxacillin MICs in *S. aureus* strains that carry *mecA* can range from <2 to >1,000 mg/L and expression of resistance can be highly heterogeneous within the same strain (194,195). *S. aureus* especially cefoxitin or methicillin- (oxacillin-) “sensitive” *S. aureus* (MSSA) remains one of the most common organisms isolated from clinical specimens worldwide.

Oxacillin-susceptible *mecA*-positive *S. aureus* (OS-MRSA) which have been reported worldwide (190,192,196–200). While such strains would be classified as cefoxitin or methicillin-sensitive *S. aureus* (MSSA) by conventional phenotypic laboratory testing, genotypically they carry the *mecA* gene and hence have been named oxacillin-sensitive, *mecA*-positive *S. aureus* (OS-MRSA) also known as cefoxitin-sensitive-MRSA. In addition to OS-MRSA, MRSA isolates that harbour a divergent *mecA* homologue termed *mecC* within the novel SCCmec XI element have been reported. Such strains remain fully susceptible to oxacillin by the MicroScan method with intermediate susceptibility to oxacillin using the disc diffusion method (201,202) with low sensitivity of chromogenic media for their detection (203) as well as negative results by both latex agglutination tests and by PCR assays for *mecA* (204,205). It is also not known if some strains of OS-MRSA could carry the *mecC* gene.

Due to apparent or phenotypic oxacillin or cefoxitin sensitivities, OS-MRSA strains can easily be missed or be under-diagnosed in busy routine clinical diagnostic microbiology laboratories (191). Clinically, it is generally believed that most OS-MRSA strains exhibit oxacillin heteroresistance, even at a low frequency, and that the use of older anti-staphylococcal beta-lactams might lead to treatment failure, however, data regarding epidemiology, virulence factors and optimal therapy for infections with OS-MRSA are limited and need further research and investigation. The aims of this study were 1) to investigate the prevalence of OS-MRSA in seven major hospitals in the Wessex region/UK from a cohort of 500 clinically significant phenotypically identified MSSA isolates by checking for the presence of both *mecA* and *mecC* genes, 2) to genetically characterise OS-MRSA strains by pulsed-field gel electrophoresis (PFGE) and compare these to common UK epidemic strains (EMRSA-15 and EMRSA-16); and 3) to determine Panton-Valentine Leukocidin (PVL; *lukFS*) gene carriage rates among these isolates.

3.1.1 Methodology

A multicentre epidemiological and non-interventional analysis involved Southampton University Hospitals NHS Foundation Trust, Hampshire Hospitals NHS Foundation Trust (Winchester and Basingstoke), Queen Alexandra Hospital (Portsmouth), Royal Bournemouth and Christchurch NHS Foundation Trust, Salisbury District General Hospital and Dorset District General Hospital.

From September 2012 to April 2013, an anonymous cohort ($n = 500$) of clinically significant, phenotypically identified and reported (cefoxitin and oxacillin sensitive) MSSA isolates were obtained from the microbiology laboratories of the participating hospitals. Investigated clinical isolates, all from infected sites, have either been part of historical collections or originated from current diagnostic submissions. Isolates from the same patients or isolates from samples with any doubts of microbiological and/or clinical significance by the participating microbiologist/ infection specialist were excluded from the study. Included isolates were transferred to the microbiology departments at Hampshire Hospitals NHS FT Winchester, and the Public Health England (PHE) Southampton Laboratory. Bacterial colonies were identified and antimicrobial sensitivities performed based on PHE “Health Protection Agency” standards and British Society of Antimicrobial Chemotherapy (BSAC) guidelines (109,206). Oxacillin MICs for all isolates were determined using the Oxoid M.I.C. EvaluatorTM (M.I.C.ETM; Thermo Fisher Scientific) by an experienced biomedical scientist in microbiology. Real-time PCR was used to detect genes encoding methicillin (oxacillin) resistance (*mecA* and *mecC*), a *S. aureus*—specific sequence in *femB*, and Panton-Valentine leukocidin (PVL; *lukFS*) Sybergreen PCR using previously published methodologies (207–209). OS-MRSA strains were genetically characterised by pulsed-field gel electrophoresis (PFGE) of *Sma* I macro-restriction fragments using the HARMONY protocol (210) and band profiles analysed using BioNumerics v3.5 (Applied Maths).

3.1.2 Results

Over the study period, 500 clinically and microbiologically significant MSSA isolates were obtained from various clinical sites and specimen types (Figure 3.1) in the participating hospitals. All 500 isolates were phenotypically susceptible to oxacillin/cefoxitin and have been reported as MSSA by the diagnostic laboratories, of these 63% exhibited an oxacillin MIC of <0.25 mg/L, for 32.5 % of isolates the oxacillin MIC values were between ≥ 0.25 and <0.5 mg/L and for the remainder of isolates 4.5 %, the MIC ranged between ≥ 0.5 and <1.5 mg/L. Despite apparent cefoxitin and oxacillin sensitivities (MIC of 0.25 mg/L), six (1.2 %) of the isolates were positive for the presence of *mecA* gene indicating OS-MRSA. These isolates were from various hospitals and all were found in cases of skin and soft tissue infections (SSTI) with or without abscess and/or foreign body in situ.

Among our cohort, no isolate harboured the *mecC* gene. Twenty-one isolates (4.4 %) carried the Panton-Valentine leukocidin (PVL; *lukFS*) genes, four from blood culture isolates and the remainder in cases with SSTI infections. None of the OS-MRSA isolates were positive for the PVL gene.

Genomic analysis by PFGE showed the OS-MRSA strains to be genetically diverse (42–82 % band identity) and distinct from the common UK epidemic MRSA-15 and 16 (

Figure 3.2).

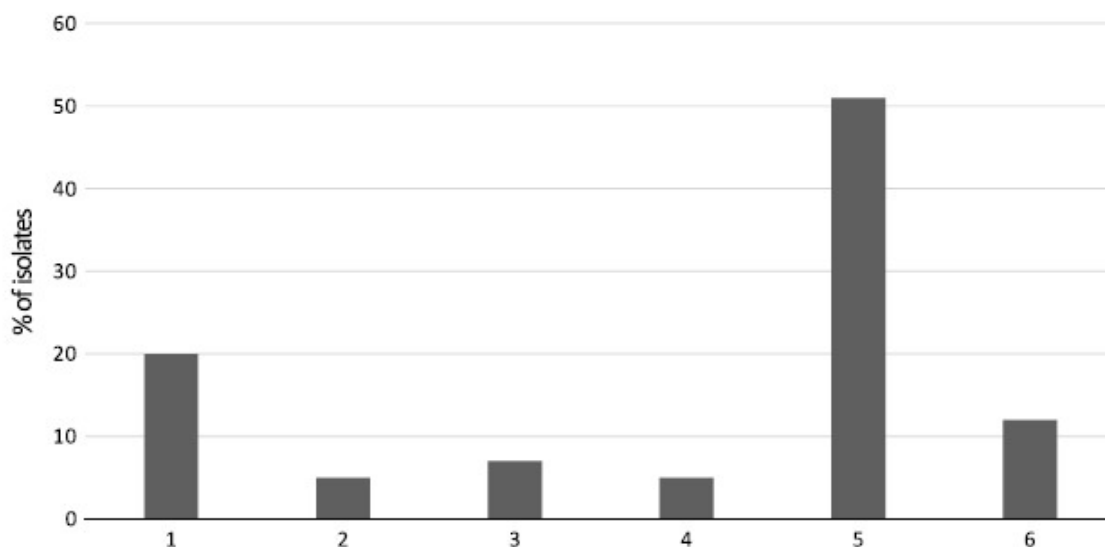


Figure 3.1 Percentage of phenotypic MSSA isolates per sample site

1. Blood culture isolates
2. Isolates from cerebrospinal fluid, cardiac valves, gastrointestinal and other sites
3. Isolates from bone and joint samples
4. Isolates from respiratory, ear, throat and ophthalmology samples
5. Isolates from skin and soft tissue (SST) and pus samples
6. Isolates from SST with associated abscesses and/ or foreign body

From Saeed K, et al. Infection. 2014;42(5).

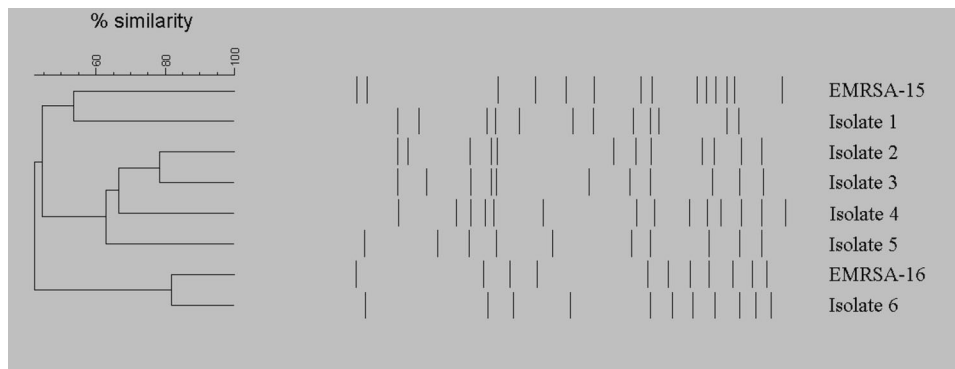


Figure 3.2 Pulsed-field gel electrophoresis (PFGE) band profiles and dendrogram (UPGA type) for OS-MRSA isolates and UK epidemic strains EMRSA- 15 and EMRSA-16

From Saeed K, et al. *Infection*. 2014;42(5).

3.1.3 Discussion

A review recently highlighted a number of hidden or cryptic resistant mechanisms in *S. aureus* that can escape routine diagnostic laboratory tests (191). This study demonstrates that some *S. aureus* strains can be misclassified and misreported as methicillin sensitive (MSSA) in routine diagnostic laboratories by conventional tests. *S. aureus* strains harbouring *mecC* gene have been widely reported (201-204, 217,218, ,211–216). None of the isolates in our cohort carried the *mecC* gene. However, we found 6 (1.2 %) strains matching the criteria for OS-MRSA in our cohort with positive PCR for the *mecA* gene, but phenotypically identified/reported as MSSA by conventional methods with low oxacillin MICs 0.25 mg/L. These findings are almost identical to what has been reported in a similar study that involved 11 hospitals in Japan (199), but lower than the % rates from a recent study involving over 1,500 isolates from 10 cities in China (200). In our study, the small number of isolates did not allow us to identify specific trends or characteristics or risks for getting infections with these strains. Even though all of the OS-MRSA strains were identified in cases of SSTI with or without abscess and/or foreign body in situ in this cohort, we do not believe that this is really specific to these strains as we have previously reported infections from other sites and sources including bacteraemia cases (190,192). OS-MRSA is not only important as it can be missed by routine or conventional microbiology tests, but also appears to be genetically diverse and distinct from the common UK epidemic MRSA strains (

Figure 3.2).

Despite sharing some genetic characteristics, these OS-MRSA isolates seem to have evolved concomitantly, but independently, from other predominant clones; for instance only one strain showed 82 % identity to MRSA-16. Just like Hososaka et al (199), none of these OS-MRSA isolates carried the genes encoding PVL, however overall the positivity rate for PVL among the 500 isolates was 4.4 %. In addition to high PVL rates among SSTI cases, we found that 4 % of the blood culture isolates harboured PVL gene, which is much higher than the reported UK national rates of 2 % (219). This highlights the potential under-diagnosis of strains harbouring these virulence genes, as despite technological abilities in some laboratories, not all isolates are routinely tested for the presence of these genes and the criteria for sending isolates to reference laboratories could be subjective.

In addition to published human reports on OS-MRSA (191,192,196,197,199,200,220), in a recent study the authors characterised *S. aureus* isolates from bovine mastitis milk samples collected from four different province/regions in China and identified the high prevalence of OS-MRSA, which may suggest a bovine link (221). However, origins and mechanisms for oxacillin susceptibility in OS-MRSA strains are yet to be fully defined. Nevertheless, chromosomal factors unrelated to the *mecA* gene, which confer different levels of the oxacillin-heterogeneous phenotype (222) or genes involved in cell wall biosynthesis and environmental factors may be implicated. Although OS-MRSA strains retained partial susceptibility to β -lactams in vitro (223) and in animal models (224), it is generally believed that most *mecA* positive, OS-MRSA strains exhibit oxacillin heteroresistance, even at a low frequency, and that the use of older anti-staphylococcal β -lactams might lead to treatment failure (223,225), ceftaroline will probably have superior activity against these strains. In our limited clinical experience, infected patients with these strains have a poorer outcome when treated only with flucloxacillin, nafcillin, oxacillin or first to third generation cephalosporin (191,203) and therefore infections with these strains should be treated with antibiotics active against MRSA (e.g. linezolid, daptomycin, glycopeptides or according to antibiotic sensitivities) or an MRSA-active antibiotic should be added to flucloxacillin, however more studies are needed to substantiate these views. Routine laboratories should refer suspected isolates to relevant reference units for further molecular characterisation, especially when patients are not responding to flucloxacillin or when there is recurrent or relapse of the infection with phenotypic MSSA isolates.

To our knowledge, this is the first prevalence study of the emerging problem of OS-MRSA infections in the UK. It highlights the need for more advanced microbiological testing e.g. PCR over

conventional microbiology methods to avoid missing and subsequently mistreating infections with OS-MRSA strains. However, there are still many unknowns regarding their dissemination, virulence mechanisms, challenges in clinical practice and antibiotic treatment strategies. Further studies are needed to identify trends and risk profiling of patients at high risk of carriage and/or infection with these strains, which could have an impact on the empirical treatment of persistent or relapsed or recurrent MSSA infections and infection prevention practices.

3.2 Cryptic resistance in *Staphylococcus aureus*: a risk for the treatment of skin infection?

Note this review has been peer reviewed and published (191).

Skin and soft tissue infections (SSTIs) are the most common of infections worldwide and their treatment is becoming an increasing challenge owing to emerging bacterial resistance to multiple antibiotics and is further complicated by the need to treat without relevant microbiological diagnosis, especially in the community. *S. aureus* remains the leading organism in SSTI (226). Since the introduction of penicillin G, we have learnt that *S. aureus* can develop resistance to antibiotics rapidly and upon exposure to new antibiotics by a variety of strategies (227) (Figure 3.3). MRSA, perhaps due to improved awareness and relative ease of detection in routine diagnostic laboratories, has been the primary focus of studies and targeted action against resistance in *S. aureus*, initially as hospital-acquired, but then as community-associated MRSA, but eventually this distinction became blurred and of no clinical relevance in certain places (228).

Familiarity with local antibiotic patterns is important for selecting empirical antibiotic therapy, and one can argue that it is relatively straight forward in clinical medicine; once a resistant organism has been isolated, antibiotic therapy can be tailored accordingly. However, the challenge with SSTIs is that not only are there major difficulties in obtaining microbiological cultures (226), but also hidden or cryptic antibiotic resistances associated with agents such as *S.*

aureus. Current limited knowledge about cryptic resistances in *S. aureus* presents a major challenge to routine diagnostic laboratories regarding definitive identification, and subsequently for clinicians to tailor antimicrobial therapy in their patients in a timely fashion.

The purpose of this review is to explore a number of challenging and easily missed cryptic antibiotic resistances in *S. aureus* in routine diagnostic laboratories, the consequences or potential implications of these on the antimicrobial therapy of *S. aureus* SSTIs in clinical practice and further research.

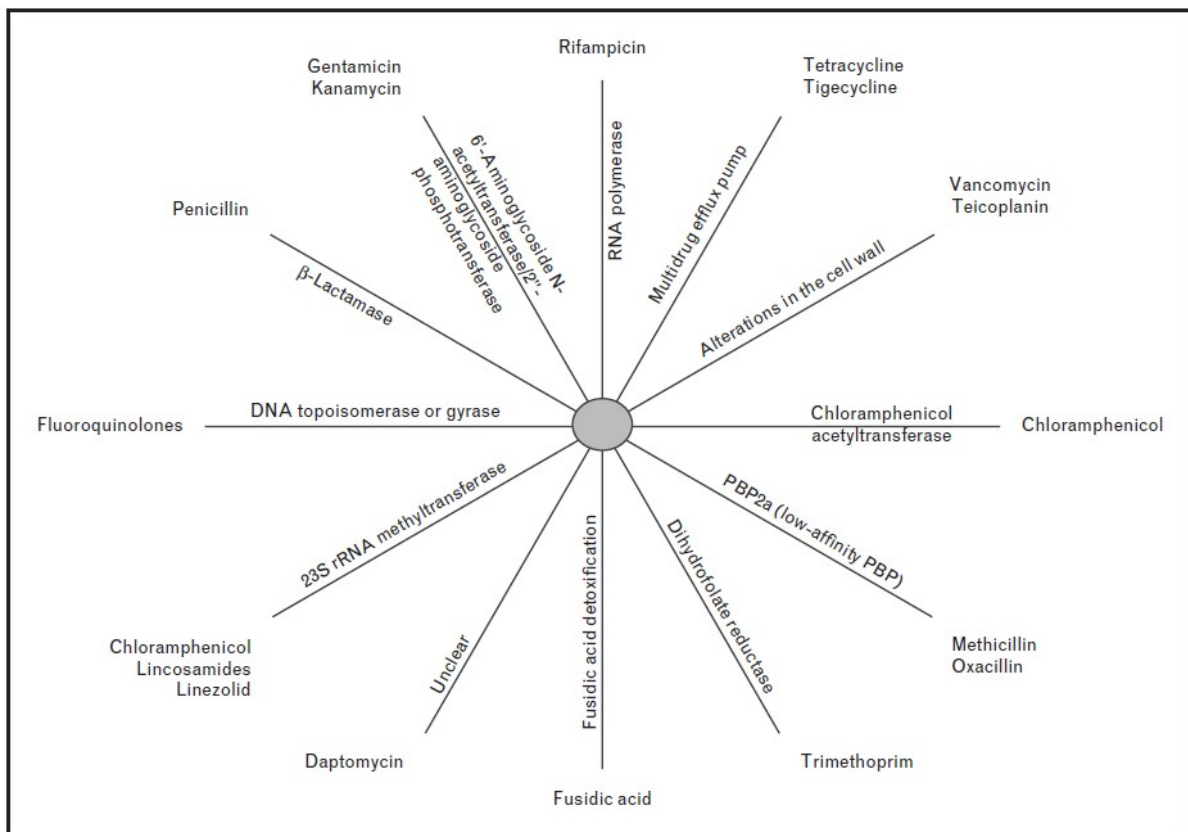


Figure 3.3 Examples of strategies used by *Staphylococcus aureus* to resist antibiotics. PBP, penicillin-binding protein

From Saeed K, et al. *Curr Opin Infect Dis* 2014;27(2):130–6.

3.2.1 Methicillin-Resistant *Staphylococcus aureus*: New Shapes, Forms, and Variants

It is known that methicillin (meticcillin) resistance in staphylococci is mediated by an altered penicillin-binding protein (PBP2a), which confers resistance to beta-lactam antibiotics and is encoded by the *mecA* gene. Staphylococcal cassette chromosome (SCC) elements are vectors

for *mecA*, and other resistance genes, with a lateral transfer event involving SCCmec reportedly responsible for the acquired resistance (229). Phenotypically, methicillin resistance expression could be homogeneous, heterogeneous or borderline (220,230,231). Despite these, the identification of MRSA in routine diagnostic microbiology laboratories can be readily achieved by a range of methods, including the use of chromogenic media, antimicrobial susceptibility testing, detection of PBP2a by latex agglutination tests and the molecular detection of the *mecA* gene (225,232–234). However, recently, the emergence of oxacillin-susceptible-*mecA*-positive MRSA (OS-MRSA) strains has been reported worldwide (190,192,196,197). These strains pose a diagnostic challenge for routine clinical microbiology laboratories, because on a culture plate they appear to be susceptible to ceftaxime with oxacillin minimum inhibitory concentration (MIC) less than 2 mg/l, but isolates harbour the *mecA* gene. The only apparent trigger to suspect and test these isolates for *mecA* gene is that a number of *S. aureus* colonies may be seen growing towards the antibiotic disc or E-test strips (Figure 3.4). Little is known about the epidemiology and virulence of these strains, but from the isolates we have detected so far, they appear to be strains completely distinct from common epidemic MRSA (190). Whereas in-vitro studies may suggest retained partial susceptibilities to β -lactams (223), in our anecdotal clinical experience infected patients with these strains have poorer outcome when treated only with anti-staphylococcus β -lactams (192)); therefore, treatment alternatives for such isolates should be broadened to include glycopeptides, linezolid, daptomycin or even ceftaroline, as this type of organism tends to be susceptible to these. Additional studies are clearly needed, with larger collections of isolates directly comparing efficiency of oxacillin versus others in order for these assumptions to be substantiated.

In addition to OS-MRSA, the description of MRSA isolates that harbours a divergent *mecA* homologue termed *mecC* within the novel SCCmec XI element is of major concern (204). Unlike MRSA or OS-MRSA, MRSA strains containing the *mecC* gene tend to produce negative results, both by a latex agglutination test and by a PCR assay for *mecA* (205). Studies suggested that they appear to be ceftaxime resistant, but were fully susceptible to oxacillin by the MicroScan method and showed intermediate susceptibility to oxacillin by the disc diffusion method (201,202). There are also reports of low sensitivity of chromogenic media for the recovery of *mecC* MRSA (203). In a study using the Vitek 2 system, there was a sensitivity of 88.7% and a specificity of 99.5% for the identification of these isolates (201); otherwise, specific PCR tests are required, which are available at certain centres (209,211,235).

A recent publication, from the UK, estimated the human *mecC* MRSA prevalence rate as a proportion of phenotypic MRSA to be 0.5% (236). These strains are also reported worldwide (203,204,211–217) and among other host species with evidence of animal-to-human transmission (237,238). Despite the absence of pyrogenic toxin superantigen-encoding genes, enterotoxin genes, most exfoliative toxin genes and PVL toxins (202,205,239), it is been shown that *mecC* MRSA is capable of causing a range of infections. Skin and soft-tissue colonization can progress to potentially fatal illness (202,239).

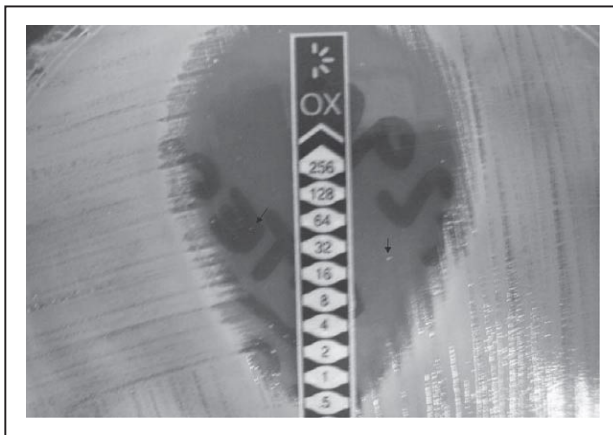


Figure 3.4 Oxacillin E-test showing phenotypically susceptible *Staphylococcus aureus* with small colonies growing (arrows) towards the E-test strip; the isolate confirmed later is an MRSA.

From Saeed K, et al. *Curr Opin Infect Dis* 2014;27(2):130–6.

These new forms of MRSA highlight a number of issues including how *S. aureus* continues to evolve its genetic machinery to avoid antibiotics and sustain its public health threat. In addition, they accentuate the urgent requirement for rapid tests to identify these cryptic resistances, as inadequate or inappropriate empirical therapy of infections with these strains can have serious adverse impacts on clinical outcome.

3.2.2 Reduced Susceptibility to vancomycin: A Hidden Challenge

True vancomycin-resistant *S. aureus* (VRSA) arises with acquisition of the *vanA* gene, which originates from enterococci (240). Sequential mutations in vancomycin-sensitive *S. aureus* (VSSA), particularly after prior exposure to vancomycin, lead to the materialization of

heteroresistant vancomycin- intermediate *S. aureus* (hVISA) and eventually vancomycin-intermediate *S. aureus* (VISA) through alterations in the bacterial cell wall, resulting in reduced autolytic activity and wall thickening preventing vancomycin from reaching its binding site. In 2006, the Clinical and Laboratory Standards Institute (CLSI) defined vancomycin-susceptible *S. aureus* as having an MIC of 2 mg/ml or less, and currently VISA and VRSA are those with MIC of 4– 8 mg/ml and 16 mg/ml or higher, respectively. In Europe, for simplicity, both the European Committee on Antimicrobial Susceptibility Testing and the British Society for Antimicrobial Chemotherapy define *S. aureus* strains as vancomycin susceptible (MIC 2 mg/ml) or resistant (MIC >2 mg/ml). Phenotypically, hVISA strains appear susceptible; however, they contain subpopulations that display variable susceptibility to vancomycin (241,242) including some with vancomycin MICs in the intermediate range, but the vancomycin MIC for the entire population of the strain remains within the susceptible range (241) (Figure 3.5), which is far from a precise definition for hVISA. Population analysis profile (PAP) testing remains the gold standard for detection of these subpopulations, wherein VSSA is defined as the area under the curve of a PAP test (PAP/AUC) ratio less than 0.9 and hVISA as a PAP/AUC ratio of 0.9 or higher (243). These analyses have never made it to routine diagnostic laboratories, and therefore, although the reported frequency of hVISA is in the range from 0.5 to 20% (241,244,245), the true prevalence and clinical impact of hVISA remains a disputed fact. Following initial reports of hVISA and VISA (241), a number of worrying and furthermore confounding clinical reports of hVISA/VISA infections became available, describing vancomycin treatment failure and increased mortality (246–249). Interestingly, other clinical studies suggested colonizing natures and attenuated virulence of hVISA/VISA isolates (250), with lower infection mortality risks (251) and reduced chances of shock in patients with hVISA infections compared with VSSA (252). A recent study and pooled data from a recent meta-analysis demonstrated similar mortality rates between VSSA and hVISA infections (253,254), but with more treatment failures among the hVISA group (253), which could be explained by the fact that VISA isolates commonly have increased polysaccharide capsules protecting the bacteria from phagocytic uptake by the infected host (255).

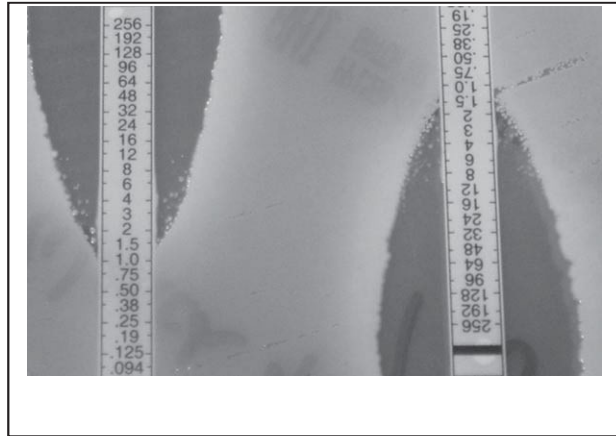


Figure 3.5 Glycopeptide intermediate *Staphylococcus aureus* containing subpopulations that display variable susceptibility to vancomycin and teicoplanin E-tests.

From Saeed K, et al. *Curr Opin Infect Dis* 2014;27(2):130–6.

In our view, there are still major uncertainties regarding what should be the optimum vancomycin susceptibility breakpoint and the optimal MIC testing method. The lack of a precise definition and simple standardized testing makes detection of hVISA a major challenge in clinical microbiology laboratories and can be repeatedly missed by the unreliable routine disk diffusion methods, and relying on vancomycin MIC alone will not be sufficient. The relevance of hVISA to clinical vancomycin failure in SSTI and other infections is still not fully understood and it is difficult to establish whether hVISA is the cause or the result of treatment failure with vancomycin or whether presence of VISA isolates can preclude or predict resistance to other antibiotics, for example daptomycin (256–258). However, for now, the clinical data currently point towards hVISA/VISA isolates possibly being less likely to cause acute clinical deterioration, but more likely to be persistent, which could have major consequences not only on individuals but also on the whole health economy.

3.2.3 Small Colony Variants of *Staphylococcus aureus*

Small colony variants (SCVs) of *S. aureus* are a naturally occurring subpopulation often associated with chronic antibiotic exposure (259). Although long-term therapy with gentamicin and antifolate agents is clearly associated with their selection, clinical reports suggest that

other drugs may also be implicated. The molecular determinants of the SCV phenotype can vary including defects in pathways involved in electron transport or thymidine biosynthesis (260) or a permanent activation of the bacterial stress (stringent) response (261). Phenotypically, SCVs are characterized by their slow growth rate and small colony size relative to the parent strain found in antibiotic-refractory infections including SSTIs (260)(262). These variants are clinically important due to their reduced membrane potential and reduced uptake of cationic antibiotics (260), which correlates them with a tendency to cause persisting and recurring infections (263).

Although reported as 'rare or uncommon', in our opinion, their true prevalence is largely underestimated in clinical specimens, as these strains often present problems for the diagnostic laboratory and often escape detection because their uncommon morphological and physiological features might be mistaken for coagulase-negative staphylococci and thus be discarded as 'skin flora'. Even though they are reported to be less virulent than are wild-type *S. aureus* (260,261), they can spontaneously revert to a normal phenotype (264) and potentially regain their ancestral virulence and can also acquire and express other classical mechanisms of resistance to antimicrobial agents.

A recent study (265) of *S. aureus* SCVs and their influence on the worsening of lung disease in US children with cystic fibrosis (CF), which included molecular diagnostic analysis, revealed that 24% of a cohort of 100 children carried *S. aureus* SCV. Furthermore, *S. aureus* SCV carriage was associated with a greater drop in lung function during the study. The study also revealed evidence that it is possible to select for the persistence of *S. aureus* SCV during treatment with trimethoprim– sulfamethoxazole. It also appeared that coinfection with *P. aeruginosa* was a cofactor in successful colonization of the CF lung by *S. aureus* SCV. More study is needed prior to recommending any changes in testing, treatment or infection control. At this time, we do not know whether SCV *S. aureus* are true pathogens and worry that treatment changes could lead to worse rather than better outcomes. At this point, it is not clear that SCVs themselves are the aetiological agent, or biomarkers thereof, therefore large-scale, multicentre studies are necessary (L. Hoffman 2013, personal communication).

Regarding SSTI, there are no large clinical trials examining antibiotic options for *S. aureus* SCV infections; in clinical practice, antibiotic choices remain largely empirical mostly with β -lactams

or glycopeptides, although they are considered to be less active against SCVs based on in-vitro testing (266,267). A recent review (268) summarized a number of small clinical studies and case reports describing the antibiotics used prior to SCV identification and for their subsequent treatment. They tend to involve aggressive polytherapy for their eradication involving rifampicin or a fluoroquinolone and quinupristin/dalfopristin, and for a very long period, a practice that could lead to the selection of more resistance that may further complicate matters for both patients and clinicians.

A potential novel strategy is photodynamic therapy, which utilizes light in combination with a light-activated antimicrobial agent, known as a photosensitiser, to generate toxic reactive oxygen species that can oxidise many biological structures and kill bacteria via several mechanisms (269). A more recent study demonstrated that the combination of methylene blue and laser light of 665 nm effectively kills *S. aureus* SCVs, suggesting that photodynamic therapy could be a promising alternative therapy for SCV superficial infections (270). The characteristics of SCVs remain a dilemma not only for the prevention of their emergence but also to treat or eradicate them even when clinically detected, and hence further clinical, multicentre and interventional studies are warranted.

3.2.4 Inducible Resistance in Clindamycin Continues to be Concealed

Clindamycin use may have added advantages over some other antibiotics because of its penetration to skin/skin structures, as well as its ability to inhibit protein synthesis and turn off bacterial toxin production. Inducible clindamycin resistance among some *S. aureus* strains is not detected by standard broth microdilution testing, automated susceptibility testing devices, E tests or the standard disk diffusion test, unless a D-zone test is performed (271). Despite increasing reports, D test is not routinely performed in some laboratories and those who perform it have shown different rates of inducible clindamycin resistance in different regions. Some clinicians tend to avoid the use of clindamycin for staphylococcal infections whenever erythromycin resistance is noted.

Clinical response to clindamycin therapy, despite the presence of inducible resistance, has been reported; others raise concerns over treatment failure and the use of clindamycin in

these situations, especially in deep seated infections, endocarditis, abscesses and osteomyelitis (271). In our opinion, clinical data regarding the risk of emergence of inducible clindamycin resistance during therapy are very limited. There are still major uncertainties about the reliability of susceptibility reports for clindamycin, the clinical importance of this inducible resistance and the efficacy of clindamycin in these cases. However, in our experience, clindamycin can still be used for shorter period (5–7 days) in mild infections even with inducible resistance isolates, but with close follow-up and therapy modification if no clinical response within 5 days or deterioration during the course. However, it should not be used or at least used as monotherapy in the case of more severe infections with these isolates.

3.2.5 Discussion

S. aureus remains the most dominant pathogen in SSTI. Treatment failure is a major concern in the management of these infections. Source control, for example incision and drainage when there is a drainable focus, remains a major factor (272). Antibiotic choice and timeliness are also very important factors in the successful therapy of these infections. The choice of empirical antibiotic depends on the clinical presentation, local epidemiology and clinician's experience with a particular agent. In general, the determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal therapy. In a recent European study (226) of severe SSTI, involving approximately 2000 cases, with clinical pictures and comorbidities similar to those which clinicians encounter in hospitals on a daily basis, microbiological diagnosis was obtained in only 50% of cases despite appropriate cultures. This yield is much lower in the primary care setting and for milder infections, as we tend to treat but not necessarily test. Interestingly, in the same study, clinicians used 54 different antibiotic agents in the initial management of skin infections; 40% of patients had modification of antibiotics of which the majorities were due to treatment failure. Furthermore, just less than 70% of cases were already on antimicrobials in the 3 months prior to hospitalization. The study highlights the complexity of SSTI even when we know the causative organisms and their antimicrobial sensitivities. We believe that this condition is even more convoluted especially when clinicians do not know the causative agents and/or when organisms escape diagnosticians, or they are armed with obscure antibiotic resistances. All lead to delays in receiving effective antibiotic therapy and ultimately to worse patient outcome, increased cost of care and longer hospital stay (226,273–278). A recent review (279) provided an update on novel anti-MRSA molecules currently under preclinical and clinical development, including new agents for treatment of SSTI. Therefore, although the antimicrobial

pipeline for *S. aureus* looks promising, *S. aureus* strains causing SSTI are evolving with regard to virulence, as well as antimicrobial resistance with cryptic resistances continuing to escape routine diagnostic tests. This means that there are still many unknowns regarding their global dissemination, virulence, threats in clinical practice and optimal treatment strategies. For now, we must rely on measures such as good antimicrobial stewardship, infection prevention and sending suspected isolates to reference laboratories for confirmation. Developing an understanding of the pathogenic consequences of cryptic resistance in *S. aureus* may ultimately provide novel preventive or treatment approaches for this significant human pathogen. Until then it is a major challenge to predict the impact of this continuous evolution on future treatment, clinical outcome and cost of care in SSTI.

3.2.6 Future work

Whole genome sequencing of the OS-MRSA isolates in Wessex cohort; this work currently been undertaken in collaboration with PHE Southampton. The aim is to further understand the mechanism(s) of this resistance which may assist in defining appropriate therapeutic strategies. Additionally, this and the potential applications of new technology and novel ways of delivering antimicrobials may assist in tackling the threat of antimicrobial resistances. Some of these applications will be addressed in the next chapter.

Chapter 4 Biomarkers, antibiotic stewardship and tackling antibiotic resistance

The twentieth century has been described as the golden age of antibiotics. Currently antibiotic prescribing tracks along a therapeutic critical point, over or unnecessary use leads to resistance selection, whereas withholding them may lead to worsening outcomes for patients or death. Hence in general and as “current best practice” is for clinicians to prescribe antibiotics whenever there is uncertainty about a diagnosis even when an infection cause has the remotest possibility i.e. “just in case” antibiotic prescribing.

Antibiotic resistance is a global issue and concern; and has many challenges, some of which are illustrated in (Figure 4.1). It is an inevitable evolutionary process; however their selection and spread of antibiotic resistance can be slowed. Reviving antibiotic pipelines and/ or development of new models of antibiotics will undoubtedly be of great value against challenging multi drug resistant organisms, however at present this is in poor supply. Therefore, other possible solutions to curb antibiotic resistance, by every prescriber, must be implemented to improve antibiotic stewardship. These possible solutions may be categorised into a number of areas including, but not limited to:

- Over use of antibiotics in human health
- Over use of antibiotics in agriculture and animal husbandry
- Poor public health or infection control infrastructure (globally) including education, surveillance, access to health care and political stability
- Bacterial evolution (post antibiotic exposure) selection pressure
- Ease of global travel
- Lack of new antibiotic discovery
- Lack of rapid diagnostics

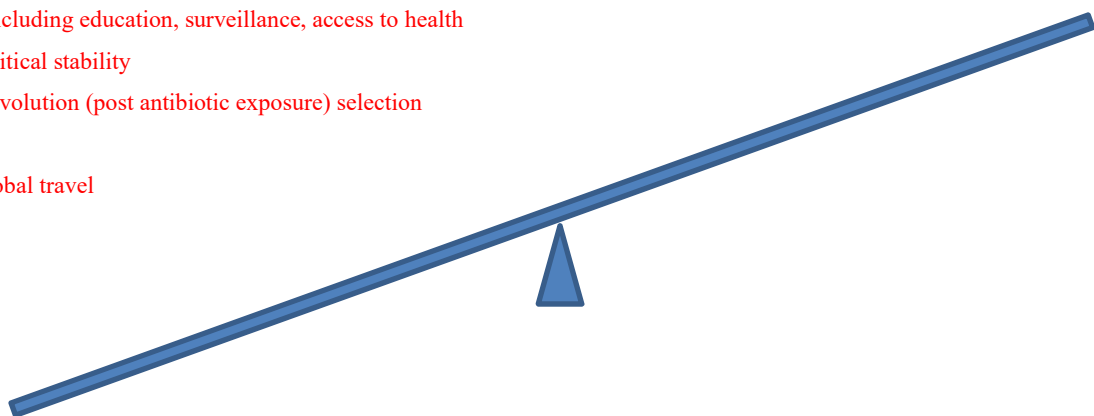


Figure 4.1 Global challenges contributing to spread of antibiotic resistance

Figure by K Saeed not published

- 1 Faster and appropriate diagnostics that can support or exclude bacterial infections which means less empirical therapy and less “just in case” prescribing.
- 2 Better antibiotic stewardship: novel methods of delivering antibiotics, multidisciplinary team management (involvement of experts).
- 3 Better infection control provision as fewer infections means less need to use antibiotics and subsequently less selection pressure.
- 4 Antibiotic alternatives to reduce selective pressure on antibiotics.

The following sections is dedicated to some aspects the first two points in the above categories highlighting the importance of procalcitonin, as a relatively novel biomarker, and a novel negative pressure therapy with installation in the delivery of high concentration of antibiotics to infected sites and the importance of these interventions in a stewardship programme.

4.1 Faster and appropriate diagnostics and antimicrobial stewardship

4.1.1 Reduction in antibiotic use through procalcitonin testing in patients in the medical admission unit or intensive care unit with suspicion of infection

Note: this original work has been peer reviewed and published (36).

Clinical signs and laboratory findings may be subtle in the early stages of infection. In the case of sepsis, multiple organ failure may already have occurred by the time the diagnosis is made and by this stage mortality is considerably greater (280,281). Hence there is almost certainly considerable overprescribing of empirical antibiotics at the point when the presence of bacterial infection is a diagnostic uncertainty. This has cost implications but more importantly represents a significant burden of antibiotic pressure on the bacterial ecology. Sepsis is defined as systemic

inflammatory response syndrome (SIRS) caused by infectious agents. Despite advances in medical technology and clinical care, mortality rates in sepsis remain high (282). Non-infectious factors such as trauma, haemorrhage, pancreatitis, collagen vascular disease or malignancy may be responsible for SIRS, and surgery can result in a similar clinical presentation leading to diagnostic difficulty and uncertainty whether to commence antibiotic treatment (280,281,283,284). Early diagnosis of infection and the prompt initiation of adequate antimicrobial therapy are important for successful outcome. Diagnostic challenges and the lack of specific early markers of infection can lead to withholding or delaying antimicrobial treatment in critically ill patients, or conversely unnecessary antimicrobial treatment in others. In less seriously ill patients presenting as emergencies to medical units, similar diagnostic challenges present themselves. It is not always apparent whether the clinical signs are manifestations of infection or some other pathological process. Common examples of this are crackles heard on auscultation of the chest and distinguishing between an infective or cardiac cause, and whether confusion in an acutely ill elderly patient is the result of infection or some other cause. There is a tendency among many clinicians to treat for infection if in doubt; just in case the cause is infective. This leads in turn to inappropriate antibiotic use, higher costs and so-called 'collateral damage' from antibiotics.

Better biomarkers for diagnosing infection would improve the appropriateness of antibiotic use (285). Recently, PCT has been found to have an important role in the diagnosis of bacterial infection (286). PCT is a prohormone of calcitonin, normally produced by thyroid gland C-cells in response to hypocalcaemia. Under normal conditions, very low concentrations of PCT in serum (<0.1 mg/L) are observed (33). In infection, the inflammatory process induces extra-thyroid production of PCT, levels of which increase after 3-4 hours, peaking at around 6 hours with a plateau of up to 24 hours (287). Multiple studies have established the utility of PCT in detecting bacterial infection and different cut-off values have been proposed for different clinical conditions (73,74,288–292). PCT might also accurately differentiate between systemic bacterial infection and non-infectious states (293). However, not all studies have recognised this differentiation (294–296).

The primary aim of this evaluation was to determine the effect of implementing rapid PCT measurement to guide antibiotic therapy in two groups of patients: medical admission unit (MAU) patients and patients on the intensive care unit (ICU) in a UK health setting. To our knowledge, PCT has

not been previously evaluated in this setting. A secondary aim was to establish the effect of this approach on reduction of antibiotic usage and potential collateral damage.

4.1.1.1 Methods

The Royal Hampshire County Hospital in Winchester is a general hospital with around 400 acute beds covering all main specialties. The MAU is a 26-bedded ward and the ICU has ten critical care beds. This evaluation aimed to assess the value of serum PCT measurements as a complementary biomarker to assist decisions regarding antibiotic therapy in MAU and ICU patients. PCT measurement was being evaluated in the microbiology laboratory as a rapid diagnostic test. Since it was being offered routinely and was being used alongside established biomarkers such as white cell counts and C-reactive protein (WCC and CRP), ethics approval was not sought and informed consent was not regarded as necessary. Clinical indications for offering the PCT test and for inclusion in this evaluation were as follows: (i) patients were on MAU or ICU at the time of the daily morning microbiology ward round (Monday to Friday); (ii) presence of one or more SIRS criteria [fever of $>38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$, heart rate of >90 beats per minute, respiratory rate of >20 breaths per minute or a PaCO_2 level of <32 mmHg and abnormal white blood cell count ($>12\,000/\text{mL}$ or $<4000/\text{mL}$ or $>10\%$ bands)]; (iii) infection was part of the differential diagnosis but inflammatory or other causes could not be excluded; (iv) patients were without significant positive cultures or a defined source of infection; (v) patients were not on antibiotics, or had not been in hospital for >12 h and not had more than two doses of antibiotics and a pre-antibiotic serum had been collected in advance for PCT testing.

The following were excluded: severely immunocompromised patients [human immunodeficiency virus (HIV), neutropenic and stem-cell transplant patients]; patients with cystic fibrosis or active tuberculosis; patients with autoimmune diseases, pregnant women and neonates; patients with significant positive cultures or a clearly defined source of infection. Whereas MAU patients had a single PCT test at admission, ICU patients often had more complex clinical courses and a number of patients had more than one PCT measurement. PCT in these patients was repeated only if the patient's clinical condition deteriorated and infection or sepsis remained part of the differential diagnosis.

PCT was measured in the microbiology laboratory during routine working hours using the Brahms Vidas EIA method (Bio-Mérieux, Basingstoke, UK). All PCT tests were initiated by the microbiology/infection ward round team on serum collected for other routine tests. Results were available within 90 min of the medical review and were communicated to the clinician in charge by clinical microbiologists. As the patient had already been reviewed clinically by the ward clinician and the microbiology/ infection team, the PCT result was used to make a final decision on whether to commence or withhold antibiotics. Patients were then followed up prospectively for seven days in the case of MAU or for the whole course of their ICU stay after this decision to determine the clinical outcome. Follow-up involved daily review on the microbiology/infection ward round, with assessment of temperature, vital signs, inflammatory markers and microbiology results if available and an assessment for clinical deterioration.

The following PCT levels (ug/L) based on previous publications (73,74,288–292) were used as cut-off concentrations above which active bacterial infection was supported:

MAU patients ≥ 0.25 ;

ICU medical patients ≥ 0.5 ;

ICU postoperative patients (within 0-48 h) ≥ 2.0 ;

ICU acute severe pancreatitis ≥ 4.0 .

Cost analysis: the daily cost of antibiotics for patients on MAU or ICU was calculated by taking the total antibiotic costs on that unit for a six-month period and dividing that number by number of patients who had been on antibiotics on the unit in that period, on the conservative assumption that a course of antibiotics was about seven days.

4.1.1.2 Results

From May to November 2009, 99 PCT tests were performed on 99 MAU patients whose average age was 71 years. PCT value aided the antimicrobial management in 85 of these (Table 4.1). In 33 cases (39%), PCT above the cut-off supported a decision to commence antibiotic therapy. PCT below the cut-off resulted in withholding or stopping antibiotics in 52 cases (61%). In the seven-day follow-up period none of the cases in whom antibiotics were withheld or discontinued required antibiotics on clinical grounds or developed sepsis. There were six deaths in the 99 MAU patients

but none of these were attributed to infection. In 14 of the 99 patients the PCT did not affect clinical management. Three of these were lost to follow-up, the remaining 11 cases had PCT values below the cut-off, but antibiotics were commenced or continued on the basis of medical prerogative despite the low PCT value. No clear clinical evidence of infection emerged in any of these patients on follow-up to conclude that the PCT value was falsely negative.

Clinical category, SIRS with:	PCT above the defined cut-off (commence antibiotics as planned: 'potentially infected patients')	PCT below the defined cut-off (withhold antibiotics: 'potentially uninfected patients')	Negative PCT value did not affect antibiotic management
Respiratory signs and symptoms, possible pneumonia or bacterial exacerbation of COPD part of the differential diagnosis (n)	12	16	9
Increased confusion, functional decline, urinary tract infection part of differential diagnosis (n)	7	13	2
Non-specific features, bone and joint (including prosthetic joints) or soft tissue-related infection part of the differential diagnosis (n)	5	11	2
Gastrointestinal or hepatobiliary infection including acute pancreatitis part of the differential diagnosis (n)	4	2	1
Non-specific site or source of infection part of the differential diagnosis (n)	5	10	0
Total no. of cases (N = 99)	33	52 ^a	14
Median PCT value (range)	1.6 (0.28–104) µg/L	0.07 (0.05–0.32) µg/L	
Median WCC value (range)	12.6 (6.6–27.8) × 10 ⁹ /L	9.9 (2–26) × 10 ⁹ /L	
Median CRP value (range)	162 (8–365) mg/L	44 (3–321) mg/L	
Positive microbiology results within 7 days of follow-up	44%	8% ^b	

SIRS, systemic inflammatory response syndrome; COPD, chronic obstructive pulmonary disorder; WCC, white cell count; CRP, C-reactive protein.

^a None of these patients suffered adverse effects as a result of withholding antibiotics within the seven days of follow-up.

^b No positive cultures from normally sterile sites, and no significant pathogens in this group.

Table 4.1 Antibiotic decisions on the basis of serum procalcitonin (PCT) value and outcome within seven days in medical admission unit

From Saeed K, et al. J Hosp Infect. 2011; 78:289-292.

In the ICU setting, during the evaluation period 42 patients with a variety of underlying problems met the inclusion criteria (Table 4.2). In these 42 patients, 87 tests were performed in association with 87 clinical reviews, of which 83 (95%) influenced antibiotic decisions. Forty one of these 83 tests yielded PCT values above the defined cut-offs (median PCT 4.56 mg/L), supporting early initiation (32 tests) or escalation (9 tests) of antibiotic regimens with or without further investigations in these potentially infected patients. Conversely, low PCT levels in 42 of the 83 tests supported the decision to withhold (27 tests) or stop (15 tests) antibiotics.

Some patients had serial tests, for example one patient had seven PCT measurements in the course of a seven-week ICU stay. In this patient, six of those tests resulted in withholding antibiotics; one test resulted in commencing antibiotics. Without the PCT guidance it is likely that this patient would have received seven courses of broad-spectrum antibiotics. In the seven-day follow-up period after each measurement, four patients died due to infection (median PCT value in these cases was 34.1 µg/L), all of whom were on antibiotics that appeared appropriate.

Clinical category. SIRS with:	PCT above the defined cut-off (supported giving antibiotics as 'potentially infected patients')	PCT below the defined cut-off (antibiotics withheld; 'potentially uninfected patients')	PCT value did not affect antibiotic management ^a
No. of tests			
Postoperative patients, infection part of the differential diagnosis	15	8	1
Alcoholic liver disease, acute pancreatitis, biliary infection as differential diagnoses	5	15	0
Respiratory deterioration, infection including hospital-acquired pneumonia part of the differential diagnosis	14	12	1
Other conditions where undefined source of infection part of the differential diagnosis	7	7	2
Total 87 tests from 42 patients	41 ^b	42	4
Median PCT value (range)	5.5 (0.61–200) µg/L	0.4 (0.12–2.96) µg/L	
Median WCC value (range)	14.1 (1–43) × 10 ⁹ /L	12.9 (4–25.4) × 10 ⁹ /L	
Median CRP value (range)	147 (11–482) mg/L	143 (13–312) mg/L	
Positive microbiology results within 7 days of follow-up	78%	12% ^c	

SIRS, systemic inflammatory response syndrome; WCC, white cell count; CRP, C-reactive protein.

^a Out of the 87 tests, four tests did not affect patients' management in ICU: these were excluded on the basis of immunosuppression, e.g. due to long term steroid therapy, leucopenia due to advanced acquired immune deficiency disorder. In one of these a low PCT value was regarded as a false negative result in a postoperative patient with massive blood loss requiring multiple blood transfusions (i.e. this result was regarded as a dilutional false negative). These have been included for cost calculation purposes.

^b During the evaluation period four patients died from infection, all of these were on apparently appropriate antibiotics with median PCT 34.1 µg/L. No patient suffered adverse effect or died as a result of withholding antibiotics on the basis of low PCT value within the follow-up period.

^c No positive cultures from normally sterile sites, and no significant pathogens in this group.

Table 4.2 Antibiotic decisions on the basis of serum Procalcitonin (PCT) value and outcome during stay in the intensive care unit

From Saeed K, et al. *J Hosp Infect.* 2011; 78:289-292.

Antibiotic savings in MAU and ICU were calculated on the basis of the average daily use of antibiotics in each unit. Average six-monthly antibiotic costs were £20,020 in MAU and £64,250 in ICU. In general, a seven-day course of antibiotic in MAU costs £87.50 and in ICU £280. Our evaluation suggests that in MAU, 99 PCT tests costing £990 resulted in the avoidance of 52 courses of antibiotics costing £4,550, a saving of £3,560 in 6 months (17.7% reduction). The cost saving would have been greater had the low PCT result in the 11 cases been acknowledged and antibiotics withheld. On ITU, 42 courses of antibiotics costing £11,780 were saved, at the expense of 87 PCT tests costing £870. This suggests a cost saving of £10,890 in six months (17% reduction). These savings do not include savings gained from staff time, intravenous giving-sets and bed-days.

4.1.1.3 Discussion

This evaluation demonstrates that there is a role for PCT testing in supporting clinical assessment and antibiotic decision-making. In this group of patients with a suspicion of infection, PCT has been highly discriminatory in separating patients who require antibiotics from those in whom antibiotics contribute little to the clinical course. In no case in either MAU or ICU did withholding antibiotics, as a result of a low serum PCT, compromise patient outcome. Many of these patients would have received empirical antibiotics in the absence of PCT testing. As our hospital is unlikely to

be significantly different from any other general hospital, this leads to the conclusion that a significant proportion of routine antibiotic use in hospitals is unnecessary. The burden of antibiotic volume is a direct pressure on the selection of antibiotic resistance mechanisms contributing to healthcare-associated infection (HAI). It is therefore apparent that judicious use of PCT in patients with suspicion of infection can result in a reduction in antibiotic use which will help to reduce HAI incidence.

To date, no single biological indicator of sepsis and infection has gained unanimous acceptance. PCT has been proposed as a promising candidate, and the results of our evaluation support this contention (73,74,280,288–292). Without the availability of PCT measurement we believe that all of the patients studied would have received antibiotics on the assumption that their SIRS were related to infection. However, once PCT measurement was initiated as part of the clinical infection assessment, the result played a key role in antibiotic decision-making. A raised PCT supported early antibiotic treatment in patients on MAU and ICU; whereas a low PCT serum level supported a decision to withhold or stop. This was achieved without adverse effect in those patients who were denied antibiotics.

The importance of antibiotic stewardship for future public health and the adverse events associated with inappropriate antibiotic use is increasingly stressed (285). MRSA infection and CDAD are important HAIs and antibiotic usage in hospitals has been shown to be a risk factor for both (297,298). On the other hand, delays in commencing antibiotic treatment in an infected patient can have serious deleterious effects (276,299–305). Anything that can improve diagnostic sensitivity, differentiating bacterial infection from non-infection, can help to improve the appropriate use of antibiotics. In this context PCT measurement appears to be a very effective tool.

During the six-month evaluation period a reduction in antibiotic costs resulting from PCT-directed antibiotic decision-making of £14,450 was established. This represented 17.7% and 17% reductions in antibiotic use for MAU and ICU respectively, although this is likely to be an underestimate. A study has demonstrated that administration of antibiotics is associated with significant hidden costs, which in some cases exceed the costs of the antibiotics themselves (306). The workload and costs for disposable materials were found to be important cost drivers in that study, accounting for 13–113% of the overall costs for treatment with intravenous antibiotics and the total acquisition costs

of medication. Based on these figures, additional savings of £1,880-£16,328 may be realised by reducing workload and reducing use of materials. On these grounds there may be a case for offering PCT as a routine or indeed point-of-care test.

In conclusion, this evaluation has some limitations in that it was carried out in a single centre and was not controlled. However, as a 'real world' investigation we believe the results are valuable. PCT, like any other biomarker, has limitations: its role in certain groups of patients such as in oncology, haematological malignancy, autoimmune disease, patients on immunosuppressive drugs, HIV disease, neonatal medicine and pregnancy have yet to be established. As a test managed by the microbiology/infection team on their daily ward rounds in conjunction with a clinical assessment, PCT has resulted in a remarkable improvement in the management of antibiotic use without compromising patient safety. It has supported the early treatment of patients with potential infections and has avoided unnecessary treatments in other cases. This reduction in unnecessary antimicrobial prescribing has the potential to safely reduce selection pressure, minimise antibiotic-associated unwanted effects and likely results in savings in antibiotic costs, staff time, intravenous lines and bed-days.

4.1.2 Procalcitonin levels predict infectious complications and response to treatment in patients undergoing cytoreductive surgery for peritoneal malignancy

This original work has been peer reviewed and published (307).

Cytoreductive surgery for peritoneal malignancy (PM) is associated with extensive tissue resection and a prolonged operating time. Splenectomy is often performed and, in the majority of cases, blood transfusions are common and hyperthermic intraperitoneal chemotherapy (HIPEC) is generally administered (308). Early post-operative supportive therapy is invariably delivered in the ICU for an average of two days. The systemic inflammatory response mounted following this extensive surgery is substantial and often manifests as SIRS. SIRS can also occur as a consequence of post-operative

infection or sepsis. In these cases, early recognition is paramount and enables prompt administration of broad-spectrum antibiotic therapy and, if required, further surgical procedures to achieve source control. In many cases, the inability to distinguish between the causes of SIRS in this patient group makes the diagnosis of post-operative infection particularly challenging.

Currently used inflammatory markers of systemic inflammation e.g. WCC and CRP, which are routinely used as surrogate markers for infection, are of limited use in this group of patients. In our experience, these markers are non-specific for bacterial infection in PM cases and elevations may lag behind clinically significant events. Furthermore, in patients with underlying medical conditions, e.g. liver disease or immunosuppression, the WCC and CRP may remain low despite the presence of infection. Conversely, following surgical procedures, WCC and CRP levels may be high in the absence of infection (6–11,34,309). Consequently, the use of these markers may result in either under or over- diagnosis of post-operative infections which may be associated with a delay in appropriate management, or the inappropriate administration of antibiotics. Minimising the inappropriate use of antibiotics is important and significantly reduces the risk of complications including the potential selection of multi-drug-resistant bacteria and CDAD.

As mentioned previously PCT, is an appealing biomarker as, not only is it a more sensitive and specific marker of bacterial infections compared with WCC and CRP, but it also rises earlier in the course of bacterial infection. There is a body of controlled studies, mostly in medical patients, that support the role of PCT in diagnosing bacterial infections as a useful antimicrobial stewardship tool (36,73,74,288–292,310).

Current evidence relating to the use of PCT measurements in surgical patients is limited but encouraging and, in the main, demonstrates that there is a transient “physiologic” PCT rise following surgery in general (33,294,311–313), though, the available evidence also suggests that PCT is a more accurate predictor of major anastomotic leak after elective colorectal resection than WCC and CRP (314). However, Meisner et al. suggested that “physiologic” post-operative induction of PCT largely depends on the type of surgery. For example, while intestinal surgery and other major operations often result in a post-operative PCT increase, in the majority of patients undergoing relatively minor surgery, involving primarily aseptic surgical procedures, the PCT remains normal (315). This led the authors to conclude that PCT should be used post-operatively for the diagnosis of infection only when the range of PCT concentrations during the normal course

of specific surgery types is considered and when PCT concentrations are sequentially assessed (315). To our knowledge, there are no published data relating to PCT dynamics and their associated clinical usefulness in PM patients undergoing cytoreductive surgery.

Our aim was to study the dynamics of serum PCT in this group of patients to: firstly establish baseline measurements for PCT in this group and observe its dynamics in the immediate post-operative period; and secondly to find out about the potential diagnostic ability of PCT in early infectious complications, compared to CRP and WCC, post cytoreductive surgery for PM, by determining area under the curve (AUC) as well as sensitivity, specificity, positive and negative predictive values. Additionally potential variations in PCT dynamics in splenectomised versus non-splenectomised patients undergoing cytoreductive surgery were evaluated.

4.1.2.1 Methods

A non-interventional, single-centre prospective study at Basingstoke and North Hampshire Hospital/Hampshire Hospitals NHS Foundation Trust, one of the two major National PM centres in the UK. From February 2014 to February 2015 serum samples were obtained on the immediate pre-operative day (Day 0), and then on Day 1 (POD1), Day 3 (POD3) and Day 6 (POD6) post-operatively, from fifty adult patients undergoing extensive cytoreductive surgery for PM. Patients younger than 18 years, pregnant patients, or those who refused consent, were excluded.

The samples were transferred to the microbiology department, initially stored at - 20 °C, and tested in batches for PCT based on our previously published methodology (80). Simultaneous CRP and WCC were measured as part of routine clinical care. As this was a non-interventional study, and PCT measurements were not performed in real-time, no clinical decisions were made based on PCT results.

In the immediate post-operative period, patients were followed up clinically for infection and assigned to either infected or non-infected groups. Patients were monitored for their outcome and

length of stay (in ICU and hospital) until discharge. PCT, CRP and WCC results were analysed in both groups (infected and non-infected), and then in splenectomy versus no splenectomy subgroups.

Infection diagnoses were made following consideration of clinical (agreement of at least 2 or 3 consultant intensive care specialists, infection specialists or surgeons), radiological, microbiological or other pathological findings.

Statistical analyses were performed using SPSS (V21). Continuous variables were expressed as means and standard deviations. Categorical variables were expressed as frequencies and percentages. Fisher's exact tests and/or Chi-Square tests were used to compare qualitative variables, and Students t-test for quantitative unpaired data. ROC was performed on the apparent fastest rising biomarker in relation to timing of clinical diagnosis of post-operative infections to determine area under the curve for PCT, CRP and WCC. Sensitivity, specificity, PPV and NPV to assist or predict the diagnosis or exclusion of post-operative infectious complications were also determined at various cut-off cut off levels for each biomarker.

The study was approved by Health Research Authority NRES Committee West Midlands - Edgbaston, UK (No. 13/WM/0510) and the HHFT Research and Development Department.

4.1.2.2 Results

A total of 50 (28 female vs. 22 male) patients were prospectively recruited and included in this non-interventional study. Demographic, clinical and surgical characteristics including baseline (preoperative) values for PCT, WCC and CRP as well as lengths of operation and hospitalisation of the patients are presented in (Table 4.3). All patients received HIPEC as part of their procedure and twenty- one patients had a splenectomy as part of their operation.

	Non-infected group N = 36	Infected group N = 14	P value when applicable
Mean Age, yrs/SD	54.8/15.2	54.3/13.1	0.871
Splenectomy, N	13	8	
Mean duration of surgery in all cases hrs	7.68/2.18	8.58/2.05	0.149
Mean duration of surgery without splenectomy hrs/SD	7.07/1.8	8.1/1.4	
Mean duration of Surgery with splenectomy hrs/SD	8.75/2.4	8.89/2.4	
Admission to ICU immediate post op, %	100%	100%	
Mean length of stay in ICU, Days/SD	1.8/0.9	2.2/0.9	0.285
Mean overall length of stay, Days/SD	20.3/8.5	25.6/14.7	0.132
Mortality, N	0	0	0.285
Mean preoperative (Day 0) PCT, µg/L/SD	0.18/0.7	0.05/0.01	0.262
Mean Preoperative (Day 0) CRP, mg/dL/SD	22.4/26	25.6/34.3	0.788
Mean preoperative (Day 0) WCC, ×10 ⁹ /L/SD	6.4/1.5	6.6/2.5	0.863
Antibiotic prophylaxis 24 h, N			
Piperacillin/tazobactam & metronidazole	1	1	
Coamoxiclav & metronidazole	30	12	
Teicoplanin, gentamicin & metronidazole	5	1	
Types of surgery, ^a N			
Complete cytoreduction: The surgery involves stripping of parietal peritoneum and resecting intra abdominal structures at fixed sites that contain tumour laden visceral peritoneum to accomplish a complete cytoreduction	18	6	
Debulking surgery: removal of as much as possible of mucous and tumour	18	8	

SD – standard deviation, WCC – white cell count, CRP – C-reactive Protein, PCT – Procalcitonin, ICU – Intensive Care Unit, N-Numbers.

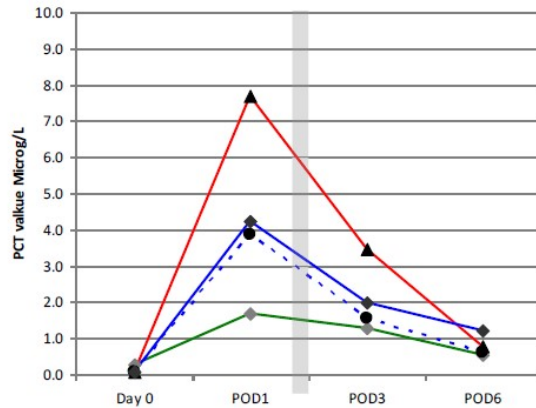
^a Surgeries may involve splenectomy or not and total abdominal hysterectomy and bilateral salpingo-oophorectomy in female patients.

Table 4.3 Baseline, Clinical, demographic characteristics and outcomes.

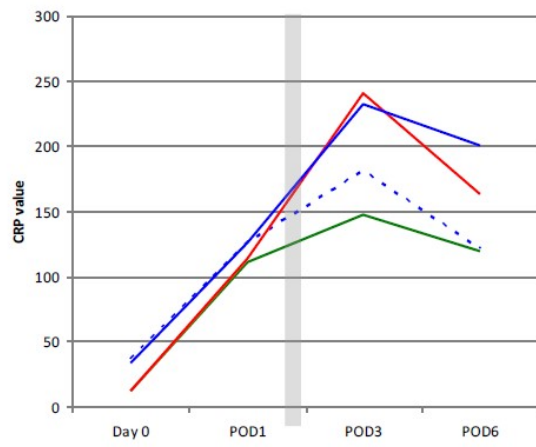
From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).

Post-operative infectious complications were diagnosed in 14 patients (28%) and included pneumonia ($n = 7$), intra-abdominal infections ($n = 3$), sepsis of uncertain source ($n = 2$), surgical site infection ($n = 1$) and urinary tract infection ($n = 1$). Timing period for diagnosis of infections were within 48 hours post-operatively, apart from the surgical site infection case which was diagnosed within 96 hours post-operatively (Figure 4.2 A-C).

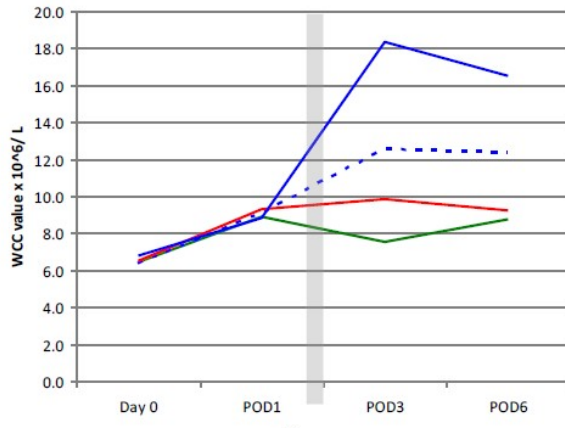
All the pneumonia cases were diagnosed based on clinical suspicion or radiological findings. One had significant positive respiratory culture for *Hafnia* species, two of these were regarded as moderate infection while the rest were severe cases of pneumonia with average PCT levels of 2.94 µg/L, WCC 10.08×10^9 /L and CRP 119. The intra-abdominal infections were intra-abdominal collections and small bowel leaks confirmed by radiological imaging and surgical corrections. These were regarded as severe infections with average PCT levels of 14.4 µg/L, WCC 7.5×10^9 /L and CRP 151.3. The two cases of sepsis of uncertain source were diagnosed clinically and no apparent source was identified despite extensive investigations. These were also regarded as severe infections with average PCT of 6.81 µg/L, WCC 6.5×10^9 /L and CRP 104.5. The surgical site infection was diagnosed on clinical examination and positive culture for *S. aureus* this was regarded as a mild infection with PCT level of 0.95 µg/L, WCC 7.8×10^9 /L and CRP of 73.



A



B



C

* The gray transparent vertical lines represent overall timing period of diagnosis of infection, apart from the surgical site infection case which was diagnosed within 96 hours post operatively.

—▲ Infection no splenectomy
 —◆ no infection no splenectomy
 —● Infection with splenectomy
 - -■ no infection with splenectomy

Figure 4.2 A: PCT, B: CRP and C: WCC dynamics pre (Day 0)* and post operatively (POD1,3 and 6) after cytoreductive surgery in infected and non-infected cases with or without splenectomy.

From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).

Finally the clinical urinary tract infection was confirmed by growing significant coliforms from the participant's urine sample. It was mild in severity with PCT value of 1.92 ug/L, WCC 5.8×10^9 /L and CRP of 121. Following appropriate therapy and management and within 24 h the average serum PCT levels in pneumonia cases came down to 1.39 ug/L while the WCC and CRP went up to 15.4×10^9 /L and 234.28 respectively. Similarly following corrections of intra-abdominal infections and clinical response to therapy average serum PCT levels came down 5.05 ug/L, but both WCC and CRP went up to 11.6×10^9 /L and 365.66 respectively.

These findings were also apparent for cases of sepsis with uncertain source where the average PCT level after treatment reduced to 2.05 ug/L, while the WCC and CRP were up to 22.05×10^9 /L and 219 respectively. Again, Serum PCT decreased to 0.23 ug/L after treating the surgical site infection, while the WCC and CRP went up with values of 9.3×10^9 /L and 114 respectively. However, Serum PCT and CRP were both came down to 0.43 ug/L and 89 respectively and the WCC remains within normal limits 6.7×10^9 /L after treating the case with urinary tract infection.

Pre-operative (Day 0) PCT, CRP and WCC were almost identical in both infected and non-infected groups (Table 4.3 and Table 4.4). However, on POD1, serum PCT levels increased sharply in patients who developed an early post-operative clinical infection [mean 2.5 ug/L, standard deviation (SD) 0.9 in the non-infected, vs. 5 ug/L,SD 1.9, in the infected group, respectively] $p = 0.058$ (Table 4.4). On POD3 and POD6 infected cases are clinically resolving and mean serum PCT levels showed 54.4% and 77.2% reduction on POD3 and POD6 respectively compared to POD1. While the WCC and CRP show an increase (delayed rise) of 61.6% and 95.7% respectively on POD3 despite appropriate management and clinically resolving infections and not returning to POD1 levels even on POD6 (Table 4.4). Additionally the physiologic increase in serum PCT on POD1 in non-infected cases appears to be reducing by 46% and 79% on POD3 and POD6 respectively. While WCC is on a very slight rise, the CRP increases on POD3 by 37% despite lack of clinical infection and only approaching its POD1 levels on POD6 (Table 4.4).

	Infection status	Mean	% Change in mean value compare to the value on POD1	Std. deviation	Std. error mean	P value
Day 0 PCT	Infected	0.0564		0.01737	0.00464	0.262
	Non-infected	0.1867		0.72505	0.12084	
Day 0 CRP	Infected	25.6364		34.37520	10.36451	0.788
	Non-infected	22.4828		26.01184	4.83028	
Day 0 WCC	Infected	6.6857		2.51850	0.67310	0.863
	Non-infected	6.4472		1.54762	0.25794	
POD1 PCT	Infected	5.7014		8.38010	2.23968	0.058
	Non-infected	2.4067		3.71097	0.61849	
POD1 CRP	Infected	120.5000		51.34461	13.72242	0.769
	Non-infected	116.3889		41.17092	6.86182	
POD1 WCC	Infected	9.0429		4.03460	1.07829	0.966
	Non-infected	9.0000		2.80540	0.46757	
POD3 PCT	Infected ^a	2.6071	↓ 54.4 ^b	3.10401	0.82958	0.165
	Non-infected	1.3636	↓ 46	2.67825	0.44638	
POD3 CRP	Infected ^a	235.9286	↑ 97.5	91.30125	24.40128	0.005
	Non-infected	159.3889	↑ 37	77.84133	12.97356	
POD3 WCC	Infected ^a	14.6929	↑ 61.6	7.03699	1.88071	0.001
	Non-infected	9.3611	↔	3.79829	0.63305	
POD6 PCT	Infected ^a	1.0300	↓ 77.2	0.60269	0.16107	0.023
	Non-infected	0.5594	↓ 79	0.65360	0.10893	
POD6 CRP	Infected ^a	184.4286	↑ 46.9	77.82595	20.79986	0.015
	Non-infected	119.9143	↔	81.78680	13.82449	
POD6 WCC	Infected ^a	13.4071	↑ 48	5.49314	1.46810	0.021
	Non-infected	10.0861	↔	3.94644	0.65774	

^a Infected cases are clinically resolving on POD3 and POD6 following appropriate treatment.

^b ↑ = increase, ↔ = almost no change & ↓ = decrease in % compared to values on POD1.

Table 4.4 Mean values for serum PCT, CRP and WCC preoperatively (Day 0) and post-operatively on POD1, 3 and 6 in infected vs. non infected cases.

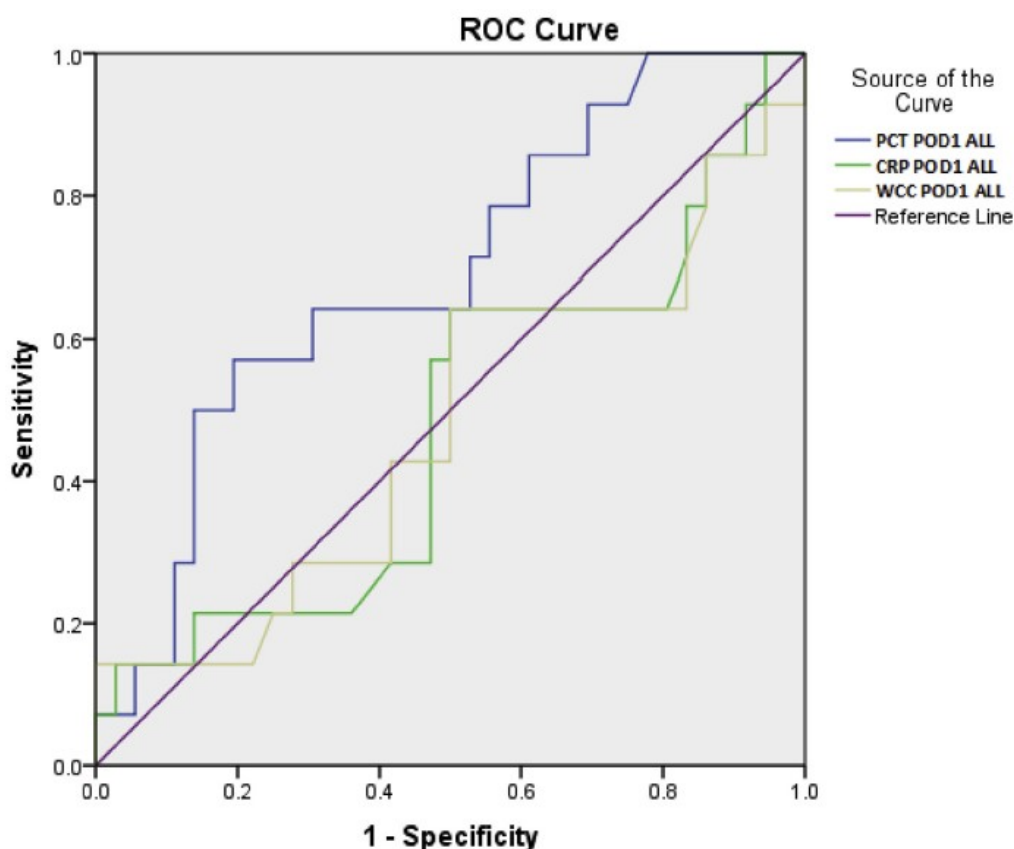
From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).

In patients who had no splenectomy, mean serum PCT levels remained below 2 ug/L (SD 2.2) when infection was not present, but increased to 8 ug/L (SD12.6) in the presence of infection. In splenectomised patients, serum PCT levels increased at similar rates on POD1 in the presence or absence of post-operative infection, and decreased at similar rates on POD3 and POD6 (Figure 4.2 A). Unlike PCT, the CRP was elevated in all patients at almost identical rates, on POD1 (Figure 4.2 B). The WCC again showed a slight increase on POD1 in both groups, but remained within normal ranges (Figure 4.2 C) despite this major surgery. Again a more noticeable increase in the CRP and WCC in patients with infection occurred 48 hours after the PCT rise, i.e. on POD3 (Figure 4.2 B and C). Subsequent to infection source control and antibiotic therapy, serum PCT levels decreased on POD3, and then further towards baseline levels on POD6, while the CRP and WCC remained elevated in the infected group despite appropriate management. As serum PCT appeared to be considerably the fastest rising biomarker in relation to post-operative infection cases, an ROC curve analysis on POD1 demonstrated that serum PCT levels had better predictor for the presence of post-operative infectious complications compared to WCC and CRP. For PCT (all patients) the AUC was 0.689 compared to 0.477 for CRP and 0.476 for WCC ($p = 0.04$) (Figure 4.3). In patients without splenectomy, the PCT-associated AUC was 0.746, and again much higher than the CRP AUC of 0.522 and WCC AUC of 0.514 ($p = 0.02$) (Figure 4.4). Sensitivity, specificity, PPV and NPV with 95% confidence interval (CI) at various cut-off levels for each of the markers are shown in (Table 4.5).

Marker and cut off level	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)
PCT \geq 0.5 $\mu\text{g/L}$	100 (77.84–100)	22.22 (10.12–39.15)	33.33 (19.57–49.55)	100 (63.06–100)
PCT \geq 1.5 $\mu\text{g/L}$	69.23 (38.57–90.91)	62.16 (44.76–77.54)	39.13 (19.71–61.64)	85.19 (66.27–95.81)
PCT \geq 2 $\mu\text{g/L}$	57.14 (28.86–82.34)	74.29 (56.74–87.51)	47.06 (22.98–72.91)	81.25 (63.56–92.79)
CRP \geq 50	78.57 (49.20–95.34)	5.56 (0.68–18.66)	18.44 (12.88–39.54)	40 (5.27–85.34)
CRP \geq 100	58.33 (27.67–84.83)	33.33 (19.09–50.22)	21.21 (8.98–38.91)	72.22 (46.52–90.31)
CRP \geq 150	28.57 (8.39–58.10)	86.11 (70.50–95.33)	44.44 (13.70–78.80)	75.61 (59.70–87.64)
WCC \geq $10 \times 10^9/\text{L}$	14.29 (1.78–42.81)	80.56 (63.98–91.81)	22.22 (2.81–60.01)	70.73 (54.46–83.87)
WCC \geq $12 \times 10^9/\text{L}$	26.67 (7.79–55.10)	65.71 (47.79–80.87)	25 (7.27–52.38)	67.65 (49.47–82.61)

Table 4.5 Sensitivity, specificity, positive and negative predictive values with 95% confidence interval (CI) at various cut off levels for each of the markers.

From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).

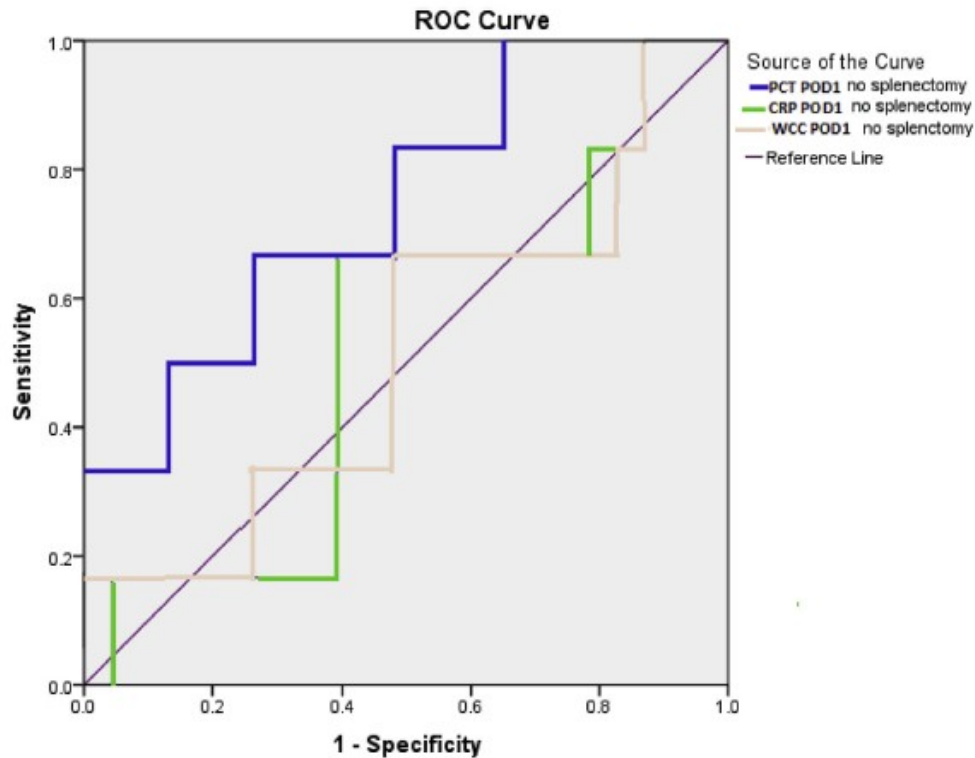


Diagonal segments are produced by ties.

Test Result Variable(s)	AUC	<i>P</i> value	<i>P</i> value for AUC difference between PCT and (CRP or WCC)
PCT POD1all	0.689	0.039	0.04
CRP POD1 all	0.477	0.804	
WCC POD1 all	0.476	0.795	

Figure 4.3 Receiver operating characteristic curves and evaluation of the value of POD1 PCT, CRP and WCC for all cases and ability to diagnose or predict early post-operative complications following cytoreductive surgery in PM cases.

From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).



Test Result Variable(s)	AUC	<i>P</i> value	<i>P</i> value for AUC difference between PCT and (CRP or WCC)
PCT POD1 no splenectomy	0.746	0.067	0.02
CRP POD1 no splenectomy	0.522	0.872	
WCC POD1 no splenectomy	0.514	0.914	

Figure 4.4 Receiver operating characteristic curves and evaluation of the value of POD1 PCT, CRP and WCC for patients without splenectomy and ability to diagnose or predict early post-operative complications following cytoreductive surgery in PM cases.

From Saeed K, et al. Eur J Surg Oncol. 2016;42(2).

4.1.2.3 Discussion

The SIRS response following complex and prolonged cytoreductive surgery can be immense. This response makes the process of diagnosing post-operative bacterial infections challenging and interferes with our ability to monitor response to therapeutic interventions such as antibiotics, using clinical or laboratory-based findings. Better and more rapid identification of patients developing infectious complications post cytoreductive surgery is crucial as it allows for timely and appropriate

management in a vulnerable patient population. The WCC and CRP are routinely used as surrogate post-operative markers of infection but they are neither sensitive nor specific.

Infection is still one of the commonest causes of post-operative morbidity and mortality, particularly after major surgery (316–320). Septic complications following colorectal resection consist mainly of surgical site infections (up to 40%), pulmonary infections (10%) and urinary infections (5%) (321). In the current study, 28% developed early infectious complications following cytoreductive surgery. Pneumonia was the commonest post-operative infection occurring in seven cases (14%). Three patients (6%) developed intra-abdominal infections requiring further surgical interventions. There was no post-operative mortality.

Intra-abdominal infections are particularly serious. Associated clinical signs are usually insensitive and do not always allow for early diagnosis. An associated mortality rate of 30% has been reported in patients with major intra-abdominal infections requiring ICU care and, in patients who develop peritonitis following abdominal surgery, associated-mortality increases further (322,323). Early diagnosis in these cases is crucial as it allows for the timely initiation of appropriate management strategies which ultimately lead to improved patient outcomes. Thus identification of a rapid and more sensitive and specific marker that could assist in the timely identification or prediction of patients with post-operative infection would be of major clinical importance. In the current study, a baseline PCT, WCC and CRP prior to surgery (Day 0) are maintained at normal levels which may indicate that the pathology itself does not lead to elevation of these markers (Table 4.3). Additionally, in our cohort, post-operative POD1 increase of serum PCT, as opposed to CRP and WCC, was associated with potentially an earlier prediction of clinical post-operative infection cases regardless of severity (Figure 4.2 A-C, Figure 4.3 and Figure 4.4), particularly in non-splenectomised patients. In cases of post-operative clinical infection, serum PCT was both faster to rise (POD1), and then fall following initiation of antibiotics or other source control measures with its levels reducing by 54.4% on POD3 and 77.2% on POD6 i.e. almost back to pre-operative levels. However, the WCC and CRP belatedly increase by 61.6% and 95.7% respectively on POD3 despite appropriate management and clinically resolving infections and still higher than mean POD1 levels on POD6 (Table 4.4). While one need to realise the limitation of a small sample size, in real life the delay rise in WCC and CRP can misleadingly trigger escalation of antibiotics and/or unnecessarily performing additional investigations.

In clinical practice we try to identify the fastest and most reliable biomarker to support the clinical decision making processes with regards to diagnosis or prediction of post-operative infections. Biomarkers assist us in planning further management e.g. additional investigations, escalation of treatment or antimicrobial therapy in order to improve patients' outcomes and experiences. Hence the earliest opportunity (POD1) was chosen for ROC analysis as PCT appeared to increase in this period. CRP or even WCC may have higher Area under the curves on POD2, POD3 or POD6 for infected cases on these days. However, we believe by then it could be either too late for patients or could even lead to unnecessary escalation of management, especially in the ICU setting as CRP and WCC continue to be high or still rising despite appropriate therapy (Figure 4.2 A-C, Figure 4.3 and Figure 4.4). In our cohort, the AUC for PCT was significantly higher than that for CRP and WCC (Figure 4.3 and Figure 4.4) (Figure 4.3 and 4.4). However, although the PPV for serum PCT at various levels was still higher than the WCC and CRP, this did not appear to be that promising, for all three markers (Table 4.5). This may be explained by the small sample size and relatively small number of infected cases. Interestingly the NPV of serum PCT, at various cut off levels, were much better than both WCC and CRP in excluding potential bacterial infectious complications. In clinical practice this could be as important and could assist in avoiding unnecessary use of resources or treatment escalation which ultimately, may lead to better patient satisfaction.

To our knowledge, there are no previously published studies or data relating to PCT dynamics pre- and post-cytoreductive surgery in PM cases to which we can compare our findings. Reith et al. reported that raised serum PCT levels were associated with post-operative complications of severe pneumonia, ischaemia and anastomotic leaks in a prospective study including 70 patients, 35 of whom underwent elective intra-abdominal colorectal surgery (not cytoreductive surgery for PM) (324). Conversely, other studies involving patients undergoing various types of elective colorectal surgery, failed to show any advantage of PCT, when compared to CRP, with regards to ability to discriminate between infected and non-infected patients (325,326). However, these latter studies were smaller and therefore the results must be interpreted with caution. Others did not show any advantage of PCT over CRP in the detection of post-operative anastomotic leaks (327). Similarly in a large prospective, observational study involving analysis from 500 patients across three centres. CRP and PCT were measured daily until the fourth postoperative day following elective colorectal surgery with anastomosis. CRP was marginally more discriminating than PCT for the detection of intra-abdominal infection (areas under the ROC curve: 0.775 vs 0.689, respectively, $P= 0.03$). PCT levels showed wide dispersion. For the detection of all infectious complications, CRP was also significantly more accurate than PCT on the fourth postoperative day (areas under the ROC curve: 0.783 vs 0.671,

$P = 0.0002$) (328). However, to our knowledge none of these cases were post cytoreductive surgery for PM case and hence results may not applicable to these groups of patients.

Elevated serum PCT levels have been observed in non-infected patients following intra-abdominal and cardiothoracic surgery, as well as in the ICU setting. The mechanisms underlying these findings are not completely understood. However, it is considered that the observed rise in PCT may be a consequence of the severe inflammatory response or secondary to transient bacterial translocation and toxin release during these operations, particularly as a consequence of intestinal manipulation or malperfusion (33,294,311–313). Similarly, our findings demonstrate that cytoreductive surgery resulted in raised serum PCT levels in patients without infection, especially on POD1. However in non-splenectomised patients, levels remained <2 mg/L. It is not clear why PCT values rose in splenectomised patients, even in the absence of infection (Figure 4.2A). This is unlikely to be due to length of the operation, as this was almost identical in patients who have splenectomy as part of their surgery or not (Table 4.3) and we could not find any literature regarding dynamics of PCT in splenectomised patients. Although we cannot explain this fully, this could be an incidental finding or hypothetically, this could occur as a consequence of the reasons outlined above or, alternatively, may be due to the abnormally high concentration of circulating white cells observed post-splenectomy, which may result in increased inflammatory markers release, including PCT. We believe further investigation is warranted to look at dynamics of PCT and other biomarkers in splenectomised patients.

Recruitment from a single centre, the small study population (including only a relatively small proportion of patients with post-operative infective complications), and the even smaller splenectomy subgroup, are limitations of this current pilot study. However, to our knowledge, this is the first prospective study assessing perioperative procalcitonin dynamics in patients undergoing cytoreductive surgery for PM and, for such surgery, represents a substantial case load. Another limitation is that due to logistical and patient consents related issues we measured PCT, CRP and WCC levels on Day 0, POD1, 3 and 6. Daily or twice daily levels of these markers would have been more ideal and might have highlighted additional findings. Despite these limitations, we believe this small cohort highlights a number of interesting findings including that baseline PCT, CRP and WCC values in PM patients prior to surgery (Day 0) are tend not to be elevated and stay within normal limits. It also shows that there is a trend for a faster rise in serum PCT compared to CRP and WCC in non-splenectomised infected patients as well as faster decline in these cases following

appropriate management and serial PCT measurements may assist in monitoring responses to therapy. Secondly the AUC for serum PCT was significantly higher than WCC and CRP in early postoperative infectious cases (POD1), particularly in non- splenectomised patients. However, the PPV of serum PCT is not very high even though it is higher than those for WCC and CRP. Interestingly the NPV of serum PCT appears to be much higher than that of CRP and WCC at various cut-off levels in excluding potential infectious complications. Finally and just like WCC and CRP there is a physiological rise in serum PCT as a result of SIRS post cytoreductive surgery in PM cases. This rise appears to be more prominent in splenectomised patients regardless of the presence of infectious complications. Therefore, serum PCT, just like WCC and CRP, need to be interpreted with extreme caution and only be used in association with other clinical, micro- biological, biochemical and radiological findings in day to day clinical practice. Further and larger studies, ideally of multicentre design, are required to identify the full potential of serum PCT alongside other traditional and more novel biomarkers in these groups of patients.

4.2 Better antibiotic stewardship: novel methods of delivering antibiotics, multidisciplinary team management

4.2.1 Negative Pressure Wound Therapy and Intra-Articular Antibiotics Instillation (NPWTia) for the Treatment of Chronic Arthroplasty-associated Infections and Implant Retention: An Alternative Approach

Note this original work (innovative practice) has been peer reviewed and published ((329)

As previously mentioned the most serious complication to arise after arthroplasty is infection with rates of 1% to 3% for primary surgery and least 4 to 8 times higher for revision procedures (135,149,330). Arthroplasty-associated infections (AAI) or PJI can carry a high morbidity rate, they can increase mortality, and are an extensive burden on the health economy (331). Over the past years there has been a significant increase in the number of joint prosthesis replacements. In 2006, about 800,000 hip and knee arthroplasties were performed in the United States and 130,000 in England (332,333). These numbers worldwide is increasing every year and therefore, even though the infection rates are low, the future true incidence of PJI is likely to increase dramatically as the number of operations continues to rise and the follow-up periods get longer (334).

Generally, there are several treatment options available for the management of PJI (335–341). The choice depends on many factors including the onset of infection, the causative bacterial agent if known, the extent of tissue damage, the quality of the implant, and presence of comorbidities and patients' and surgeons' preferences. DAIR is not a widely considered option for chronic PJI. The reason for this again could be multifactorial including low success rates of around 30% for this type of procedure (342–344). This has led to the need for more innovative technology in the treatment of this type of infection. The main aim of this type of orthopaedic surgery is to not only meet the urgent needs of patients with chronic PJI, but also to reduce cost from complex and frequently ineffective revisions and re-revisions. In this manuscript we would like to report:(i) A successful management of a chronic PJI after surgical debridement and using VAC ULTA/VeraFlo system to deliver Negative Pressure Wound Therapy and intra-articular

antibiotics instillation (NPWTiai) with implant retention, (ii) work plan, antibiotic choice, and concentration that was selected in this case and briefly comment on other potential antibiotics that can be used via NPWTiai, and (iii) in addition, what impact this technique could have on cost savings to the health economy. To our knowledge this is the first that there has been a detailed description of antibiotic choice, concentration, and dose frequency used with this novel technology.

4.2.2 Brief Case Information

In January 2012 at the Royal Hampshire County Hospital, Winchester, UK, VAC ULTA/VeraFlo system was applied to deliver NPWTiai to a 75-year-old lady with a chronically infected right total hip replacement. The patient had had multiple revision operations, initially for mechanical reasons, then multiple re-revisions due to repeated AAI. Her last re-revision was in 2008 due to an infective process without positive microbiological culture; this is not unusual after receiving many weeks of broad spectrum systemic antibiotic therapy. Despite aseptic procedures and perioperative antibiotics, this was complicated by another clinical infection and it was decided to use prolonged antibiotic therapy in the community to suppress the infection. However, as in most cases with time, the patient suffered further break-through infection with localized and occasionally systemic symptoms. A clinical management plan was agreed by the patient, the orthopaedic surgeon, and infection team to undergo further surgical debridement followed by NPWTiai aiming to eradicate the infection and implant retention (Figure 4.5, Figure 4.6, Figure 4.7, Figure 4.8 Figure 4.9).

4.2.3 VAC ULTA/VeraFlo System

The VAC ULTA/ VeraFlo system [KCI Medical Products (UK) Ltd., Wimbourne, Dorset, UK] is an integrated wound management system that provides cyclic multiphase negative pressure wound therapy (NPWT) with an installation of a topical solution which may include a chosen antibiotic as outlined in the work plan below. The advantage of traditional NPWT has been published before (345–347). The VAC ULTA/ VeraFlo system has additional advantages over traditional NPWT, this is probably due to its special VeraFlo dressings, automatic volumetric fluid delivery pump that allows for volume and pressure accuracy with a homogenous and uniform distribution of solutions

throughout the entire wound bed, during its instillation and hold phases, and also equal vacuum distribution, during its vacuum phase, which are central for promoting adequate microcirculation and granulation (Figure 4.7) (348).



Figure 4.5 Wound prior to surgery 11 Jan 2012



Figure 4.6 Wound after debridement and washout, intra operative samples grew *Pseudomonas aeruginosa*

From Saeed K, et al. Tech Orthop. 2013;28(2).

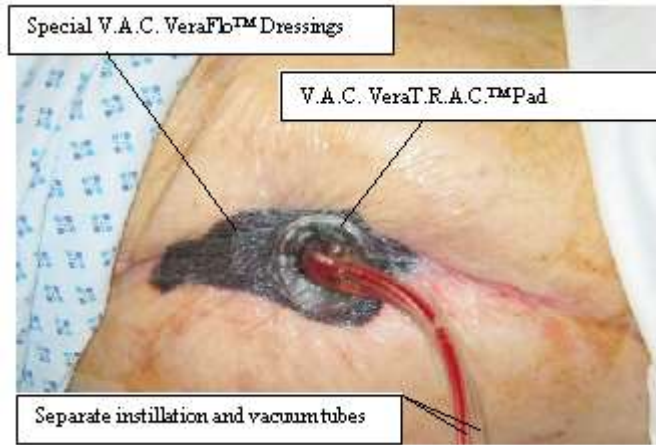


Figure 4.7 Wound is covered with V.A.C. ULTA/VeraFlo™ system



Figure 4.8 The wound 10 days after the operation and the start of NPWT



Figure 4.9 Wound four months after the procedure

From Saeed K, et al. Tech Orthop. 2013;28(2).

4.2.4 Work Plan and Methodology

4.2.4.1 Debridement Phase

Before the procedure, antibiotics were withdrawn from the patient for at least 4 weeks. The patient then underwent surgical debridement, wound clearance with the implant left in situ (Figure 4.6). About 400 mL of pus was evacuated from around the implant; this plus other intraoperative tissue samples were sent to the microbiology department at the Royal Hampshire County Hospital. All samples were cultured and grew *P. aeruginosa*, sensitive to piperacillin/tazobactam, ciprofloxacin, ceftazidime, gentamicin, and meropenem. The wound was left open by the surgeon and covered by VAC ULTA/VeraFlo system while still in theatre (Figure 4.7). Treatment was then commenced using intra-articular gentamicin by VAC ULTA/VeraFlo system and systemic intravenous piperacillin/tazobactam as a targeted therapy.

4.2.4.2 VAC ULTA/VeraFlo Cycles

This system was used to deliver NPWTia, continuously for 24 hours a day over 3 weeks using regular cycles, which can be altered by the operator. After applying Veraflo dressings and connecting the system to instillation fluid and the drainage bags, the system performs an automatic seal check and calculates the exact volume required to be instilled to the wound cavity. Each cycle is composed of 3 phases, with approximately 12 cycles being used each day, that is, around 2 hours (120 min)/cycle as below:

Instillation phase: This usually takes <1 minute and the fluid volume used usually depends on wound size and capacity, which can be automatically calculated by the system itself. In this case - 125 mm Hg pressure, with medium intensity was used and the (instillation) solution was composed of 5 mg/kg gentamicin/500 mL sodium chloride 0.9%.

Hold phase: This allows the solution to have adequate contact with the wound bed; in this case it was kept for 20 minutes.

Vacuum phase: Finally the extraction of the instilled solution through a separate vacuum tube, this phase was sustained for about 100 minutes to complete a full NPWTiai cycle, before automatically starting the next instillation phase

4.2.4.3 Dressing Change and Review Instillation Fluid Volume with Time

The entire dressing, including the sponge and tubing, was changed every third or fourth day with the dressing foam from the deep portion of the wound sent for microbiological culture at the time of dressing change to check for bacterial growth. As the wound size got smaller the fluid volume in the instillation phase was reviewed. In this case negative culture was achieved with the first dressing change that is, only 48 hours after surgery and commencement of gentamicin by NPWTiai.

4.2.4.4 Review Need for NPWT and NPWTiai

On the basis of the wound progress (Figure 4.8), it was decided to stop the NPWTiai after 3 weeks, but to continue for an additional 3 weeks with ordinary NPWT.

4.2.4.5 Patients Outcome and Follow-Up

After a single surgical debridement and implant retention, the patient received 3 weeks intravenous piperacillin/tazobactam and 3 weeks of gentamicin by NPWTiai while in hospital. This was followed by a further 3 weeks of ordinary NPWT and oral ciprofloxacin in the community. The patient was followed up and monitored regularly, by district nurses and appeared fortnightly in the orthopedic/infection clinic, for wound healing, localized and systemic signs and symptoms of infection, and up to the time of writing this report (July 2012) she remained well and no symptoms and signs of infection has been reported to suggest deep seated or implant re-infection (Figure 4.9).

4.2.5 Antibiotics that can be potentially used in NPWTiai

An equivalent to 5 mg/kg in 500 mL sodium chloride 0.9% gentamicin was used through the VAC ULTA/VeraFlo system, to target *P. aeruginosa*. This concentration allowed for localized high levels

of up to 500 times in vitro minimum inhibitory concentration (MIC) against *P. aeruginosa*, without any systemic exposure to the drug. As a precautionary measure, the patient's serum gentamicin levels were monitored at 6, 12, 24, 36, and 48 hours. No gentamicin was detected in the patient's circulation, a potential advantage especially in cases with poor renal function. The solution was replaced every 24 hours, this was recommended by pharmacy colleagues due to concerns about gentamicin instability at room temperature.

Antibiotics*	Dosage and suspension*	Comments
Vancomycin	2 g / L of Sodium Chloride 0.9%	Active against Gram positive organisms only including MRSA strains. Change bag at least every 24 hours. Serum levels can be measured if concerns about renal functions
Flucloxacillin	4-6g / L Sodium Chloride 0.9%	Please note penicillin allergy Change bag at least every 24 hours
Meropenem	4-6g in 500ml Sodium Chloride 0.9%	Change bag at least every 4 hours due to poor stability in room temperature. It should be used for confirmed resistant Gram negative infections, particularly extended spectrum B lactamse producers
Ceftazidime	2-4 g/ L Sodium Chloride 0.9%	Change bag at least every 24 hours. Can be used for treating infections with Gram-negative bacilli including sensitive <i>Pseudomonas</i>
Daptomycin	05-1g/ 0.5 L Sodium Chloride 0.9%	Active against Gram positive organisms only including MRSA strains. Change bag at least every 12 hours. Please monitor Creatinin kinase.
Clindamycin	0.9-1.2 g/ L Sodium Chloride 0.9%	Active against some Gram positive organisms (Sensitive staphylococci and streptococci) and some anaerobes. Change bag at least every 24 hours.
Colisitin	2-4 megaunit / L Sodium Chloride 0.9%	Change bag at least every 24 hours. Can be used for confirmed resistant Gram negative infections, particularly extended spectrum B lactamse producers and some metallo-beta lactamse producers. Serum levels can be measured if concerns about renal functions

Table 4.6 Potential antibiotics that may be used via NPWTi

* These choices are based on agreements among multi-disciplinary team and expertise. Antibiotics are not licensed to be used via NPWTi, please seek legal advice and consent from patients or guardians prior to applications.

From Saeed K, et al. Tech Orthop. 2013;28(2).

As with any novel technique, there are no recommended standards, agreed choices, or dosage in the literature regarding using antibiotics through NPWTi. Planning an appropriate antibiotic choice, deciding the concentrations and frequency of dosing via NPWTi were based on communication

within a multidisciplinary team that included orthopedic surgeons, clinical pharmacists, clinical microbiologists and infection specialists. Culture-directed antibiotics may increase the success, as in this case, a detailed history of possible drug allergies and ongoing clinical monitoring are needed to avoid serious allergic or toxic reactions to the solution in use. In general the chosen antibiotic should be soluble in saline or the reconstituted solution, preferably with a long stability at room temperature, it should be non-irritant to local tissue, and solutions used should be compatible with the dressings. In addition to gentamicin, the antibiotics detailed in (Table 4.6) may potentially be used via NPWTi VAC ULTA/VeraFlo system either empirically or as targeted therapy in presence of positive microbiological culture, allowing MICs up to around 1000 seconds times higher than the recommended in vitro susceptibility testing advocated by various microbiology and infectious diseases societies worldwide .

4.2.6 Cost Implications and Potential Savings from Implant retentions

8. In our institution, the basic cost of using the VAC ULTA/ VeraFlo system is around £1512.67 for 3 weeks, with current exchange rate (of £1 to \$1.55) this is almost equal to \$ 2345 for 3 weeks (Table 4.7). The economic cost of infection-related revision arthroplasty varies and has been reported as being as much as \$50,000/procedure (336,349–351). This means the basic cost of 100 revisions of PJI would be around \$5,000,000 in the United States, a figure which will not be greatly different in the UK. A greater number of studies are needed to determine implant retention rates with this technique. A report in Germany suggested success rates of about 80% using debridement and NPWTi (352). However, even with potential 50% to 80% implant retention rates with this novel technique, a crude evaluation would point to potential savings of about 45% to 75% from limiting operative expenditure in any institute (
9. Table 4.8).

Item	Cost	Cost for 3 weeks
Rental price of the V.A.C. ULTA/VeraFlo™ unit	£20.27/DAY	£425.67 for 3 weeks
Medium Veraflo dressings	316/ BOX OF 5 (£63.20 each)	£568.80 for 3 weeks
Veralink cassettes	£149/ BOX OF 5 (29.80 each)	£268.20 for 3 weeks
1000ml Canisters	£250/ BOX OF 5 (£50 each)	£250 for 3 weeks
Total cost		£1512.67 for 3 weeks or \$ 2345 for 3 weeks

Table 4.7 Itemised costs of V.A.C. ULTA/VeraFlo™ system

From Saeed K, et al. Tech Orthop. 2013;28(2).

	Total Cost of 100 Revision TKR if without using V.A.C. ULTA/VeraFlo™	V.A.C. ULTA/VeraFlo™ for 3 weeks at \$ 2,345 with 50% implant retention rate i.e. only 50 revisions would be done out of 100 chronic infections	V.A.C. ULTA/VeraFlo™ for 3 weeks at \$ 2,345 with 80% implant retention rate i.e. only 20 revisions would be done out of 100 chronic infections
Total Cost of Revision at \$50,000/ Joint	5,000,000	2,500,000	1,000,000
V.A.C. ULTA/VeraFlo™ for 3 weeks at \$ 2,345 in 100 patients	0	234,500	234,500
Savings in (\$) (saving from implant Retention – total cost of V.A.C. ULTA/VeraFlo™ for 3 weeks in 100 patients)	0	2,265,500	3,765,500
Savings in %	0%	45.3	75.3

Table 4.8 Cost of revisions in chronic infected arthroplasty and potential savings from 50 to 80 % implant retention rates using V.A.C. ULTA/VeraFlo™ system to deliver NPWTai

From Saeed K, et al. Tech Orthop. 2013;28(2).

4.2.7 Discussion

PJI are challenging problems for patients, surgeons, and the healthcare system. The commonly used treatment strategies for this type of infection are (i) debridement, antibiotics, and implant retention, (ii) I-stage revision surgery with systemic antibiotic therapy, (iii) 2-stage revision surgery with systemic antibiotic therapy, (iv) removal of infected implant without replacement with systemic antibiotic therapy, and (v) prolonged suppressive systemic antibiotic therapy in some cases(335–341). Revisions are associated with loss of bone stock, protracted immobilization or

rehabilitation and perioperative complications, especially in patients with significant comorbidities. Re-revision is associated with 3 times the risk of implant failure, a more complicated surgical course, repeated episodes of general anaesthesia, more frequent unplanned debridement before reimplantation, more frequent periprosthetic fractures, and more often required prolonged antibiotics after reimplantation and a protracted period of rehabilitation (352–354). The economic cost of infection-related revision varies and has been reported to have reaching up to \$50000 per patient (336,350–352). Therefore, on the basis of 1% to 3% infection rates and the predicted 4 million operations by year 2030, the conservative cost of primary revisions due to infection in the United States would be between \$200,000,000 to \$600,000,000 per year, a substantive impact on the health budgets (3).

A minimally invasive surgical approach which can result in implant retention is thus an attractive form of treatment. Although debridement, antibiotics, and implant retention tends to be tried in acute PJI, implant retention is not a widely considered option for chronic PJI as success rates are much lower (342–344). This may be due to that fact that conventional ways of delivering antibiotics may fail to achieve adequate concentration at the site of infection, which in itself is a very complex issue as bacteria in biofilms have significant tolerance to antimicrobial agents compared with planktonic forms of bacteria (355–358). However, in our case the NPWTai proved successful in managing a complex *P. aeruginosa* biofilm infection without removal of the implant. Before this approach, our patient had undergone almost all other treatment modalities used for management of PJI, but without success. The system allowed for delivering high concentration of antibiotics locally and directly to the implant, avoiding the "collateral" damage of systemic antibiotics such as antibiotics toxicity, antibiotic-associated *C. difficile* colitis, and selective pressure on normal flora. The underlying mechanism of the success this time is not fully understood; one could postulate that the NPWTai may have disturbed the biofilms, creating a more aerobic environment in the wound and allowing for more membrane permeability which in turn means that the locally delivered high concentration of gentamicin is able to work better against the *P. aeruginosa*. There is evidence in animals that intra-articular injections of antibiotics create concentrations that far exceed those achieved by intravenous administration (359). Intravenous antibiotics, generally used for 6 weeks after revision arthroplasties, can produce synovial fluid concentrations as high as 20% to 50% serum levels when gentamicin and cephalosporins are used, respectively (360,361). These levels are too low to be effective particularly in cases of resistant organisms (362). Antibiotic concentration many

times higher than MICs can be achieved and maintained for weeks with this novel technique even in cases with highly resistant organisms.

Implant retention is not only ideal for patients, but may also prove advantageous for institutes' economics. Our evaluation provides a basic comparison between costs of NPWT*iai* via VAC ULTA/Vera Flo system versus cost of revisions in PJI, suggesting a potential saving of about 45% to 75% from limiting operative expenditures (

Table 4.8), these evaluations do not account for additional savings from prevention of repeated procedures, bed days, prolonged intravenous and oral antibiotics, staff time, and other hidden costs that tend to be more difficult to calculate, for example, negative psychological effects that are associated with revisions and re-revisions.

This report highlights that NPWT*iai* could be added to the armamentarium of orthopaedic surgeons as an alternative approach in managing acute and chronic PJI especially where implant retention is intended or unavoidable because of patient comorbidities. However, there are still many unanswered questions in regards to standardization and duration of the antimicrobial therapy and NPWT*iai* therapy and formal cost benefit analysis. Further evaluations and larger studies with simultaneous controls and comparative groups and/or wounds are needed to address many of these questions.

Conclusion

Modern medicine constantly challenges us to seek new diagnostic and therapeutic techniques to improve the care of our patients and at the same time to reduce cost of care and the burden on health economy. PJI poses a significant burden on patients, surgeons, and the healthcare economy. Therapeutic success rates for PJI can be improved tremendously if the condition is diagnosed early and accurately, regardless of the treatment option chosen by patients and surgeons.

Misdiagnosis of PJI can result in inappropriate treatment of a patient or, conversely, prolonged use of unnecessary antibiotics or unnecessary surgery, adding considerably to health care costs and exposing patients to avoidable unwanted or harmful effects. Any diagnostic technique that could be added to the armaments of doctors and surgeons and could complement other techniques, including traditional culture use in the diagnosis of PJI and assist us in reaching safer decisions are most welcome. The fact that culture-negative PJI cases occur, biomarkers and molecular techniques, such as PCR identification of bacterial DNA with other clinical diagnostics will remain helpful in the diagnosis of PJI. Therefore, if novel technology, such as PCR or biomarker assays, is available in a healthcare setting, I would recommend its application whenever PJI is suspected. However, we must take to account other relevant clinical, microbiological, biochemical, and histopathological findings.

Furthermore, establishing the presence or absence of bacterial infection would be clinically very useful to ensure those patients who suffer from a bacterial infection get antibiotics in a timely manner while those without bacterial infection are not exposed to unnecessary antibiotics. As discussed before antimicrobial resistance is a natural phenomenon; it is an evolutionary process enabling microbes to survive exposure to antimicrobial substances. Detecting these resistances in most routine diagnostic laboratories has been done through traditional culture and antibiotic sensitivities. However these traditional tests can be misleading and unable to detect “cryptic resistance” or hidden resistant mechanisms. Again the novel PCR technology and molecular methods could assist scientists in overcoming these challenges, which would have major therapeutic and public health implications. I speculate that with the refinement of the PCR technologies and the advances in sequencing tools and microarrays, these tests would be subjected

to well designed, multicentre systematic studies, allowing for not only obtaining timely true positive or negative results, but also for determining the antimicrobial susceptibilities of the detected strains.

The use of novel biomarkers, like PCT, have supported local antibiotic stewardship, however, there are still many unanswered questions related to PCT and other novel markers, as well as questions regarding the application of novel technologies and delivery routes of antimicrobials to infection sites and their impact on stewardship programmes, effects on patients microbiome and costs of health care provision; more research and studies are required to address these questions.

Appendix A Synovial fluid Procalcitonin and the diagnosis of potential implant related infections

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EBJIS 2014 Utrecht/ The Netherlands

Synovial fluid procalcitonin and the diagnosis of potential implant related joint infections

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Introduction

Prosthetic joint infection (PJI) is a major burden for individual patients as well as the global health care industry. While reports suggest that a small minority of joint arthroplasties will become infected, appropriate recognition is critical to preserve or restore adequate function, prevent excess morbidity and reduce cost to the healthcare economy. Ambiguous diagnosis in PJI cases creates significant psychological stress for patients and surgeons. Any test that can assist in the diagnosis of PJI would be of major value to provide early efficacious treatment.

Procalcitonin (PCT) is a 116 aa peptide - Precursor of the hormone Calcitonin. PCT is secreted by almost every single organ or tissue and can be easily detected in sera during systemic bacterial infection. However, in more localised infections, the levels of serum PCT may not be elevated (Figure 1). Hence, serum PCT lacks sensitivity in the diagnosis of PJI, particularly when there are no systemic features [1-4].

Here we highlight that measurement of synovial PCT may support the diagnosis or exclusion of PJI subject to larger studies.

Methodology

This non-interventional, unblinded comparative analysis was performed at the Royal Hampshire County Hospital (Hampshire Hospitals NHS Foundation Trust), Winchester, UK.

Using a standard quantitative PCT enzyme immunoassay kit (Brahms Diagnostica, Berlin, Germany) on a mini -VIDAS analyser (BioMérieux, Marcy l'Etoile, France), we measured synovial PCT level retrospectively in 17 patients.

The patients were categorized into two groups, PJI case (n = 8) and Aseptic loosening or non infected cases (n=9), based on IDSA definitions.

Cases of aseptic loosening, but clinicians give antibiotics for > 5 days preoperatively and cases where the presence of PJI deemed possible without meeting the criteria in the IDSA guidelines were excluded.

By using standard quantitative mini VIDAS Enzyme immuno assay (BRAHMS UK Limited and BioMérieux, Basingstoke, UK, synovial PCT level was measured according to our previously published methodology [5].

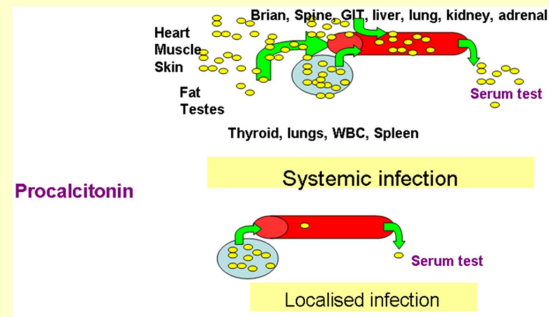


Figure 1: During systemic infections PCT is secreted by almost every single organ or tissue and can be easily detected in sera, which is not the case in localised infections.

Results

-17 cases with either predefined PJI or non-infected (aseptic) loosening. Infecting organisms were *Staphylococcus aureus* * (n=5), *Enterococcus spp.*♦ (n=1), *Escherichia coli*♦ (n=1) and Coagulase-Negative *Staphylococcus* ♦♦(n=1) (Figure 2).

-Median synovial PCT in the PJI group was 3.15 µg/L vs. 0.05µg/L in the aseptic loosening.

- Only two cases with PJIs had concomitant positive blood cultures. Highest synovial PCT levels were seen in *Staphylococcus aureus* infections regardless of presence of concomitant positive blood cultures.

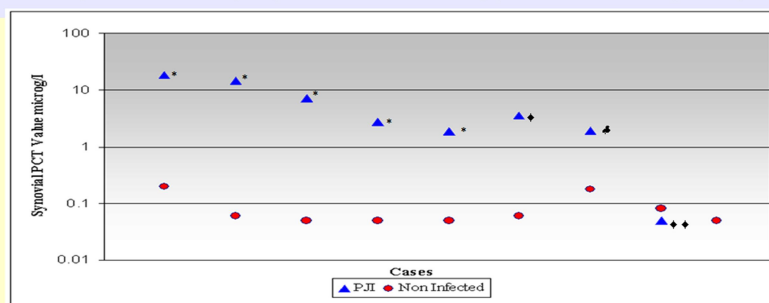


Figure 2: Synovial PCT values in PJI and Non infected cases

Conclusions

In this small cohort, we have demonstrated that synovial PCT can be found in quantifiable concentrations with a trend of higher values in PJI vs. aseptic loosening cases. Highest synovial PCT levels were seen in *Staphylococcus aureus* infections regardless of presence of concomitant bacteraemia and / or prior antimicrobial therapy. The lowest value was in a case of Coagulase-Negative *Staphylococcus* (CoNS) PJI. Synovial PCT warrants further and larger studies to confirm its usefulness in facilitating the diagnosis or exclusion of PJI.

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