CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY

CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY

Dissertation submitted to the Tamil Nadu Dr.M.G.R Medical University in partial fulfilment of the requirement for the M.S Degree Examination

Branch II (Orthopaedic Surgery)

April 2016

CERTIFICATE

This is to certify that the dissertation **titled "CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY"** is a bonafide work of **Dr. ELVIS BENJAMIN**, in the Department of Orthopaedics Surgery, Christian Medical College and Hospital, Vellore in partial fulfilment of the rules and regulations Of the Tamil Nadu Dr.M.G.R Medical University for the award of M.S Degree Branch II (Orthopaedic Surgery), under the supervision and guidance of **Prof. Dr. ALFRED JOB DANIEL** during the period of his post-graduate study from April 2014 to April 2016.

This consolidated report presented herein is based on bonafide cases, studied by the candidate himself.

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CERTIFICATE

This is to certify that the dissertation titled "CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY" is a bonafide work of Dr. Elvis Benjamin, in the Department of Orthopaedics Surgery, Christian Medical College and Hospital, Vellore in partial fulfilment of the requirement for the M.S Degree (Final) Branch II (Orthopaedic Surgery) examination Of the Tamil Nadu Dr.M.G.R Medical University to be conducted in April 2016.

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I hereby declare that this dissertation titled "CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY" was prepared by me in partial fulfilment of the regulations for the award of the M.S Degree (Final) Branch II (Orthopaedic Surgery) Of the Tamil Nadu Dr.M.G.R Medical University, Chennai towards examination to be held in April 2016. This has not formed the basis for the reward of any degree to me before and I have not submitted this to any other university previously.

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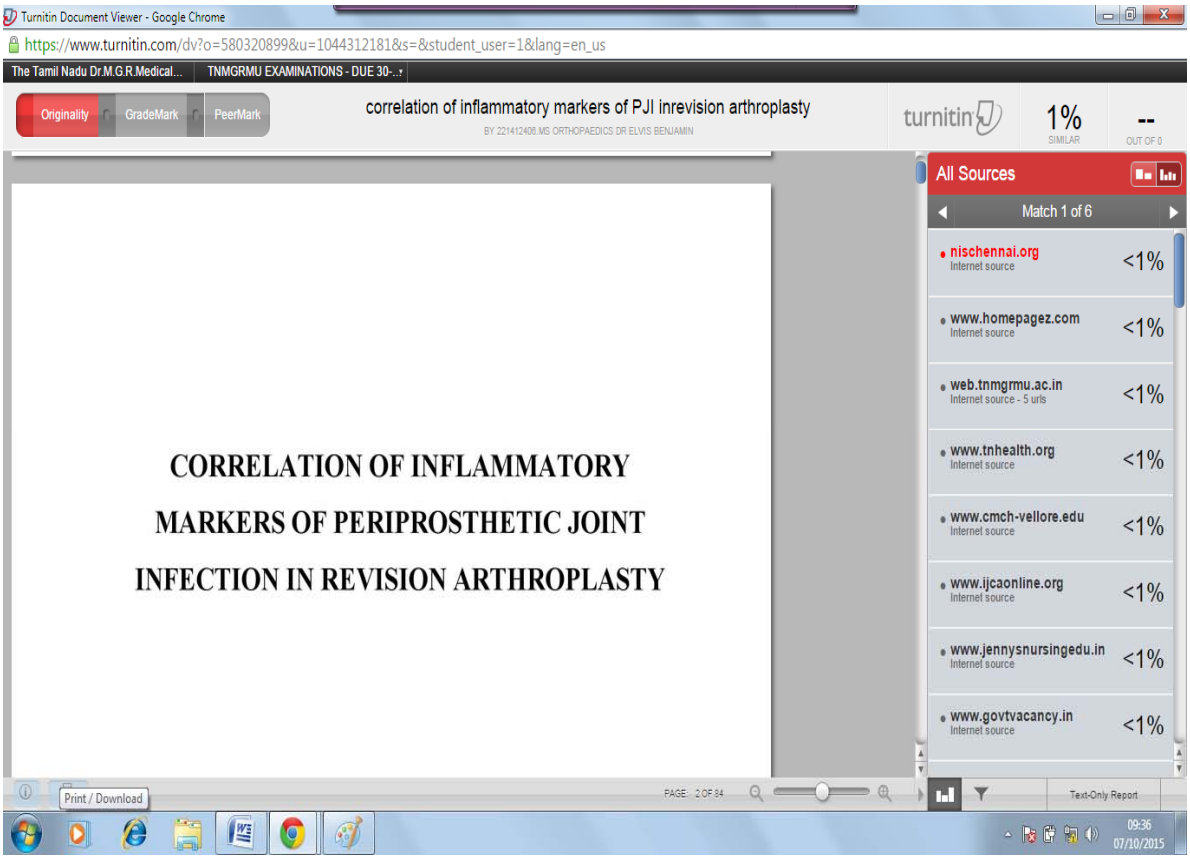
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TITLE OF ABSTRACT:

CORRELATION OF INFLAMMATORY MARKERS OF PERIPROSTHETIC JOINT INFECTION IN REVISION ARTHROPLASTY

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DEPARTMENT OF ORTHOPAEDICS, CHRISTIAN MEDICAL COLLEGE VELLORE.

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DEGREE AND SUBJECT:
M.S DEGREE, Branch II ORTHOPAEDIC SURGERY

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OBJECTIVE:

To investigate the correlation between the inflammatory markers for the diagnosis of periprosthetic joint infection in patients undergoing revision arthroplasty. HYPOTHESIS: IL-6 is an accurate, marker of periprosthetic joint infection as compared to conventional markers.

METHODS:

This study is a prospective, observational, cross sectional study, which included patients who were planned for revision hip or knee arthroplasty, either for loosening, implant exchange or because of periprosthetic joint infection. Preoperative inflammatory markers were sent for peripheral total white blood-cell count, the erythrocyte sedimentation rate, serum C-reactive protein levels and serum interleukin-6 . The diagnosis of periprosthetic joint infection was confirmed based on the diagnostic criteria laid by Parvizi J, et al. We analysed the sensitivity, specificity, positive predictive Value, negative predictive value and accuracy of each marker in aseptic loosening and periprosthetic joint infection and investigated the correlation

RESULTS:

This study demonstrated that CRP was the most accurate marker of deep infection in revision arthroplasty (Periprosthetic Joint Infection) with Sensitivity of 100%, specificity of 95%, Interleukin-6(IL-6) as a new marker was found to be less accurate than CRP, with sensitivity of 75%, specificity of 75% and Accuracy of 75%. Combination of both CRP & IL-6 can be more useful in identifying patients with deep periprostheticjoint infection, with Sensitivity of 75%, specificity of 100% and accuracy of 92.8% ,while both ESR and TLC are not useful in the diagnosis periprosthetic joint infection due to low diagnostic value.

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INTRODUCTION

Despite the wide variety of tests available for diagnosing periprosthetic joint infection (PJI), numerous problems face surgeons attempting to differentiate between septic and aseptic failure of arthroplasty components. Tests currently in use are either highly sensitive (and less specific) (eg, erythrocyte sedimentation rate [E.S.R], C-reactive protein [CRP], and serum white cell counts [TLC] ; fluorodeoxyglucose–positron emission tomography scans , bone scans , and polymerase chain reaction) or highly specific (and less sensitive) (e.g., Gram stain) ; they require specialized knowledge (e.g., frozen section), require time before results can be assessed (e.g., cultures) , or are prohibitively expensive for routine clinical application (e.g., fluorodeoxyglucose–positron emission tomography, bone scans, and polymerase chain reaction).In an effort to address this lack, we investigate the role of IL-6 (INTERLEUKIN-6), protein profiling might play in improving surgeons' ability to diagnose PJI quickly and accurately.

The erythrocyte sedimentation rate, the C-reactive protein serum level, and the white blood-cell count are routinely used to diagnose periprosthetic infection. In the present study, the diagnostic accuracy of the interleukin-6 serum level will be compared with the accuracy of these standard tests for the evaluation of a group of patients who had had a hip or knee arthroplasty or other implant and were undergoing a reoperation for the treatment of an infection or another implant-related problem. Standard radiographs and the laboratory blood analyses that are used as first-line tests to determine the presence of periprosthetic infection, namely,

the erythrocyte sedimentation rate, C-reactive protein serum level, and white blood-cell count, are not consistently reliable. The white blood-cell count is rarely elevated in the presence of a chronic periprosthetic infection and both the erythrocyte sedimentation rate and the C-reactive protein level are nonspecific markers of inflammation that may be elevated in association with any of several chronic inflammatory conditions.

Accumulating evidence suggests that interleukin-6 (IL-6), a factor produced by monocytes and macrophages that also functions as a hepatocyte-stimulating factor and induces the production of major acute-phase proteins, including C-reactive protein may be a valuable marker of infection following major surgery. Recently, it was established that serum IL-6 levels quickly return to normal after total joint surgery and are not elevated in patients with aseptic loosening.

IL-6 is a 26-kilodalton pleiotropic cytokine that functions as a pro inflammatory and anti-inflammatory molecule, a modulator of bone resorption, a promoter of haematopoiesis, and an inducer of plasma-cell development. IL-6 is produced by stimulated macrophages and monocytes when tissue is injured. The serum IL-6 level in normal individuals is approximately 1 pg/mL with slight elevations during the menstrual cycle, modest elevations of up to 10 pg/mL in patients with certain cancers (for example, melanoma), and large elevations of 30 to 430 pg/mL for as long as three days after surgery.

IL-6 plays an important role in modulating immune function as it is a primary stimulator of other acute-phase proteins such as C-reactive protein, serum amyloid A protein, haptoglobulin, protease inhibitors (for example, α-antitrypsin and α1-antichymotrypsin), complement factors, and fibrinogen and functions to regulate pyrexia by pituitary hormones.

Accumulating evidence indicates that the serum IL-6 level can be a valuable marker of inflammation in association with trauma, sepsis, meningitis, malaria, arthritis, and shock as well as after major cardiac and abdominal surgery. Elevated IL-6 levels have been found in association with bacterial meningitis and acute viral infections of the central nervous system. In addition, several investigators have described elevated IL-6 levels in patients with sepsis and documented bacteraemia (including neonatal bacterial infection), which in some cases is associated with morbidity and mortality. In a study of patients undergoing lung and heart-lung transplantation, elevated IL-6 levels appeared to be indicative of infection, and an abnormally high baseline with several sharp spikes appeared to be indicative of rejection. The erythrocyte sedimentation rate and the C-reactive protein level are widely used as serum markers for assessing bacterial infection in patients managed with total joint arthroplasty.

Previous studies have indicated that IL-6 concentrations are associated with inflammatory activity and exhibit more rapid increase and quicker return-to-normal values than either the C-reactive protein level or the erythrocyte sedimentation rate, suggesting that the IL-6 level may be a superior indicator of postoperative inflammatory response. IL-6 levels peak in the first six to twelve hours after

surgery and fallback to their baseline range by forty-eight to seventy-two hours postoperatively. After surgery, the erythrocyte sedimentation rate typically increases, with a peak at five to seven days postoperatively, and then slowly decreases to preoperative levels in approximately three months; however, some studies have shown that the erythrocyte sedimentation rate can remain elevated above baseline for as long as one year. C-reactive protein is an acute-phase reactant that is produced by the liver in response to inflammation, infection, and neoplasm its serum levels are elevated to their peak values two to three days after surgery and return to normal values approximately three weeks after surgery.

The present study investigates that the serum IL-6 level can be a more accurate marker than either the erythrocyte sedimentation rate (ESR), the C-reactive protein (CRP) or serum white cell counts (WBC) level for the detection of periprosthetic joint infection.

AIMS AND OBJECTIVES

The aim of this study is to find the correlation between the inflammatory markers, for the diagnosis of periprosthetic joint infection, in patients undergoing revision arthroplasty. Our working hypothesis is that Interleukin-6 is a better sensitive & specific marker for diagnosis of periprosthetic joint infection than the conventional markers i.e. Erythrocyte Sedimentation Rate (ESR), C - reactive protein (CRP) & Total Leukocyte Count (TLC). The diagnosis of periprosthetic joint infection is the most challenging complication of total joint arthroplasty. The diagnosis between aseptic loosening and periprosthetic joint infection is often difficult and presents a dilemma to the clinician as the treatment differs. However, infection is still the cause of failure after 1% to 2% of primary total hip arthroplasties and the rate of failure due to infection is even higher after revision procedures.

We would be investigating the role of interleukin-6 as a diagnostic marker of periprosthetic joint infection in patients undergoing revision hip or knee arthoplasty. To determine whether assessment of the interleukin-6 level can be used to detect peri prosthetic infection, we are proposing a prospective, cross section study design to study a series of patients who will be undergoing a revision total hip or knee arthroplasty.

We propose to preoperatively estimate interleukin-6, erythrocyte sedimentation rate (ESR), C- Reactive Protein(CRP) and blood cell counts (Total

blood count), joint aspirate examination for Total Leukocyte Count & percentage of polymorph nuclear Cells (PMN%) and analyse the sensitivity, specificity, positive predictive Value, negative predictive value of each in aseptic loosening and periprosthetic joint infection. Periprosthetic Joint Infection (PJI) will be defined as per the guideline on the Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection (41) which states the following criteria:

- Two positive periprosthetic cultures with phenotypically identical organism,
- Or a sinus tract communicating with the joint,
- Or having 3 of the following minor criteria:
    - Elevated serum CRP & ESR;(ESR>30mm/hr;CRP>10mg/L)
    - Elevated synovial fluid WBC count;(>3000cells/μL)
    - Elevated synovial fluid Polymorhonuclear cells percentage (PMN% >80%)
    - Positive histological analysis of periprosthetic tissue; (Histopathological analysis should show at least five polymorphnuclear leukocytes per high-power field of the periprosthetic tissue specimens)
    - A Single positive culture

OBJECTIVE: The objective of this study is to investigate the correlation between the inflammatory markers for the diagnosis of periprosthetic joint infection in patients undergoing revision arthroplasty.

HYPOTHESIS: Interleukin-6 is an accurate, i.e. specific and sensitive, marker of periprosthetic joint infection in revision arthroplasty as compared to conventional markers i.e. E.S.R, C.R.P and T.L.C.

REVIEW OF LITERATURE

Implantation of joint prostheses is becoming increasingly common, especially for the hip and knee. It provides significant reduction in discomfort and immeasurable improvement in mobility for patients.(1)(2). It has been estimated that around 800,000 hip and knee prostheses implantation procedures are performed only in USA every year, including both primary and revision surgery(3). From reviewing the worldwide literature it is seen that 1 to 5% of these prostheses become infected, and as the number of these arthroplasty surgery increases so does the number of cases that evolve with infection(3). Periprosthetic joint infection occurs less frequently than mechanical loosening does, but infection is considered to be most devastating of prosthesis related complications. The main factors predisposing towards periprosthetic joint infection that have been cited in literature are advanced age, obesity, malnutrition, HIV infection at advanced age, diabetes mellitus, presence of distant infectious foci (1)(2). Patients with rheumatoid or psoriatic arthritis are estimated to be three to eight times greater risk of postoperative joint infection than other patients. Prolonged duration of surgery, performing bilateral arthroplasty, and blood transfusion are other factors related to occurrence of periprosthetic joint infection. Other factors that delay wound healing, such as hematoma, wound abscesses, cellulitis or necrosis, also increase the risk of infection. It is important to emphasize that the presence of the joint prosthesis leads to functional loss among the local granulocytes that accumulate around the implant, which become partially degranulated with diminished production of

superoxide dismutase and loss of defence capacity against bacteria, particularly against *Staphylococcus aureus.* Thus, the presence of the implant decreases the size of the bacterial inoculum needed for infection to occur, by more than 100,000-fold(4). Joint prostheses can become infected through three different routes: direct implantation, hematogenic infection, and reactivation of latent infection. Microorganisms may penetrate the wound during the operation from both endogenous and exogenous sources. Examples of such sources include patient's cutaneous microbiota, microbiota of members of the surgical team, environment, and even contaminated implants. Bacteraemia from distant infectious foci may cause prosthesis contamination through a haematogenous route. The primary foci most frequently reported in the worldwide literature are the respiratory, cutaneous, urinary, dental, and gastrointestinal tracts (2)(4).

CHRONOLOGICAL PRESENTATION OF INFECTION

The characteristic signs of periprosthetic joint infection can be divided into acute manifestations which are severe pain, toxaemia, high fever, rubor, heat and surgical wound discharge, and chronic manifestations which are formation of sinus or fistulae, progressive pain, and purulent secretions without fever. Most widely used classification system is the one proposed by Fitzgerald Jr. et al, who had divided infections in arthroplasty as follows(5).

(i) **Acute postoperative infections** occurring within three months of the surgery. The etiological agents are generally of hospital origin, especially *S. aureus* and *S. epidermidis*;

(ii) **Deep late infections** that appear between three months and two years after the surgery. The etiological agents are considered to be of nosocomial origin, since the contamination probably occurred during the act of prosthesis implantation and generally consist of bacteria from the normal microbiota of the skin, such as *S. epidermidis*(6)

(iii) **Late hematogenic infections** that occur more than two years after the surgery. The etiological agents are of community origin and are determined by the apparent source of bacteria; dental infections are associated with bacteraemia due to *S. viridans* and anaerobic bacteria, while cellulitis and skin abscesses are associated with *S. aureus* or streptococci. *Enterobacteriaceae* originate from the gastrointestinal and genitourinary tracts(7).

C- Reactive protein and Erythrocyte Sedimentation Rates, despite being non-specific, have shown sensitivity varying from 91% to 93%, respectively and specificity varying from 86% to 83%, respectively, in patients with knee arthroplasty and appears to be an useful screening tool(8)(9).

RADIOLOGICAL SIGNS OF INFECTION

The X-Ray is the main imaging method used in diagnosing the periprosthetic joint infection. The signs suggesting infection, in case of cemented prostheses, are wide band of radiolucency at the cement –bone interface or at the metal bone interface in case of uncemented prostheses, associated with bone destruction(10)(11). Though it is generally not possible to differentiate between septic and aseptic osteolysis based on a single x ray, previous radiographs are needed for comparison (10)(12)(13)(14). In aseptic loosening, the evolution is slow, while in case of infectious loosening, the loosening is rapid and aggressive with greater bone destruction (15).

A computed tomography (CT) scan may help in distinguish between septic and aseptic loosening. Presence of periosteal reaction or accumulation of soft tissue adjacent to area of osteolsysis is highly suggestive of periprostheticjoint infection (16)(17)(18).

Ultrasonography may also be used to identify the presence of soft tissue fluid collections(18).

The role of magnetic resonance imaging (MRI) is very limited because of the artefacts which are generated by joint prostheses. The techniques of reducing artefacts on MRI exists (19) but they are not enough to enable adequate evaluation around the prosthesis (20)(21)(22).

Methods derived from nuclear medicine can also be used(23). Three phase bone scintigraphy has high sensitivity but low specificity. Bone scan has high negative predictive value, that is loosening is ruled out if result is normal. Positron emission tomography using fluorodeoxyglucose (FDG-PET) presents a very divergent results in the literature, with accuracies ranging from 43% to 92%(24)(25)(26), therefore it is not considered a reliable method for evaluation of periprosthetic infection. Scintigraphy using labelled leukocytes provides excellent results, with accuracy of more than 90% and this is scintigraphy method of choice for evaluating periprosthesis joint infection. But this method is limited due to low availability in clinical practice.

HAEMATOLOGICAL TESTS FOR PERIPROSTHETIC JOINT INFECTION

Measurements of the Westergren erythrocyte sedimentation rate, the rate at which red blood cells sediment from whole blood, and of the level of C-reactive protein, a protein produced in the liver, are serologic tests that may be an important part of a diagnostic workup of patients with suspected periprosthetic infection. The erythrocyte sedimentation rate and the C-reactive protein level normally rise rapidly after joint arthroplasty, reaching peak levels several days after the operation, with the C-reactive protein level peaking slightly earlier than the erythrocyte sedimentation rate(27-29). In the absence of an inflammatory arthropathy or infection, the serum level of C-reactive protein usually returns to normal by about three weeks after the arthroplasty(29), although values may take

longer to normalize after knee arthroplasty than after hip arthroplasty(28).The erythrocyte sedimentation rate decreases more slowly than does the C-reactive protein level, may show some diurnal variation, and may remain slightly elevated for six weeks after the arthroplasty(29). Elevations in the erythrocyte sedimentation rate and especially in the C-reactive protein level after three months suggest the possibility of infection(6,30), but these levels need to be interpreted along with other findings. C-reactive protein levels and erythrocyte sedimentation rates may be slightly elevated in patients in whom heterotopic ossification has developed, are less predictive of infections in patients with underlying inflammatory arthropathies, may be elevated in patients with other postoperative complications such as bronchopneumonia(31), and sometimes may not be elevated in the presence of periprosthetic infection. Measurements of the erythrocyte sedimentation rate in particular may have a high frequency of false-positive results(32). If inflammatory arthropathies were excluded, the erythrocyte sedimentation rate was found to have sensitivity of 82% and a specificity of 85%. The predictive value of a negative test was only 58%, while the predictive value of a positive result was 95%. The C-reactive protein level was found to be a better indicator of infection than the erythrocyte sedimentation rate, with the C-reactive protein level having a sensitivity of 86%, a specificity of 92%, and predictive values for negative and positive tests of 74% and 99%, respectively. While neither the erythrocyte sedimentation rate nor the C-reactive protein level is diagnostic of infection, values that increase (or fail to decrease) three months after an arthroplasty should raise the suspicion of infection and prompt additional diagnostic studies. Another serologic

test that has shown promise for diagnosing infection is measurement of the serum level of interleukin-6 (IL-6), a factor produced by monocytes and macrophages. In a recent study, the serum level of IL-6 was found to be consistently elevated (>10 pg/mL [>10 ng/L]) in patients with periprosthetic infection, and it had a higher predictive value than most other serologic markers(33). A potential advantage of measuring the IL-6 level is that the level returns to normal soon (within forty-eight hours) after the operation and is not likely to be elevated in patients with aseptic loosening. However, it may be elevated in patients with an underlying inflammatory arthropathy.

JOINT ASPIRATE IN DIAGNOSIS OF PERPROSTHETIC JOINT INFECTION

One of the most important tests in the evaluation for potential periprosthetic infection is culture of the fluid aspirated from the joint. In 1993, Barrack and Harris reported on a series of 270 consecutive patients who had undergone aspiration and culture shortly before revision total hip arthroplasty, even when the clinical features did not necessarily suggest infection(34) The results of 291 successful aspirations in 260 patients were evaluated. Six hips (2%) were eventually found to be infected. The cultures of the aspirates had six true-positive results, four false-negative results, and thirty-three false-positive results. The high frequency of false-positive results yielded a sensitivity of only 60% and a positive predictive value of only 15%, giving the impression that culture of aspirated fluid is a relatively poor test, at least when performed in a consecutive series of patients

who had not been screened for features suggestive of infection. In a later study, however, Barrack et al. performed cultures of aspirated fluid obtained from sixty nine patients with a symptomatic total knee replacement(35) . Twenty of the knees were ultimately diagnosed as being infected, whereas forty-nine were considered to be not infected. Some patients underwent multiple aspirations, but the initial series of cultures yielded eleven true-positive results, forty- seven true-negative results, two false-positive results, and nine false-negative results, with sensitivity and specificity values of 55% and 96%, respectively. The predictive value of a positive result in this series of knee arthroplasties was 85%, which was considerably better than the 15% predictive value of a positive result in the 1993 study of hip arthroplasties. There are several possible reasons for the difference in the predictive values between the above studies (34,35). One possible reason is that one study dealt with hips and the other, with knees. False-positive test results may be more common in fluids aspirated from hips than in those aspirated from knees. On the other hand, the prevalence of infection in the second study (29%) was much higher than that in the first (2%), presumably because the test was applied to all patients undergoing revision arthroplasty in the first study but was limited to patients with “symptomatic” knee replacements in the second. The important effect of prevalence on calculations of predictive values is illustrated by using the Bayesian equation to calculate the positive predictive value. Including prevalence in the calculation yields a positive predictive value of only 15% in the 1993 study of hip fluid aspirations but a value of 72% in the 1997 study of knee aspirations. These calculations illustrate that the predictive value of a positive result of a

culture of joint fluid is higher if the study is not used as a screening test for infection but is used instead as a confirmatory test for patients in whom clinical findings (or prior laboratory test results) have already raised the suspicion of infection.

JOINT FLUID ANALYSIS FOR DIAGNOSIS OF PERIPROSTHETIC JOINT INFECTION

Several studies have indicated that cell counts of fluid aspirated from around total joint prostheses can also provide useful information, although the literature is somewhat difficult to interpret, in part because authors have used different units of volume to express values. For example, in a prospective study, Spangehl et al. included cell counts among other tests to diagnose infections at the sites of total hip arthroplasties(36). Use of 50×109cells/L, (50,000 cells/μL), as a cut-off point for the diagnosis of infection yielded a sensitivity of only 36%, reportedly because of frequent false-negative results, and use of 80% neutrophils as a cut-off resulted in a positive predictive value of only 52% because of a high frequency of false-positive findings (37). Kersey et al. prospectively analyzed the white blood-cell count and differential of fluid from seventy nine knees (seventy-four patients) prior to revision arthroplasties performed because of aseptic failure (38). Patients who were thought to have an infection were excluded. The mean white blood-cell count in the joint fluid was 782/mL (<1/μL), with a mean differential of 13% neutrophils, but eight uninfected knees had a leukocyte count of >2000/mL (2/μL). Four of those knees were affected by rheumatoid arthritis, and three of the knees with rheumatoid arthritis had >50% neutrophils. The authors

concluded that synovial white blood-cell counts and differential counts from uninfected sites of total knee replacements are similar to the counts in fluid from knees without an implant, and they suggested that <2000 white blood cells/mL and <50% neutrophils suggests the absence of infection (38). It should be noted, however, that Kersey et al. did not include patients with infection in their series, and it is recognized that other conditions, such as crystalline arthropathies, can be associated with a high concentration of neutrophils in the joint fluid.

In 2003, Mason et al. retrospectively reviewed data on 440 revision total knee arthroplasties and identified eighty-six patients who had presented with clinical features suspicious for infection and had therefore undergone joint fluid aspirations (39).The mean white blood-cell count for the fifty knees that were found to be uninfected was 645±878/mL (about 6/μL),whereas the mean count for the thirty-six infected knees was 25,951/mL (260/μL). There was a mean of 72.8%±28.6% neutrophils in the infected knees and 27%±24% in the uninfected ones. The authors suggested that the optimum criteria for diagnosing infection included a white blood-cell count of >2500/mL and >60% neutrophils (39). Trampuz et al(40) prospectively evaluated synovial fluid specimens from ninety-nine patients with septic failure of a total knee prosthesis and from thirty-four patients with an infection at the site of a total knee arthroplasty.

Using receiver operator characteristic curves, the authors estimated that a synovial fluid leukocyte count of 1.7 × 10/μL or a differential count of >65% neutrophils was the optimum cut-off for a diagnosis of infection(40). The disparity in reported cell concentrations suggests that some authors may not have reported

the correct units of volume. Setting aside the inconsistencies in units, there are still discrepancies with regard to the level at which the cell count in fluid from the site of a prosthetic joint may be considered abnormal. From a practical standpoint, we consider a white blood-cell count of >500/μL as suggestive of periprosthetic infection. The current definition of Periprosthetic Joint Infection(PJI) is defined as per the guideline on the Proceedings of the International Consensus Meeting on Periprosthetic Joint Infection , which mentions the two minor criteria as synovial fluid count > 3000 cells/μL and PMN%(>80%)(41).

FROZEN SECTION ANALYSIS OF PERIPROSTHETIC TISSUE

The most frequently used intra operative test for infection is the interpretation of frozen sections of tissue obtained from the joint capsule or periprosthetic membrane. Sometimes these specimens show marked acute inflammation and are essentially diagnostic of ongoing infection. Other times, there is essentially no inflammation, an observation that suggests the absence of infection. However, implant membranes sometimes have a low concentration of neutrophils or contain lymphocytes and plasma cells without neutrophils. The importance of this borderline inflammation is not obvious, and many investigators have attempted to establish histological criteria that are diagnostic of infection. As will be described below, these authors have used different criteria for the histological diagnosis of infection, have employed different reference standards with which to compare the histological results, and have arrived at different conclusions, especially with respect to the importance of lymphocytes and plasma

cells. Some authors have prospectively tested consecutive patients (thereby using frozen sections as a screening test), whereas others have evaluated frozen sections only when there was a suspicion of infection at the time of the operation (thereby using frozen sections as a confirmatory test). As was true of the cultures of aspirated fluid described above, analyzing frozen sections from all patients undergoing revision arthroplasty is likely to reduce the specificity and predictive value of positive results compared with the values derived when frozen sections are analyzed only when there is clinical suspicion of infection at the time of surgery. Perhaps the first study of the use of frozen sections to diagnose an infection at the site of an arthroplasty was reported by Charosky et al. in 1973.

Those authors described the results of analysis of frozen sections of implant membranes obtained from twenty patients, ten of whom had intra operative cultures that were positive for organisms and ten of whom had negative cultures. Of the ten with positive cultures, five had acute inflammation that was "2+ or greater" (not otherwise defined) and the other five had chronic inflammation that was "2+ or greater." The authors concluded that acute inflammatory changes or "severe chronic inflammation" were presumptive evidence of infection. In 1995, Athanasou et al. (42) reported on a prospective study in which frozen sections from several different sites were obtained during each of 106 hip and knee revision arthroplasties performed between 1991 and 1993, and the results were compared with those of intraoperative cultures. In an evaluation of ten high-power fields with maximal inflammation, the author's quantified inflammatory cells into four tiers (absent, one, one to five, and more than five cells per field). Of note, lymphocytes

and plasma cells were included along with neutrophils, but neutrophils entrapped in fibrin adherent to the surface of the membrane were excluded. Intraoperative cultures were considered positive if organisms grew on direct plating or if a similar strain grew on enrichment in more than one culture; single isolates from only one culture were considered to be negative findings.

On the basis of the culture results, twenty-four arthroplasty sites were determined to be infected and eighty-four were considered to be not infected. Compared with these culture results, the frozen-section analysis yielded two false-negative and three false-positive results—a sensitivity of 90%, a specificity of 96%, and positive and negative predictive values of 88% and 98%. The authors noted that there were occasional lymphocytes in the thirty-six uninfected cases .These cells were often perivascular and were not regarded as suspicious for infection. In addition, three patients with underlying rheumatoid arthritis had numerous lymphocytes and plasma cells, and five patients with aseptic loosening and abundant metal particles also had moderate numbers of lymphocytes. While these patients were recognized as probably not having an infection, the authors noted that: “in the absence of rheumatoid disease, plasma cells were a good marker of infection, being noted in eight of the infected cases.” Of the two patients who were considered to have a “false-positive” frozen section on the basis of a negative intra operative culture, one had loosening eighteen months later and was found to have an infection at the repeat revision arthroplasty. The second patient also had a clinical course suggestive of infection, which again emphasizes the limitation of using intra operative culture results as a reference standard. In 2000, Pandey et al.

(43) reported a study that appears to have overlapped, in part, with the study by Athanasou et al. (42) .Pandey et al. retrospectively reviewed the results of histologic tissue analysis and intraoperative cultures of specimens from 617 revision arthroplasties performed between 1992 and 1996 at several hospitals affiliated with the Oxford Skeletal Infection Research and Intervention Service. Although there was overlap among the authors of the two studies (43)(42), different criteria were used for the histologic diagnosis of infection. At least ten high power fields were evaluated, and an average score for the various inflammatory cells was calculated (43). One inflammatory cell per high-power field in at least ten fields was considered to be consistent with infection. For the intraoperative cultures, isolation of the same organism from three or more culture specimens was considered diagnostic of infection. Organisms were considered contaminants if different strains grew in different broths and there was no growth on direct plating. A single isolate was considered to be unimportant. Of the 617 revision arthroplasty sites, 526 were clinically suspected to be aseptic and ninety-one were suspected to be infected. Eighty-one were proven to be infected according to the microbiologic criteria noted above. Five hundred and twenty-one cases had no growth on culture and had negative histological findings as only scattered lymphocytes were present (true-negative histological findings).Both the cultures and the histological analysis showed features of infection in seventy-nine cases (true-positive histological findings). Two cases had “significant growth of organisms” on culture but negative histologic findings (false-negative histological findings), and ten cases had negative cultures but acute inflammation in the peri-

implant membrane. Seven of the ten patients had received preoperative antibiotics, and all ten were treated clinically as if they had an infection. Finally, five cases showed inflammation in the tissue but negative cultures. Two of these patients had rheumatoid arthritis and loosening developed within two years.

As described above and in additional studies(44–46), criteria for interpreting microscope slides of frozen sections are not yet uniform. Considering a low number of neutrophils (for example, one cell per high-power field (43) or even lymphocytes or plasma cells (42) to be diagnostic of infection will provide maximum sensitivity but will be associated with false-positive diagnoses and hence decreased specificity. Use of more stringent criteria (for example, tenpolymorph nuclear leukocytes per high-power field in at least ten high-power fields (47) will improve specificity at the expense of sensitivity . Numeric criteria are complicated even more by differences in the visual field size of different microscopes. While most authors have used 10× ocular and 40× objective lenses (yielding a nominal net magnification of 400×), other differences in microscope and camera configurations can vary the visual field by as much as two fold. Therefore, the number of inflammatory cells per high-power field should be recognized as only an approximation. Partly on the basis of the studies described above, we currently interpret a frozen section as being suggestive of infection if it contains at least five neutrophils in each of three 400× high-power microscopic fields located beneath the surface of the membrane. In the appropriate clinical setting, even fewer neutrophils should raise the suspicion of infection. Neutrophils entrapped in superficial fibrin or adherent to endothelial cells (marginating) are not thought to

be diagnostic of infection, but neutrophils in fibrous tissue between the capillaries that compose granulation tissue may be predictive of infection. Frozen sections of tissue from a patient with an underlying inflammatory arthropathy such as rheumatoid arthritis are especially difficult to interpret because, in these patients, acute inflammation involves peri implant membranes even in the absence of infection. Lymphocytes and plasma cells have been seen in biopsy specimens from patients who have been treated with antibiotics for infection, but these cells are currently thought to be nonspecific and in general not predictive of active infection. Inflammation is not uniformly distributed around the prosthesis, so frozen-section analysis of biopsy specimens taken from several different sites increases the sensitivity compared with that of an analysis of a single biopsy specimen. It is also important for the tissue submitted for frozen-section analysis to adequately represent the fibrous membrane and not contain only superficial fibrin. Although we continue to use the same histological criteria for diagnosing active infection at the second stage of a two-stage revision arthroplasty done because of infection, the predictive value of these observations in this clinical context (after the use of local and systemic antibiotics)requires further study (as described below). Communication and feedback between the surgeon and pathologist are key to help both physicians to determine the clinical importance of inflammation in any given case.

Periprosthetic Joint Infection (PJI) is defined as per the guideline by. Parvizi J, Gehrke T, Chen AF in the "Proceedings of the International Consensus on Periprosthetic Joint Infection" Which states the following criteria*:*

- Two positive periprosthetic cultures with phenotypically identical organism,
- Or a sinus tract communicating with the joint,
- Or having 3 of the following minor criteria:
    - Elevated serum CRP & ESR;(ESR>30mm/hr;CRP>10mg/L)
    - Elevated synovial fluid WBC count;(>3000cells/µL
    - Elevated synovial fluid PMN%; (>80%)
    - Positive histological analysis of periprosthetic tissue; (Histopathological analysis should show at least five polymorph nuclear leukocytes per high-power field of the periprosthetic tissue specimens)
    - A Single positive culture

MICROBIOLOGIC CULTURES OF TISSUE

The results of culture of tissue and/or fluid obtained during revision arthroplasty are usually considered the gold standard for determining the presence or absence of periprosthetic infection. While the clinical utility of intraoperative culture is clear, when viewed in the context of extended follow-up, the test still can yield false-negative and false-positive results. Other authors have described cases in which, despite the presence of acute inflammation in the peri prosthetic membrane and a clinical postoperative course consistent with infection, the intraoperative cultures remained negative. Some of the patients with negative cultures may have taken perioperative antibiotics. In a prospective study involving revision arthroplasty in 297 patients with a total of forty-one infections, Atkins et al. noted that only 65% of all samples obtained from the infected joints were culture positive(74). They recommended obtaining five or six culture specimens from each patient and suggested that the cutoff for a definite diagnosis of infection be growth of the identical organism on culture of three or more specimens. In general, it is recommended that surgeons take special precautions to minimize tissue contamination, such as obtaining multiple samples from deep tissues, using clean instruments for tissue retrieval, transferring tissue to the culture bottle without allowing contact with the operative field or gloves, and transferring of the culture samples to the laboratory for processingas quickly as possible. To minimize the incidence of false-negative cultures, representative samples should be obtained with sharp dissection, administration of antibiotics should be discontinued at least

two weeks prior to the surgery, and intra operative antibiotics should be withheld until the tissue samples are retrieved. Communication between the microbiologist and the orthopaedic surgeon is critical for isolation of rare and difficult-to-isolate organisms. The use of sonication may help to identify organisms that are adherent to implants or are contained within biofilm(75–77).

DIAGNOSING INFECTION AT THE TIME OF REIMPLANTATION

The understanding of the sensitivity and specificity of various observations and laboratory tests for the diagnosis of periprosthetic infection has been based mostly on the evaluation of patients who have undergone primary hip or knee arthroplasty. Criteria for diagnosing persistent infection at the time of reimplantation in a two-stage revision arthroplasty are even more ill-defined. To present knowledge, the use of frozen sections for diagnosing persistent infection at the time of reimplantation has been evaluated in only a single study(78). Using intraoperative cultures as the gold standard and the morphologic criterion of ten neutrophils or more in each of five high powered fields, Della Valle et al. recognized only one of four persistent infections in a series of sixty-four cases(sensitivity 25%)(78). While specificity was 95%, the sensitivity of frozen section interpretation in this clinical setting seems to be lower than that in the setting of primary arthroplasty. Reducing the number of inflammatory cells needed to diagnose infection would be expected to increase sensitivity but might reduce specificity. Additional studies are needed to help clarify the most effective tests for diagnosing infection in this setting.

ENDOTOXIN

Lipopolysaccharide is a component of the cell wall of gram negative bacteria. It can be released during episodes of infection, it is pyrogenic; and, when present in high enough concentrations, it can induce the release of interleukins, tumor-necrosis factor, and other cytokines from monocytes and macrophages. Although "endotoxin" strictly refers to lipopolysaccharide from gram-negative organisms, similar molecules may also be associated with gram-positive organism(79). Although endotoxin is usually neutralized before causing systemic symptoms, there is increasing evidence that it may adhere to orthopaedic biomaterials, including particles of wear debris, and may enhance the inflammatory reaction to particles that is usually associated with aseptic loosening(80–82) . Therefore, contamination of implants or instruments with bacterial endotoxin might yield an inflammatory reaction similar to that seen around infected implants. The potential clinical importance of endotoxin in periprosthetic infection and in cases of "aseptic" loosening requires further study.

MOLECULAR TECHNIQUES

With the advances in molecular biology, several sophisticated techniques are being developed for the diagnosis of periprosthetic infection. One such technique is the use of the polymerase chain reaction (P.C.R) for detecting

evidence of organisms(83–85). The technique relies on the use of forward and reverse primers designed to match specific sequences of target DNA.

The most common target gene for bacterial identification is the 16S rRNA gene that is conserved in nearly all species of bacteria. For example, Tunney et al.(75) used polymerase chain reactions to test for evidence of bacteria in fluids obtained by sonication of 120 hip implants retrieved at revision arthroplasty. The implants were first placed in a water bath and then exposed to ultrasound to disrupt any biofilm and dislodge organisms. With use of primers for the 16S rRNA gene, 72% of their cases were interpreted as positive. The main problem with this technique is related to the apparently high prevalence of false-positive results, which have several possible sources(86–88). First, polymerase chain reactions detect bacterial DNA from both viable and necrotic organisms, so traces of only a few necrotic bacteria dislodged by sonication from an implant surface may yield a positive test result. Second, one of the reagents employed in polymerase chain reactions (Taq polymerase) is derived from recombinant technology involving use of Escherichia coli organisms. Trace levels of DNA from the Escherichia coli contaminating the Taq polymerase reagent can also yield false-positive results of the polymerase chain reaction. Finally, the broad sensitivity of polymerase chain reactions directed against the 16S rRNA detects even trace contamination by clinically irrelevant organisms that occurs after specimen acquisition. One way to improve the specificity of polymerase chain reactions is to use primers and probes directed against a specific organism, or group of organisms, most likely to be involved in clinically important orthopaedic infections. Thus, combinations of

specific polymerase chain reaction assays may ultimately prove to be more useful than broad-spectrum, so-called “universal” bacterial assays. Other new techniques that may have a role in diagnosing infection include the use of microarray (89)and proteomics technologies. A microarray allows isolation and evaluation of numerous mRNA genes with a single test. Proteomics allows simultaneous isolation and evaluation of numerous proteins. The premise of these techniques is to identify organism-specific genes or proteins. The challenge for all of the new molecular tests will be to distinguish clinically important infections from trace levels of necrotic bacteria or contaminants and to provide that information quickly enough to be of practical help in guiding patient care.

INTERLEUKIN-6

Interleukin-6 (IL-6) is a 26-kilodalton pleiotropic cytokine that functions as a proinflammatory and anti-inflammatory molecule, a modulator of bone resorption, a promoter of hematopoiesis, and an inducer of plasma-cell development(48–50) .IL-6 is produced by stimulated macrophages(51) and monocytes when tissue is injured (52). The serum IL-6 level in normal individuals is approximately 1 pg/mL (53), with slight elevations during the menstrual cycle , modest elevations of up to 10 pg/mL in patients with certain cancers (for example, melanoma) (54), and large elevations of 30 to 430 pg/mL for as long as three days after surgery (55).IL-6 plays an important role in modulating immune function (48) as it is a primary stimulator of other acute-phase proteins such as C-reactive protein, serum amyloid A protein (52), haptoglobulin, protease inhibitors (for

example, α-antitrypsin and α 1-anti chymotrypsin), complement factors, and fibrinogen (56) and functions to regulate pyrexia by pituitary hormones (52). Accumulating evidence indicates that the serum IL-6 level can be a valuable marker of inflammation in association with trauma, sepsis, meningitis, malaria, arthritis, and shock as well as after major cardiac and abdominal surgery (57–60). Elevated IL-6 levels have been found in association with bacterial meningitis and acute viral infections of the central nervous system(61–63). In addition, several investigators have described elevated IL-6 levels in patients with sepsis and documented bacteremia (including neonatal bacterial infection), which in some cases is associated with morbidity and mortality(57,64–73).

MATERIALS AND METHODS

STUDY POPULATION:

This study included patients who were planned for revision hip or knee arthroplasty, either for loosening, implant exchange or because of periprosthetic joint infection, between 1st July 2014 to 1st August 2015. These patients were approached for recruitment in the study, from Orthopaedic units I, II & III. Written informed consent was taken after completely explaining the nature of study.

STUDY DESIGN AND METHODOLOGY:

We conducted a prospective, observational, cross sectional study, which included patients who were planned for revision hip or knee arthroplasty, either for loosening, implant exchange or because of periprosthetic joint infection (PJI). Preoperative patient blood samples were sent for peripheral total white blood-cell count (TLC), the erythrocyte sedimentation rate (E.S.R), serum C-reactive protein levels (C.R.P), serum interleukin-6 (IL-6). Synovial fluid aspiration was done (under radiological guidance) and sent for analysis (for total leukocyte counts and percentage of polymorph nuclear cells). Peroperatively, frozen section histopathological biopsy of the periprosthetic tissue, periprosthetic tissue cultures (three in number), and fluid aspirate if not sent earlier, were send.

The diagnosis of periprosthetic joint infection was confirmed based on the diagnostic criteria laid by Parvizi J, et al.which stipulates:

- Two positive periprosthetic cultures with phenotypically identical organism,
- Or a sinus tract communicating with the joint,
- Or having 3 of the following minor criteria:
    - Elevated serum CRP & ESR;(ESR>30mm/hr;CRP>10mg/L)
    - Elevated synovial fluid WBC count;(>3000cells/μL)
    - Elevated synovial fluid PMN%; (>80%)
    - Positive histological analysis of periprosthetic tissue; (Histopathological analysis should show atleast five polymorph nuclear leukocytes per high-power field of the periprosthetic tissue specimens)
    - A Single positive culture

We analysed the sensitivity, specificity, positive predictive Value, negative predictive value and accuracy of each marker in aseptic loosening and periprosthetic joint infection and investigated the correlation.

STUDY POPULATION INCLUSION AND EXCLUSION CRITERIA:

INCLUSION CRITERIA:

1. Patient admitted for revision hip or knee arthroplasty, implant exchange, irrespective of the hospital where the previous hip arthroplasty done.(Total Joint Arthroplasty done elsewhere, i.e. outside Christian Medical College Vellore will be included)
2. Adult patient i.e. more than 18 years of age
3. Both male and female patients will be included

EXCLUSION CRITERIA:

1. Paediatric age group
2. Critically ill patient i.e. patient admitted in intensive care unit for any reason.
3. Patients not willing for the study
4. Patients on treatment with DMARD (Disease-modifying anti rheumatic drugs).

DATA SOURCES / MEASUREMENT

INTERLEUKIN-6 ASSAY:

SOURCE OF DATA: data regarding serum values interleukin-6 was obtained from the microbiology laboratory of Christian Medical College.

METHOD OF ASSESSMENT:

- Assessment regarding interleukin-6 was done under the expert guidance of a Professor from the department of microbiology, Christian Medical College Vellore.
- The quantitative assessment of IL-6 was done by "QUANTAKINE HS ELISA" kit, manufactured by R&D systems, Minneapolis, MN 55413, USA.

ERYTHROCYTE SEDIMENTATION RATE (E.S.R):

SOURCE OF DATA:

- Data regarding blood values of E.S.R was obtained from the department of Clinical Pathology, Christian Medical College Vellore.

METHOD OF ASSESSMENT:

- Assessment of blood values of E.S.R was done by Westergren method as per the standard Operating Protocol**.**

C-REACTIVE PROTEIN (C.R.P):

SOURCE OF DATA:

- Data regarding blood values of C.R.P was obtained from the Department of microbiology, Christian Medical College Vellore.

METHOD OF ASSESSMENT:

- Assessment of blood values of C.R.P was done by as per the standard Operating Protocol of department of Microbiology, Christian Medical College Vellore.

TOTAL LEUCOCYTES COUNT (T.L.C):

SOURCE OF DATA:

- Data regarding blood values of T.L.C was obtained from the department of Clinical pathology, Christian Medical College Vellore.

METHOD OF ASSESSMENT:

- Assessment of blood values of T.L.C was done by as per the standard Operating Protocol of department of Clinical Pathology, Christian Medical College Vellore.

SAMPLE SIZE:

Based on the literature(1)the sample size was calculated to compare the mean interleukin-6 difference between infected and non-infected group:-

$N= 2[Z_{1-\alpha/2}+Z_{\beta}] \times SD^2 / (MEAN_1 - MEAN_2)$

Where,α=5%; Z=1.96

β=80%; Z= 0.84

MEAN (INFECTED) = 37.4, SD (INFECTED) =37

MEAN (NON INFECTED) = 3.4, S D (NON INFECTED) = 4

N= 12(IN EACH ARM OF INFECTED AND NON INFECTED)

A sample of 24 cases (12 infected and 12 non infected) were needed to detect a difference of 34 units in interleukin-6 level among infected and non-infected group with an error of 5% and power of 80%. Mean and standard deviation of the Interleukin-6, ESR, CRP& Total Leucocyte Count (TLC) was presented. ROC (Receiver Operating Characteristic) curve was evaluated and cut off was estimated by diagnostic accuracy. The levels of these parameters among infected and non-infected group was analysed using Independent T test/Mann Whitney μ test to determine the presence of a significant difference between patients with and without infection. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of each each marker of inflammation was also calculated.

RESULTS

The total number of patients assessed during the above mentioned time period was 28, which included 17 male and 11 female patients (Figure1) the mean age was 51 yrs., ranging from 21 yrs. to 79 yrs. and median age of 51.5 yrs. (Table 1). Patients were included from all the three orthopaedics units of our institution, unit I, II & III with 4, 5 & 19 patients included respectively (Figure 2). The average duration of stay in hospital was 19.59 days, minimum of 8 days and maximum of 81 days with standard deviation of 14.62 and median of 15 days. The duration since the last surgery was a mean of 54.5 months, median of 30 months and maximum of 180 months with a standard deviation of 49.86. Out of 28 patients, 8 patients had previous surgery done in our institution, while rest had undergone previous surgery elsewhere (Figure 3).

Age	21 to 79 yrs
Average age	51.0 yrs
Median age	51.5 yrs
Standard deviation	14.48 yrs
Minimum age	21 yrs
Maximum age	79 yrs

Table 1: Age distribution

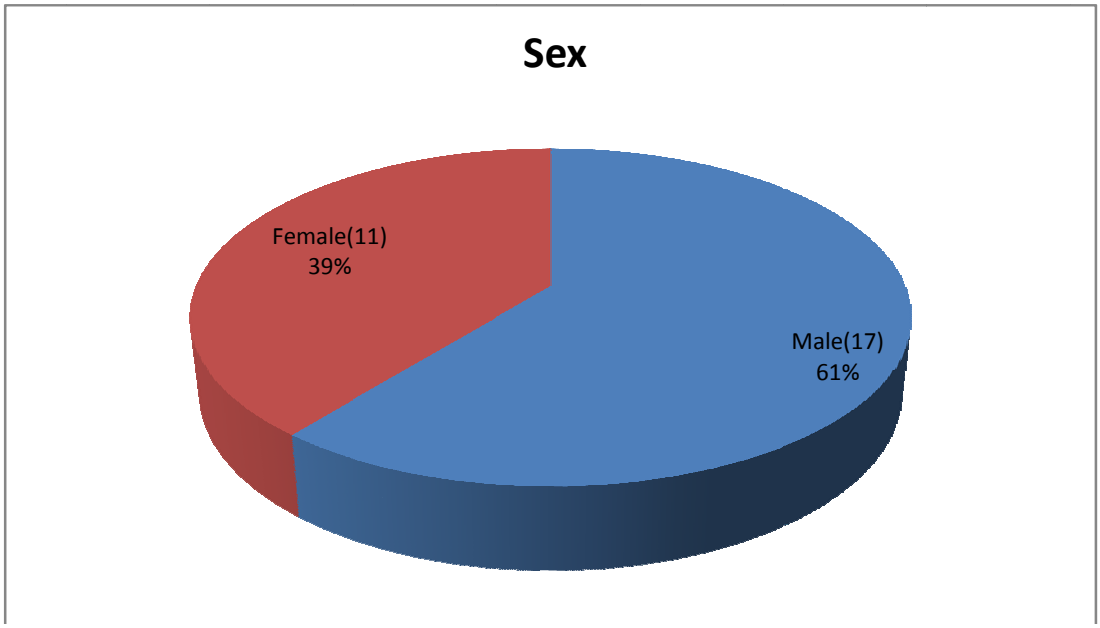

Figure 1: Sex Distribution

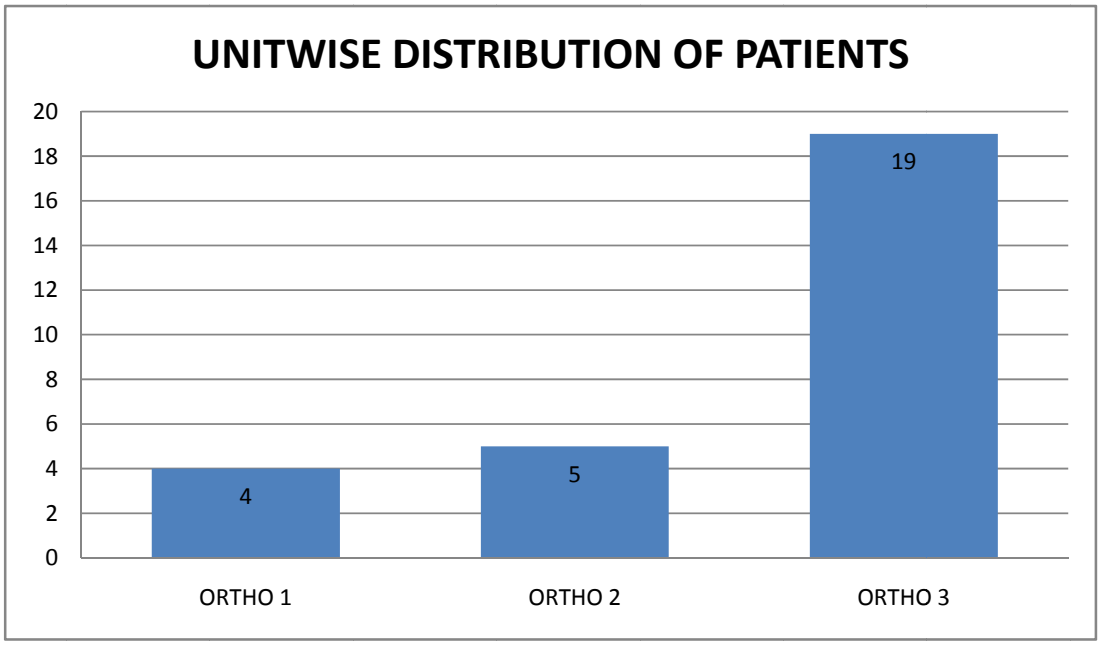

Figure 2: Patient Distribution

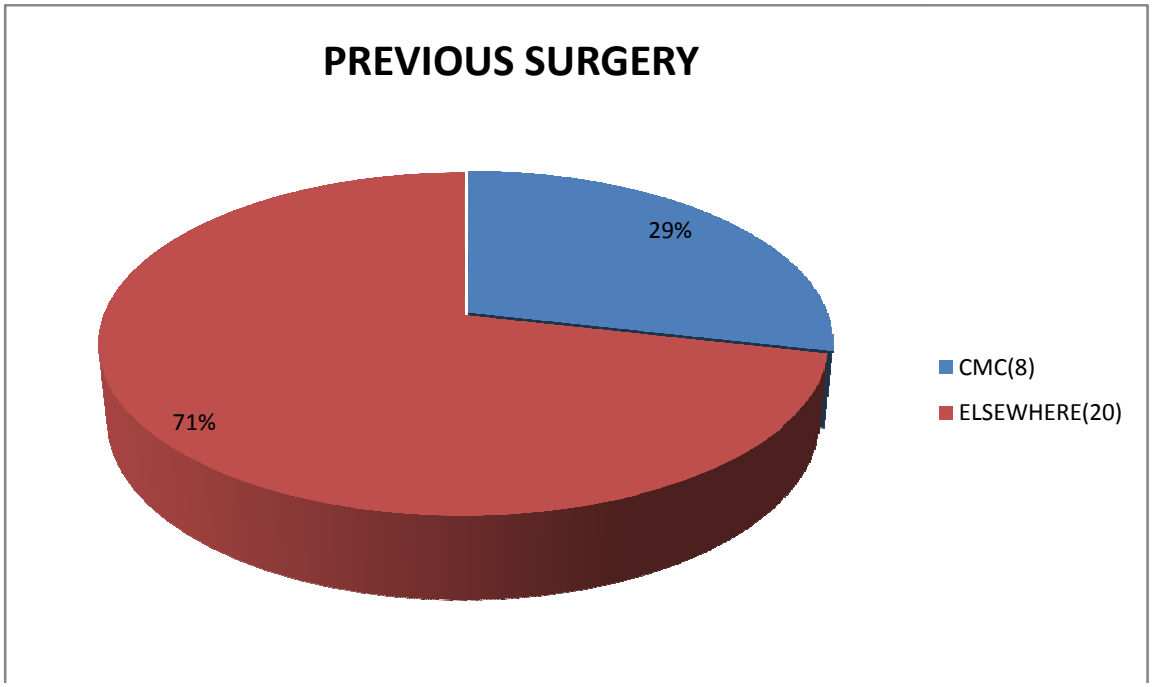

Figure 3: Previous Surgery

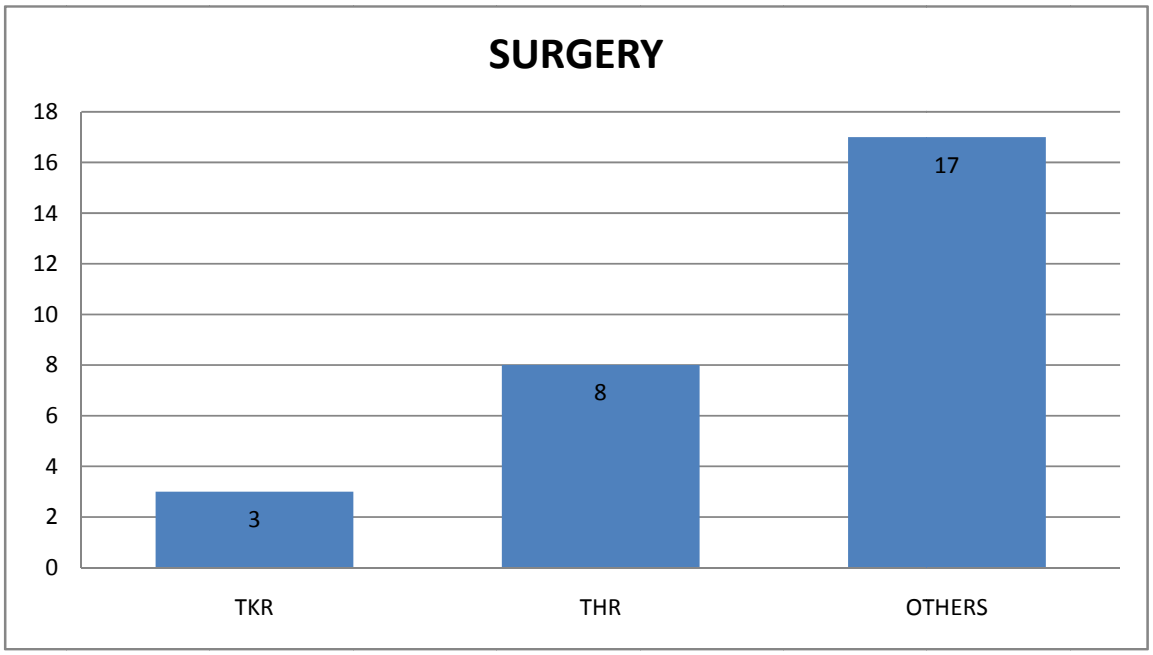

Figure 4: Previous surgery

Patients were analysed on the type of previous surgery which they underwent. Out of 28 patients, 3 patients had primary total knee replacement, 8 had primary total hip replacement while rest were categorised as “others” which were 17 patients (Figure 4). The “others” were, hemiarthroplasty, bipolar hemiarthroplasty, neck of femur screw osteosynthesis for neck of femur fractures, acetabulum fractures post internal fixation and dynamic hip screw for intertrochanteric fractures. The distribution of the previous surgery is illustrated in Figure 5.

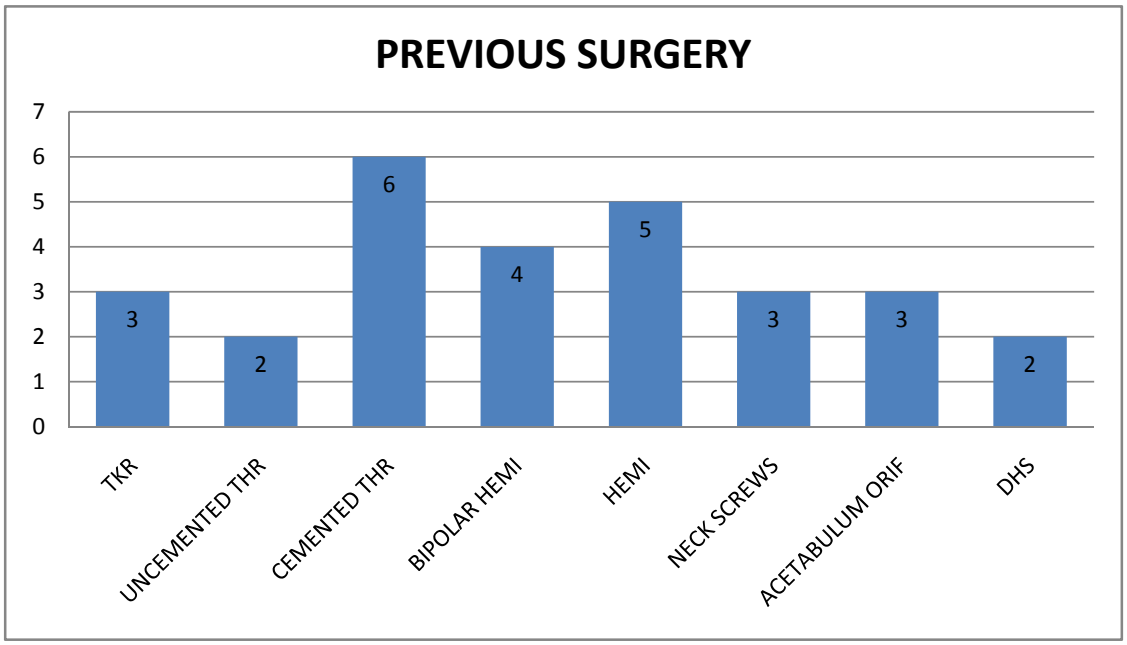

Figure 5: Distribution according to Previous Surgery

The final result: 8 out of 28 patient were infected and were referred to as PJI (Periprosthetic Joint Infection) while 20 patients were found to have aseptic loosening, as per the guideline byParvizi J, et.al (41). (Figure: 8). The intraoperative cultures were positive for Coagulase negative staphylococcus for three patients, E.coli for three patients, MRSA (methicillin-resistant Staphylococcus aureus) for one patient, Pseudomonas aeruginosa for one patient, Klebsiellaspp for one patient, Staphylococcus aureus for one patient, Proteus vulgaris for one patient and Acinetobacterbaumanii for one patient (Table: 2 & 3).The most common organisms grown in the periprosthetic culture were Coagulase negative staphylococcus and E.coli. Analysis of the major and minor criteria showed that two patients out of 28 had discharging sinuses (Figure: 7) and three patients had major criteria present while eight patients had minor criteria positive (Figure: 6).

ORGANISM	FREQUENCY
Coagulase negative staphylococcus	3
E.coli	3
MRSA	1
Pseudomonas aeruginosa	1
Klebsiellaspp	1
Staphylococcus aureus	1
Proteus vulgaris	1
Acinetobacterbaumanii	1

Table 2 : Culture report

HOS NO.	ORGANISM 1	ORGANISM 2	ORGANISM 3	ORGANISM 4
043098g	Coagulase negative staphylococcus (doubtful)	acinetobacterbaumanii	nil	nil
884297f	nil	nil	nil	nil
955665f	MRSA	E. coli	nil	nil
203426g	Pseudomonas aeruginosa(Sc)	E.coli (Sc)	Proteus vulgaris (Sc)	klebsiellasp (Sc)
190866g	nil	nil	nil	nil
213543g	nil	nil	nil	nil
126167g	nil	nil	nil	nil
191744g	E. coli	nil	nil	nil
146556d	nil	nil	nil	nil
808653f	nil	nil	nil	nil
018576g	nil	nil	nil	nil
110056g	nil	nil	nil	nil
350008a	Staphylococcus aureus	nil	nil	nil
768562d	Coagulase Negative staphylococcus	nil	nil	nil
061726g	nil	nil	nil	nil
150351g	nil	nil	nil	nil
077575g	nil	nil	nil	nil
824070b	nil	nil	nil	nil
377023f	nil	nil	nil	nil
920695c	nil	nil	nil	nil
054113g	Coagulase Negative staphylococcus	nil	nil	nil
032981g	nil	nil	nil	nil
463422b	nil	nil	nil	nil
047746g	nil	nil	nil	nil
842263c	nil	nil	nil	nil
028135g	nil	nil	nil	nil
879424f	nil	nil	nil	nil
865918f	nil	nil	nil	nil

Table 3: Detailed Culture report

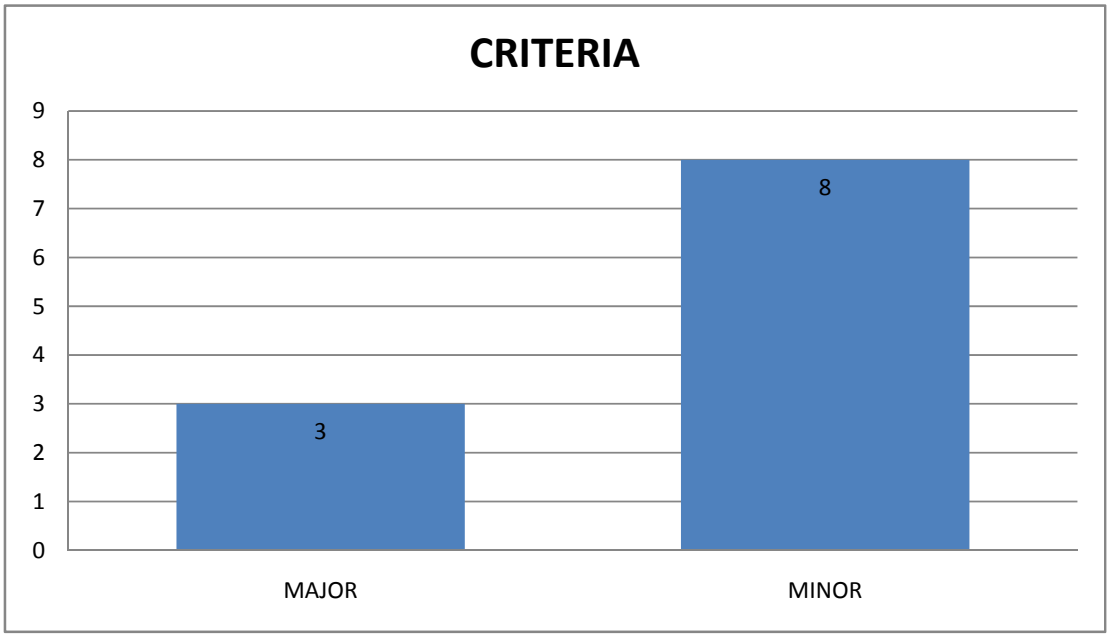

Figure 6 :Distribution of major and minor criteria

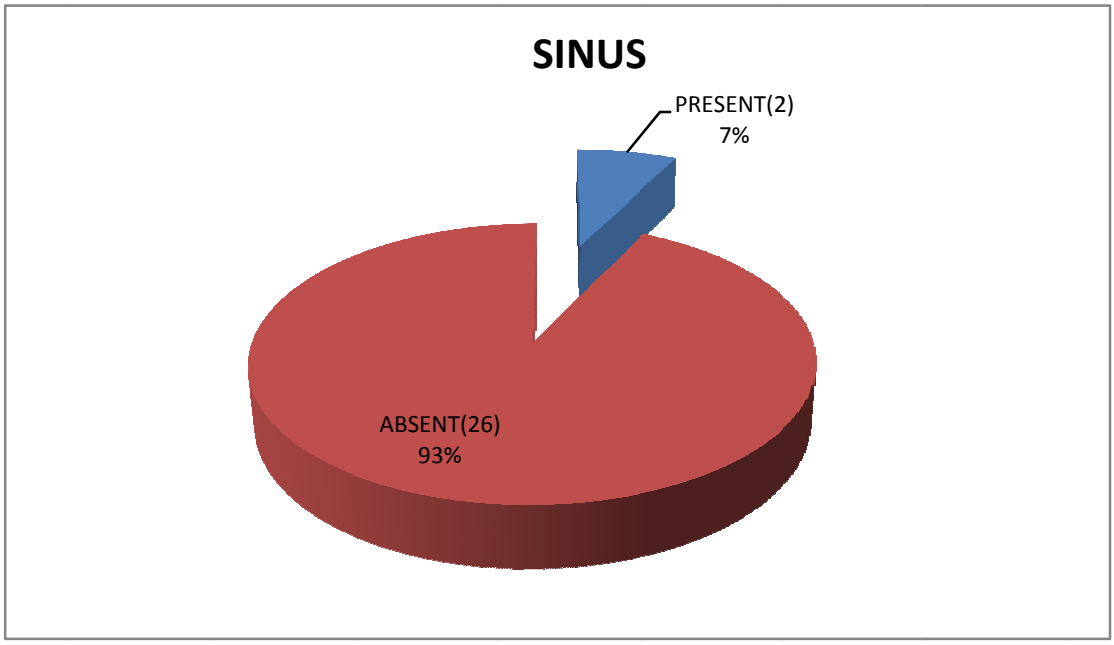

Figure 7:Sinus distribution

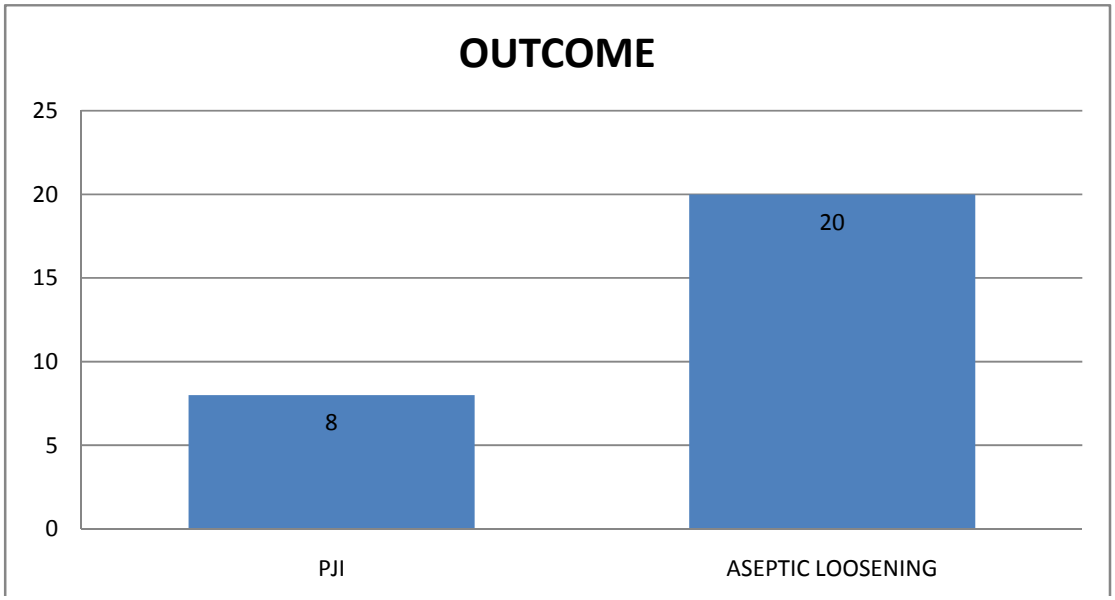

Figure 8: Final Outcome (PJI=Periprosthetic Joint Infection)

RADIOGRAPHIC RESULTS

The evaluation of patients with loose or painful prosthetic joint was followedup with radiographic studies, after a physical examination. There were very few and nonspecific changes which suggested infection on a plain radiograph. These included foci of osteolysis, periosteal reaction and bone resoprtion in the absence of wear by implant. In this study, however, majority of patients did not have any obvious radiographic findings suggestive of infection or showed features which were indistinguishable from aseptic loosening. The assessment of aseptic loosening was based on specific zones around acetabular and femoral components in which changes developed. The femoral component and associated interfaces were divided into seven zones ,as described by Gruen et al(74),while the acetabular components were divided into three zones, as described by DeLee and Charnley(75). The cementless femoral and acetabular components were classified as bone ingrowth, stable fibrous fixation or unstable as described by Engh et al(76).

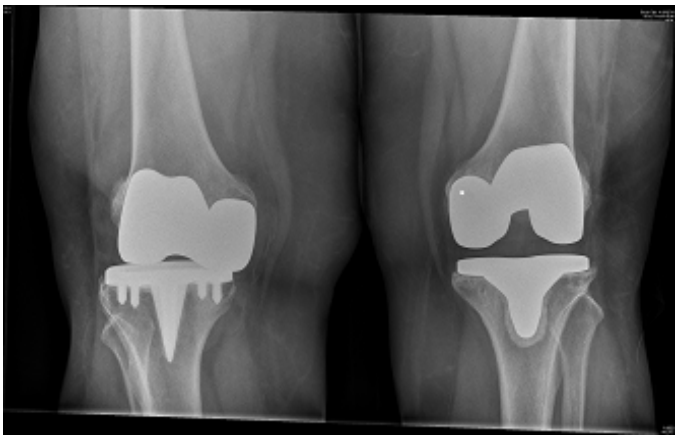

Figure 9(a): right knee aseptic loosening with implant failure of TKR components

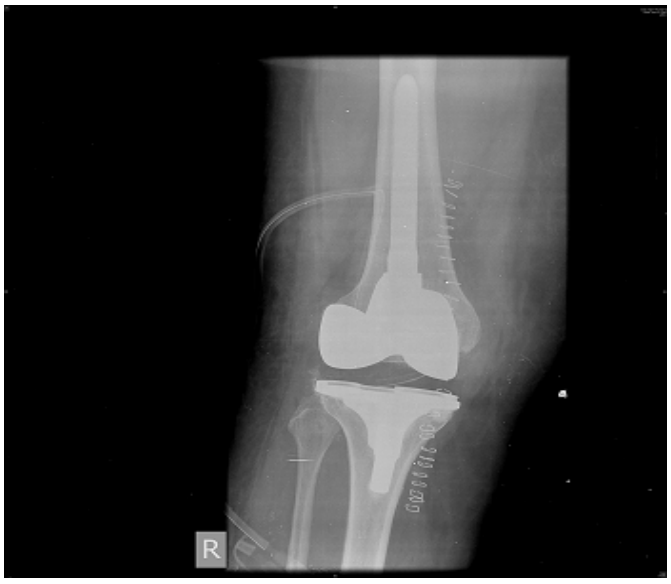

Figure9 (b): patient underwent revision total knee arthroplasty

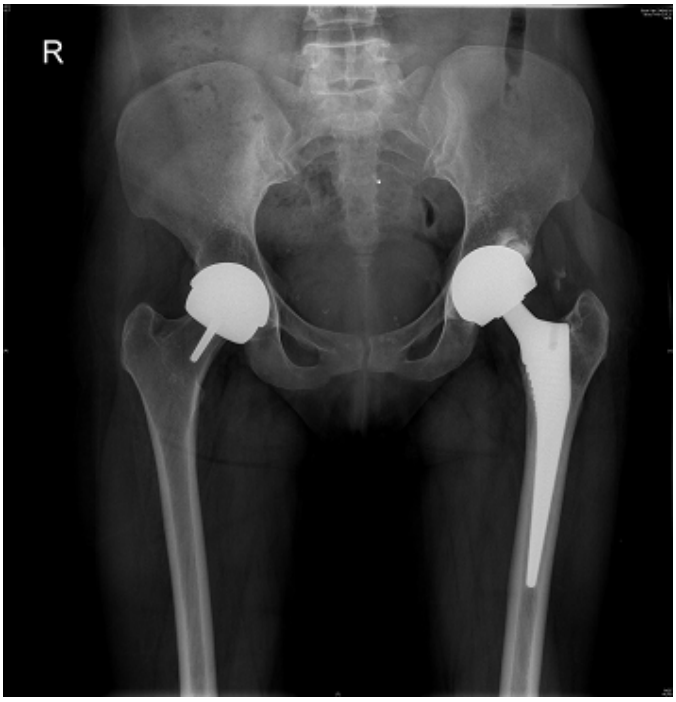

Figure 10(a): Middle aged patient with left hip aseptic loosening and status right ASR implant in situ

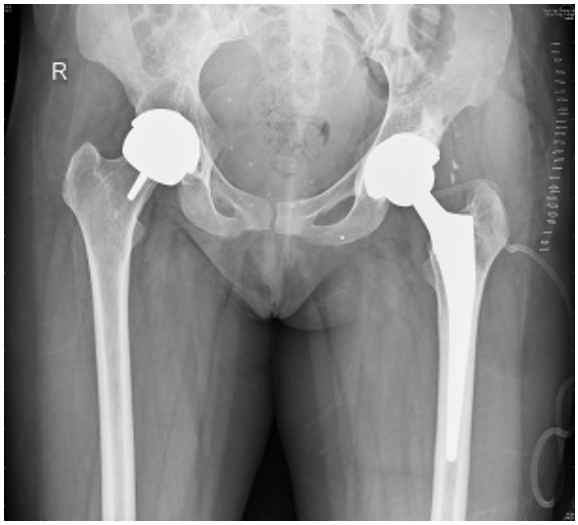

Figure 10(b): patient underwent left revision Total Hip Replacement

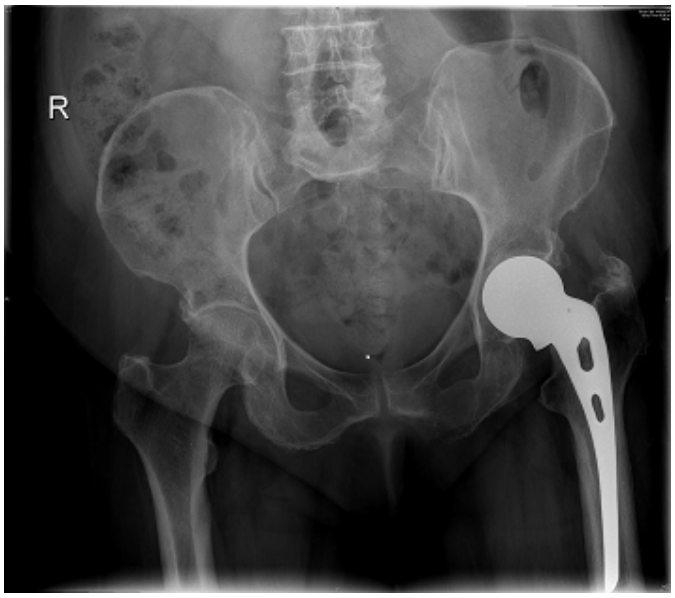

Figure 11(a): patient with aseptic loosening of left hemiarthroplasty component

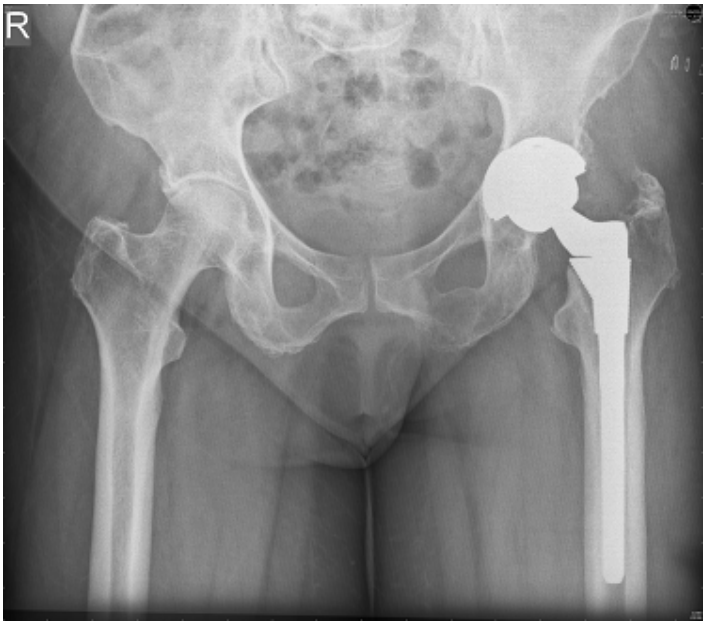

Figure 11(b): underwent left hip revision arthroplasty with SROM hip prosthesis

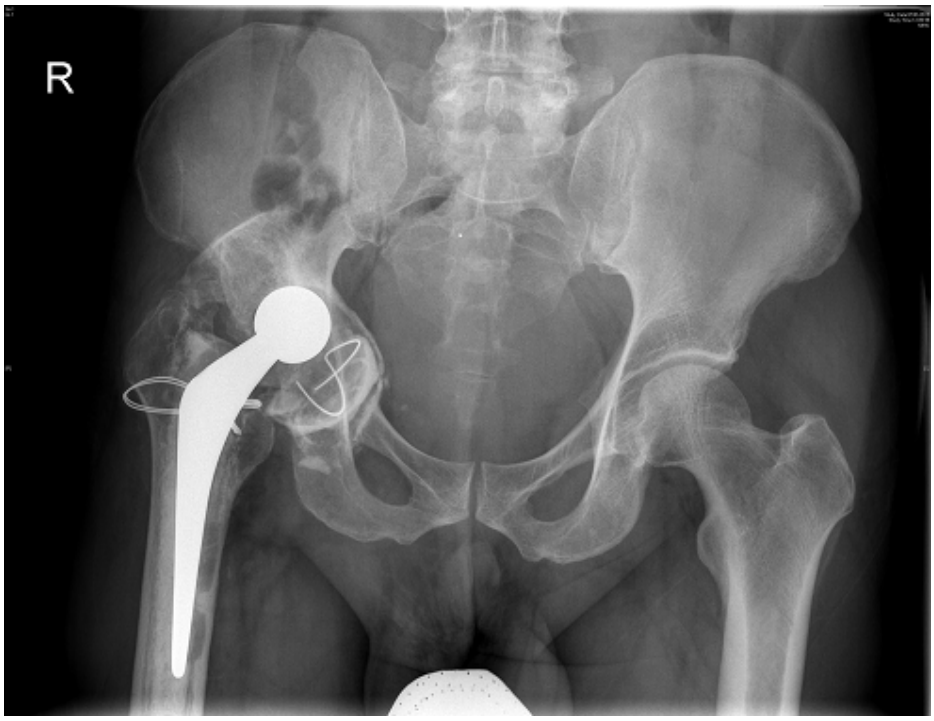

Figure 12(a): right cemented THR with aseptic loosening

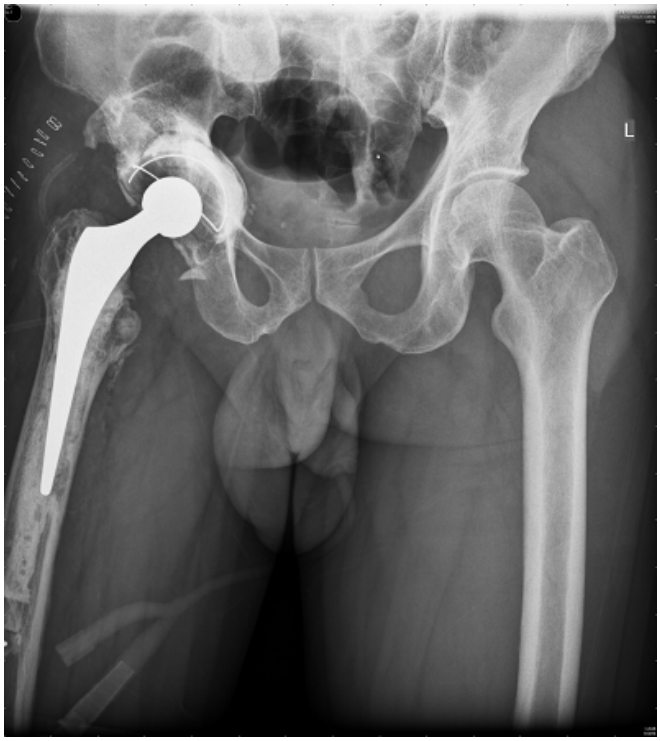

Figure 12(b): patient underwent cemented revision Total hip arthroplasty

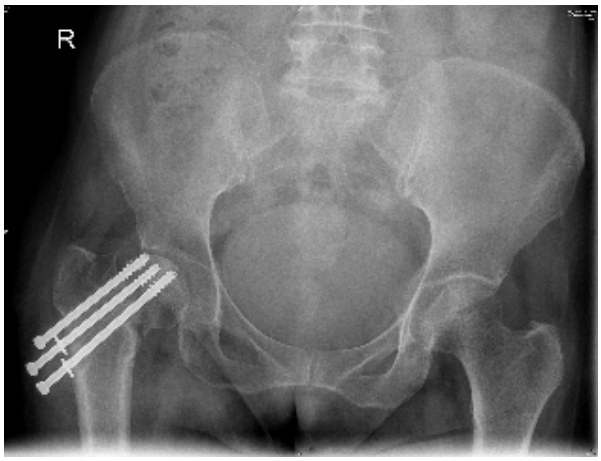

Figure 13(a): middle aged patient with right neckof femur fracture non-union with multiple screws in situ, (b) underwent right hip implant exit and revision to total hip arthroplasty

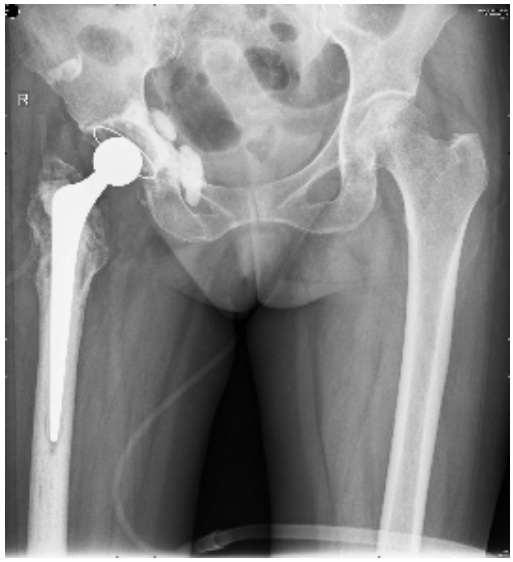

Figure 13(b)

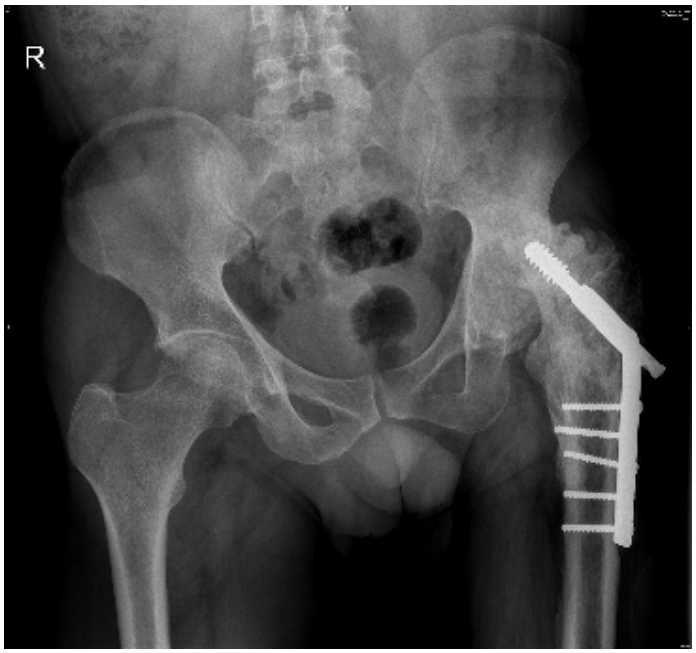

Figure 14(a): left hip chronic arthritis and Avascular Necrosis of femoral head with Dynamic Hip Screw implant in situ

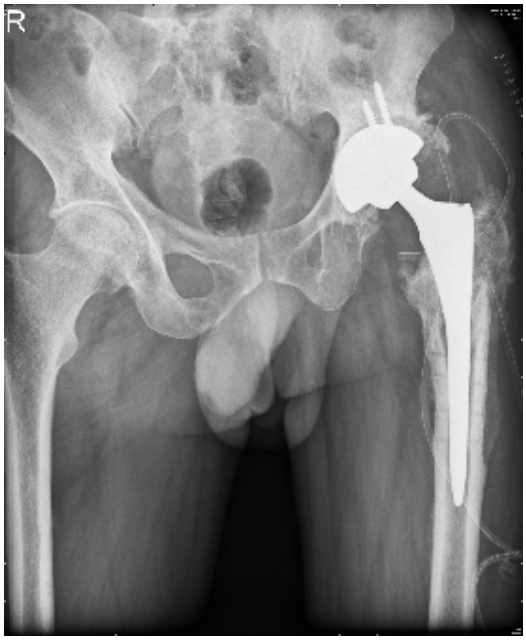

Figure 14(b): patient underwent left hip DHS implant exit and revision to Total Hip Arthroplasty

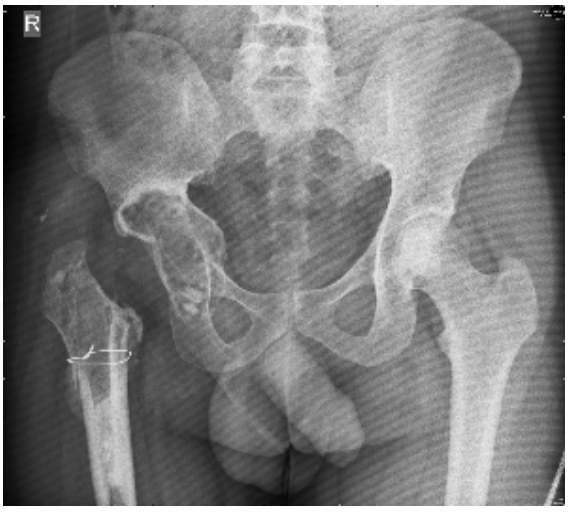

Figure 15(a): status right hip excision arthroplasty (b) underwent right hip revision to total hip arthroplasty

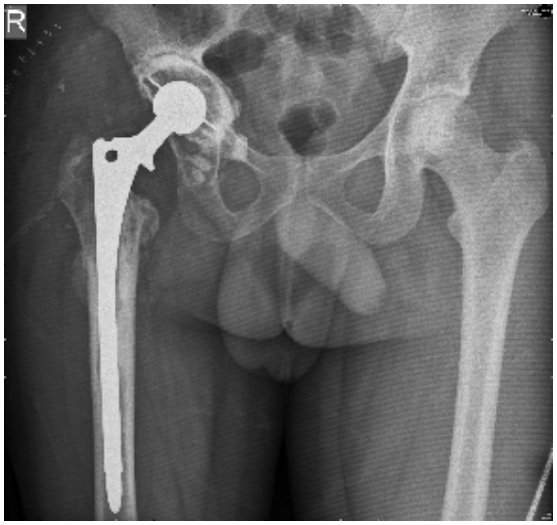

Figure 15(b)

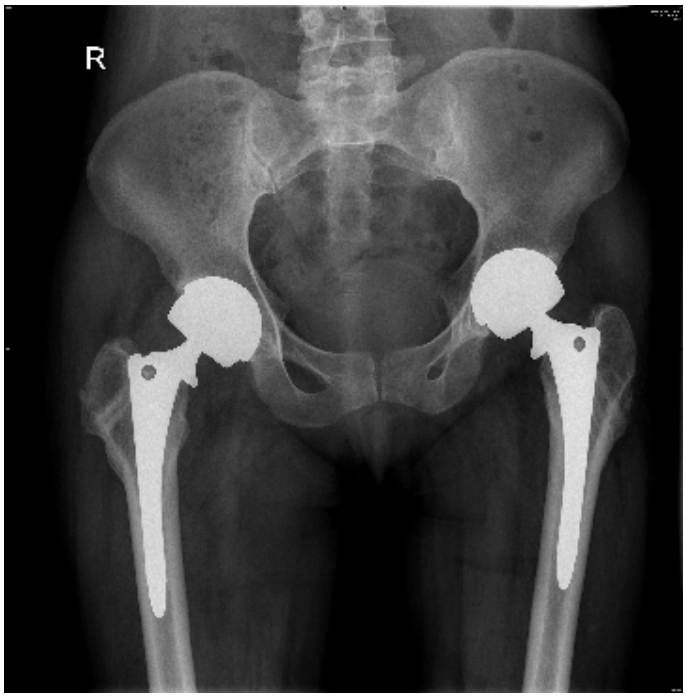

Figure 16(a): status bilateral total hip arthroplasty with right periprosthetic joint infection

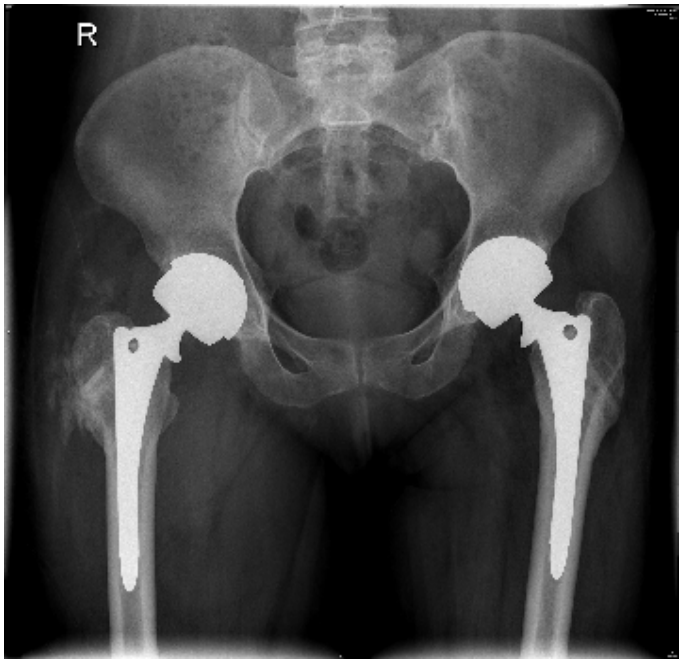

Figure 16(b): underwent right hip debridement and washout

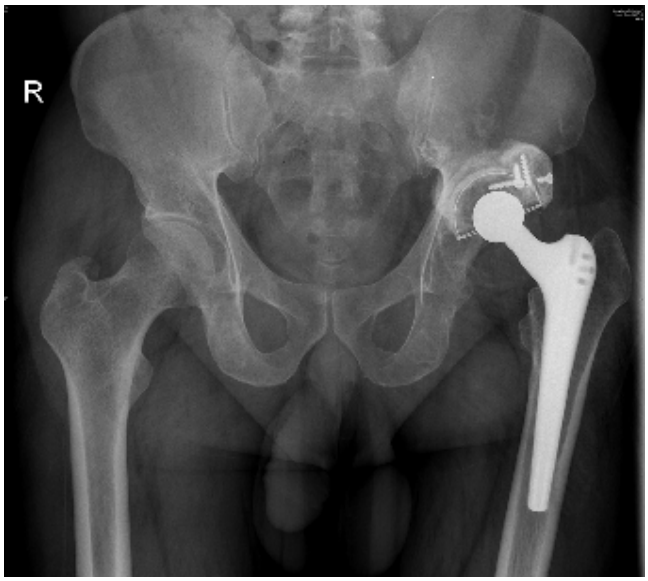

Figure 17(a): left total hip replacement with cortical iliac crest graft with aseptic loosening of acetabular components

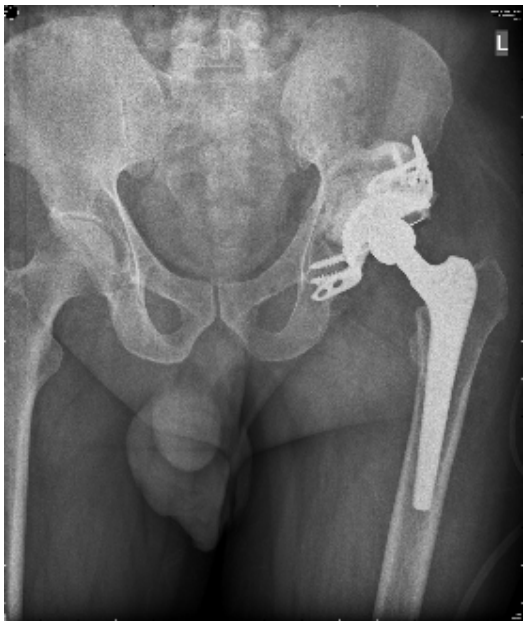

Figure 17(b): patient underwent left hip revision arthoplasty – with placement of acetabular contour cage

HISTOPATHOLOGICAL EXAMINATION (HPE) ANALYSIS

The values of neutrophils counts per high power field, on histopathological examination, between infected and non-infected cases were analysed. Based on the contingency table (table: 4) the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated (Table: 4). Fisher's exact test was used to determine direct statistical comparison between the tests. Our study uses the cut-off value more than five neutrophil counts per high power field, on histopathological examination, of the periprosthetictissue. The analysis demonstrated that histopathological examination of periprosthetictissue had Sensitivity of 75%, specificity of 100%, positive predictive value of 100%,negative predictive value of 90.9% and Accuracy of 92.85%.

STATUS	**Positive (HPE)**	**Negative(HPE)**	**TOTAL**
PJI	6	2	8
ASEPTICLOOSENING	0	20	20
TOTAL	6	22	28

Table 4: Contingency table- Histopathologicalanalysis (HPE)

HISTOPATHOLOGICAL EXAMINATION (HPE) AND INTERLEUKIN-6 ANALYSIS

The analysis of neutrophils counts per high power field, on histopathological examination and serum Interleukin-6 Levels ,between infected and non-infected cases , wasdone by the contingency table(table:4), the sensitivity, specificity, positive predictive value(PPV) and negative predictive value(NPV) were calculated (Table:5). Fisher's exact test was used to determine direct statistical comparison between the tests. Our study uses the cut-off value more than five neutrophil counts per high power field, on histopathological examination, of the periprosthetic tissue and Interleukin-6 serumcut-off value which was >5.51pg/mL. The analysis demonstrated that combined histopathological examination (HPE) of periprosthetic tissue and serum IL-6 had Sensitivity of 62.5%, specificity of 100%, positive predictive value of 100%,negative predictive value of 86.95% and Accuracy of 89.28%.

STATUS	**Positive (HPE+IL-6)**	**Negative (HPE+IL-6)**	**TOTAL**
PJI	6	2	8
ASEPTICLOOSENING	0	20	20
TOTAL	6	22	28

Table 5: Contingency table-Histopathologicalanalysis (HPE) and IL-6 combined

TOTAL LEUCOCYTE COUNT ANALYSIS

The serum values of Total Leucocyte Count between infected and non-infected cases were analysed. Based on the contingency table (table: 6) the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated (Table: 7). The ROC (Receiver Operating Characteristic) curves were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results identify the cut-off value for serum total leucocyte count was 10,000 cells/ml. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.5719 (Figure:18) with standard error of 0.1273, with Sensitivity of 50%, specificity of 70%, likelihood ratio of 1.67, positive predictive value of 40% , negative predictive value of 77.8% and Accuracy of 64%.

STATUS	**Positive (TLC)**	**Negative(TLC)**	**TOTAL**
PJI	4	4	8
ASEPTICLOOSENING	6	14	20
TOTAL	10	18	28

Table 6: Contingency table- TLC

TOTAL LEUCOCYTE COUNT

Prevalence		Pr(A)	29%	13%	48.7%
Sensitivity		Pr(+|A)	50%	15.7%	84.3%
Specificity		Pr(-|N)	70%	45.7%	88.1%
ROC area	(Sens. + Spec.)/2		.6	.388	.812
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)		1.67	.636	4.37
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)		.714	.337	1.51
Odds ratio	LR(+)/LR(-)		2.33	.468	11.8
Positive predictive value		Pr(A|+)	40%	12.2%	73.8%
Negative predictive value		Pr(N|-)	77.8%	52.4%	93.6%

Table 7: Analysis result -TLC

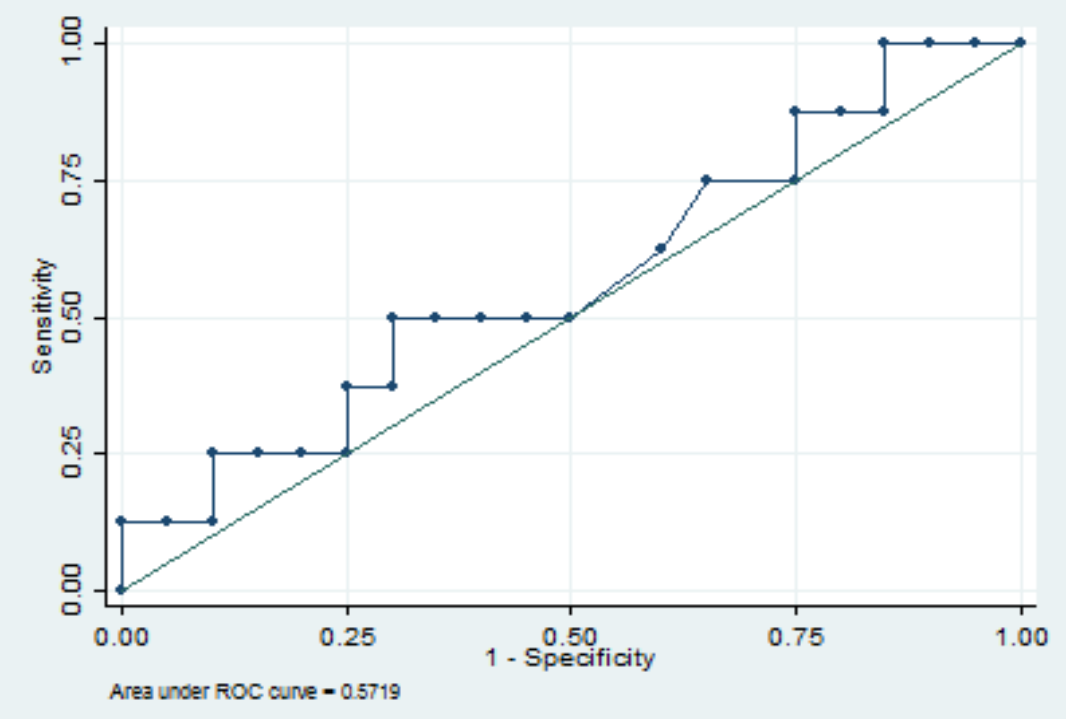

Figure 18: ROC CURVE-TLC

ERYTHROCYTE SEDIMENTATION RATE (ESR) ANALYSIS

The serum values of Erythrocyte Sedimentation Rate (ESR) between infected and non-infected cases were analysed. Based on the contingency table (Table: 8) the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The ROC (Receiver Operating Characteristic) curves were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results were based on the cut-off value for serum ESR which was 30 mm/hr. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.6906 (Figure:19) with standard error of 0.1299, with Sensitivity of 87.5%, specificity of 55%, likelihood ratio of 1.94, positive predictive value of 43.8% negative predictive value of 91.7% and Accuracy of 64% (Table:9).

STATUS	Positive (ESR)	Negative(ESR)	TOTAL
PJI	7	1	8
ASEPTICLOOSENING	9	11	20
TOTAL	16	12	28

Table 6: Contingency table- ESR

ERYTHROCYTE SEDIMENTATION RATE- ESR

Prevalence	Pr(A)	29%	13%	48.7%
Sensitivity	Pr(+|A)	87.5%	47.3%	99.7%
Specificity	Pr(-|N)	55%	31.5%	76.9%
ROC area	(Sens. + Spec.)/2	.713	.547	.878
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	1.94	1.12	3.37
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	.227	.0348	1.48
Odds ratio	LR(+)/LR(-)	8.56	1.09	.
Positive predictive value	Pr(A|+)	43.8%	19.8%	70.1%
Negative predictive value	Pr(N|-)	91.7%	61.5%	99.8%

Table 9: ANALYSIS RESULT-ESR

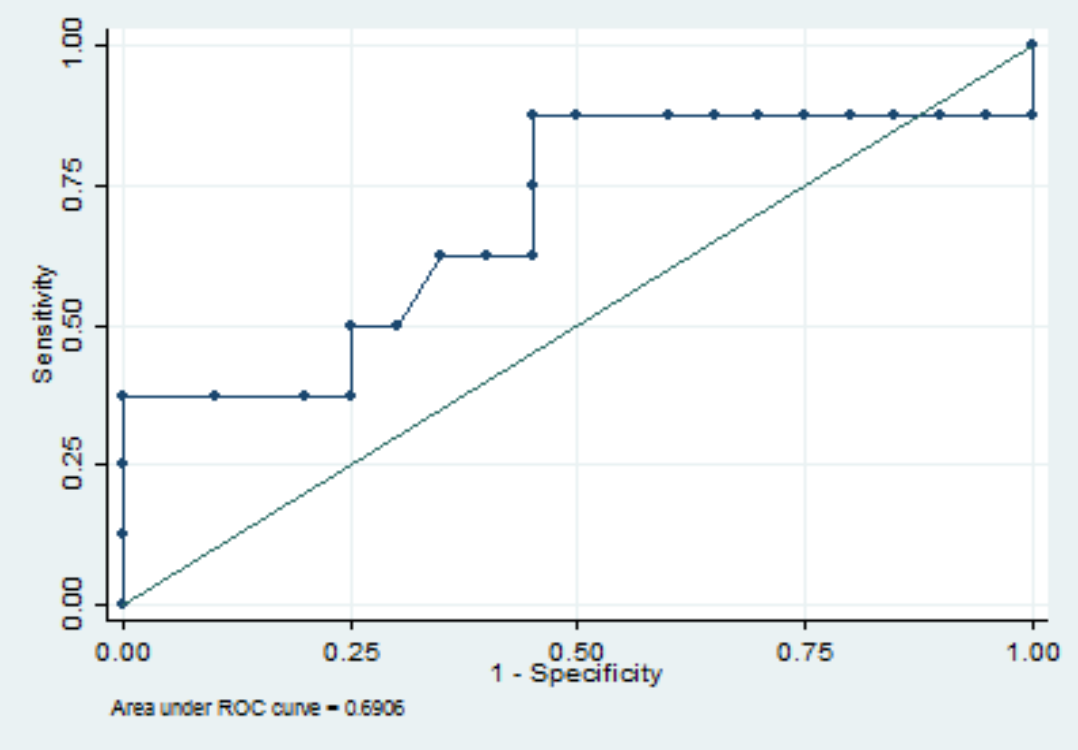

Figure 19: ROC CURVE- ESR

C-REACTIVE PROTEIN (CRP) ANALYSIS

The serum values of C - reactive protein (CRP) between infected and non-infected cases were analysed. Based on the contingency table (Table: 10) the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The ROC (Receiver Operating Characteristic) curves (Figure: 20) were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results were based on the cut-off value for serum CRP which was >10mg/L. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.9875 with standard error of 0.0149, with Sensitivity of 100%, specificity of 95%, likelihood ratio of 20, positive predictive value of 88.9% , negative predictive value of 100% and Accuracy of 96.42% (Table:11).

STATUS	Positive (CRP)	Negative(CRP)	TOTAL
PJI	8	0	8
ASEPTICLOOSENING	1	19	20
TOTAL	9	19	28

Table 10: Contingency table- CRP

C-REACTIVE PROTEIN

Prevalence	Pr(A)	29%	13%	48.7%
Sensitivity	Pr(+|A)	100%	63.1%	100%
Specificity	Pr(-|N)	95%	75.1%	99.9%
ROC area	(Sens. + Spec.)/2	.975	.926	1
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	20	2.96	135
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	0	.	.
Odds ratio	LR(+)/LR(-)	.	19.7	.
Positive predictive value	Pr(A|+)	88.9%	51.8%	99.7%
Negative predictive value	Pr(N|-)	100%	82.4%	100%

Table 11: analysis result-CRP

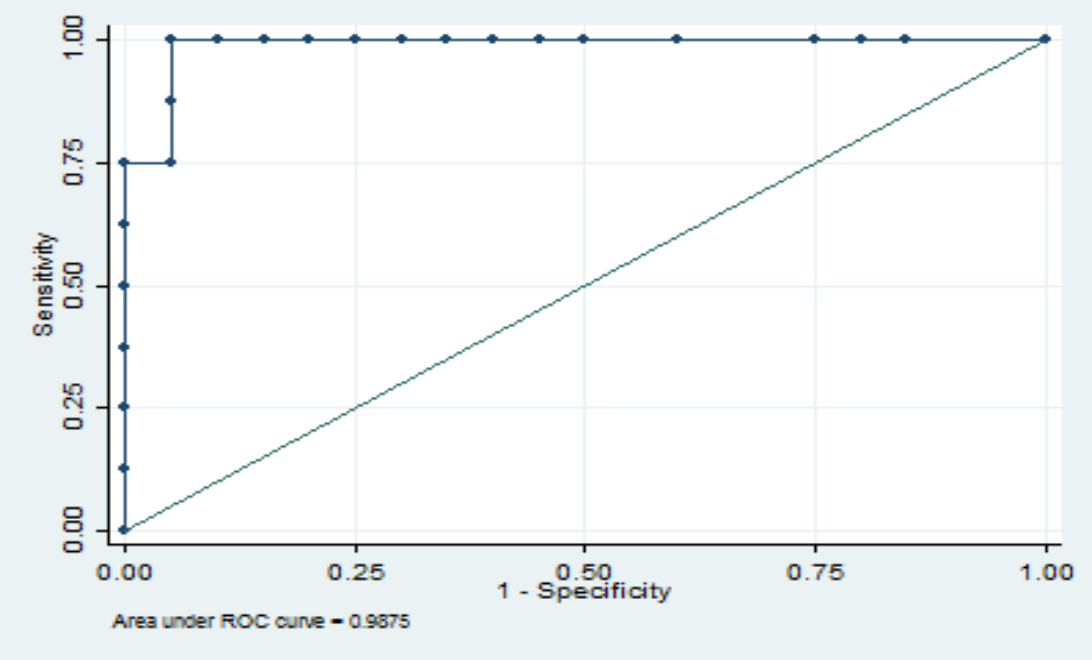

Figure 20: ROC CURVE- CRP

INTERLEUKIN – 6 (IL-6) ANALYSIS

The serum values Interleukin-6 (IL-6) between infected and non-infected cases were analysed. Based on the contingency table (Table: 12) the sensitivity, specificity, positive predictive value (PPV) and negative predictive value were calculated. The ROC (Receiver Operating Characteristic) curves (Figure: 21) were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results were based on the cut-off value for serum IL-6 which was >5.51pg/mL. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.7531 with standard error of 0.1006, with Sensitivity of 75%, specificity of 75%, likelihood ratio of 3, positive predictive value of 54.5% , negative predictive value of 88.2% and Accuracy of 75% (Table:13).

STATUS	Positive (CRP)	Negative(CRP)	TOTAL
PJI	6	2	8
ASEPTICLOOSENING	5	15	20
TOTAL	11	17	28

Table 12: Contingency table- IL-6

INTERLEUKIN-6

Prevalence	Pr(A)	29%	13%	48.7%
Sensitivity	Pr(+|A)	75%	34.9%	96.8%
Specificity	Pr(-|N)	75%	50.9%	91.3%
ROC area	(Sens. + Spec.)/2	.75	.562	.938
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	3	1.27	7.08
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	.333	.0978	1.14
Odds ratio	LR(+)/LR(-)	9	1.48	52.6
Positive predictive value	Pr(A|+)	54.5%	23.4%	83.3%
Negative predictive value	Pr(N|-)	88.2%	63.6%	98.5%

Table 13: Analysis result –IL6

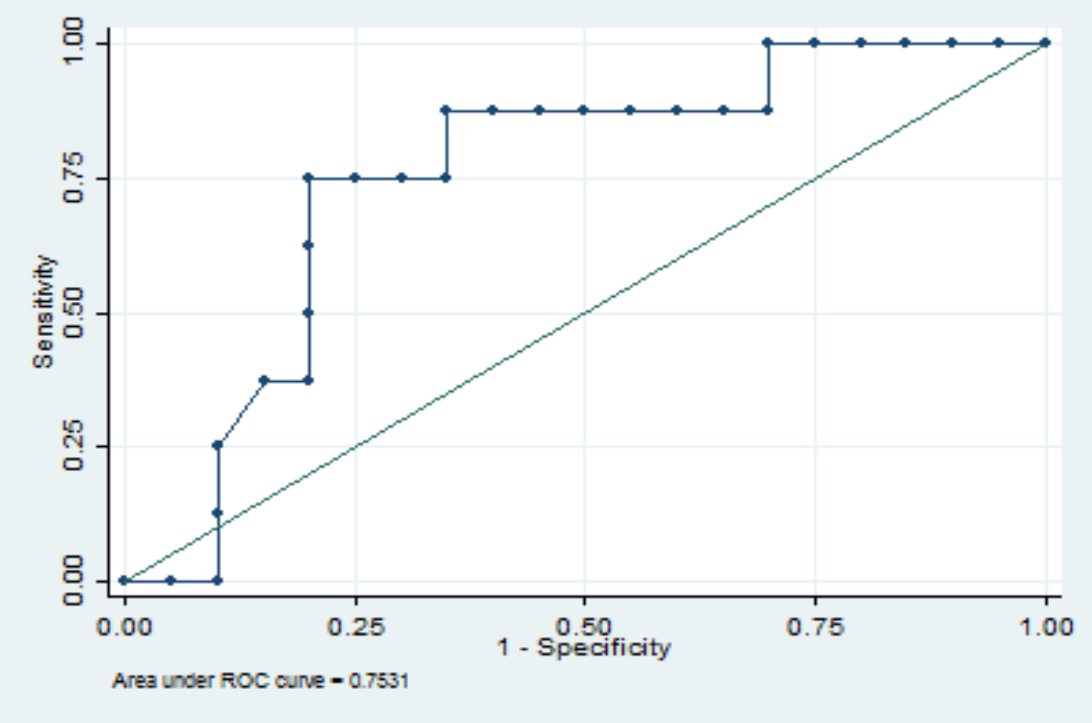

Figure 21: ROC CURVE- IL-6

INTERLEUKIN-6 OR C-RP EITHER ELEVATED

The serum values of Interleukin-6 and C - reactive protein (CRP) between infected and non-infected cases were analysed for either of the two values to be raised or positive. Based on the contingency table (Table 14) sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.The ROC (Receiver Operating Characteristic) curves were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results were based on the cut-off value for serum CRP which was >10mg/L and Interleukin-6 > 5.51 pg/mL. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.85, Sensitivity of 100%, specificity of 70%, likelihood ratio of 3.33, positive predictive value of 57.1% , negative predictive value of 100% and accuracy of 78% (Table:15).

STATUS	Positive (CRP/ IL-6)	Negative (CRP/IL-6)	TOTAL
PJI	8	0	8
ASEPTICLOOSENING	6	14	20
TOTAL	14	14	28

Table 14: Contingency table- IL-6 or C-RP EITHER ELEVATED

INTERLEUKIN-6 OR CRP EITHER ELEVATED

Prevalence	Pr(A)	29%	13%	48.7%
Sensitivity	Pr(+|A)	100%	63.1%	100%
Specificity	Pr(-|N)	70%	45.7%	88.1%
ROC area	(Sens. + Spec.)/2	.85	.747	.953
Likelihood ratio (+)	Pr(+|A)/Pr(+|N)	3.33	1.71	6.51
Likelihood ratio (-)	Pr(-|A)/Pr(-|N)	0	.	.
Odds ratio	LR(+)/LR(-)	.	4.03	.
Positive predictive value	Pr(A|+)	57.1%	28.9%	82.3%
Negative predictive value	Pr(N|-)	100%	76.8%	100%

Table 15: Analysis result: INTERLEUKIN-6 OR CRP EITHER ELEVATED

INTERLEUKIN-6 & C-REACTIVE PROTEIN BOTH ELEVATED

The serum values of Interleukin-6 and C - reactive protein (CRP) between infected and non-infected cases were analysed when both of the two values was raised or positive. Based on the contingency table (Table: 16) sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The ROC (Receiver Operating Characteristic) curves were used to examine the relationship between sensitivity and the false –positive rate (specificity). Fisher's exact test was used to determine direct statistical comparison between the tests. Our results were based on the cut-off value for serum CRP which was >10mg/L and Interleukin-6 > 5.51 pg/mL. The ROC curve analysis demonstrated an Area Under the Curve (AUC) of 0.875, Sensitivity of 75%, specificity of 100%, positive predictive value of 100% , negative predictive value of 90.9% and accuracy of 92.85% (Table:17)

STATUS	Positive (CRP& IL-6)	Negative (CRP& IL-6)	TOTAL
PJI	6	2	8
ASEPTICLOOSENING	0	20	20
TOTAL	6	22	28

Table 16: Contingency table- IL-6 & C-RP BOTH POSITIVE/RAISED

BOTH INTERLEUKIN-6 & CRP ELEVATED

Prevalence	Pr(A)	29%	13%	48.7%
Sensitivity	Pr(+|A)	75%	34.9%	96.8%
Specificity	Pr(-|N)	100%	83.2%	100%
ROC area	(Sens. + Spec.)/2	.875	.715	1
Likelihood ratio(+)	Pr(+|A)/Pr(+|N)	.	.	.
Likelihood ratio(-)	Pr(-|A)/Pr(-|N)	.25	.0753	.83
Odds ratio	LR(+)/LR(-)	.	10.2	.
Positive predictive value	Pr(A|+)	100%	54.1%	100%
Negative predictive value	Pr(N|-)	90.9%	70.8%	98.9%

Table 17: Analysisresult BOTH IL-6 & CRP ELEVATED

FINAL ANALYSIS

	CUT OFF	***SENSITIVITY***	***SPECIFICITY***	***PPV***	***NPV***	***AUC***	***ACCURACY***
IL-6	5.51 pg/mL	75%	75%	54.5%	88.2%	0.7531	75%
CRP	>10 mg/mL	100%	95%	88.9%	100%	0.9875	96.42%
ESR	>30 mm/hr	87.5%	55%	43.8%	91.7%	0.6906	64%
TLC	>10000 cells/ml	50%	70%	40%	77.8%	0.5719	64%
CRP/IL-6 EITHER ELEVATED	>10g/mL or pg/mL	100%	70%	57.1%	100%	0.85	78%
CRP & IL-6 BOTH ELEVATED	>10g/mL or pg/mL	75%	100%	100%	90.9%	0.875	92.85%

Table 18: Result: Analysis of the inflammatory markers for the diagnosis of PJI

DISCUSSION

This prospective study was designed to analyse the correlation of inflammatory markers of periprosthetic joint infection in patients undergoing revision arthroplasty. The correlation of TLC(Total Leucocyte Count), ESR(Erythrocyte Sedimentation Rate), CRP (C Reactive Protein) and a new marker Interleukin-6(IL-6) in the infected and non-infected cases were analysed. The final analysis revealed CRP was the most accurate marker of deep infection in revision arthroplasty(Periprosthetic Joint Infection) with Sensitivity of 100%, specificity of 95%, likelihood ratio of 20, positive predictive value of 88.9%,negative predictive value of 100% and Accuracy of 96.42%. Interleukin-6(IL-6) as a new marker was found to be less accurate than CRP, with sensitivity of 75%, specificity of 75%, likelihood ratio of 3, positive predictive value of 54.5% , negative predictive value of 88.2% and Accuracy of 75%. Analysis of ESR as a marker revealed a less accurate value as compared to CRP and IL-6,with Sensitivity of 87.5%, specificity of 55%, likelihood ratio of 1.94, positive predictive value of 43.8% negative predictive value of 91.7% and Accuracy of 64%.Finally the least accurate marker was TLC, with sensitivity of 50%, specificity of 70%, likelihood ratio of 1.67, positive predictive value of 40%, negative predictive value of 77.8% and Accuracy of 64%. Further analysis revealed that combination of both CRP & IL-6 was more useful in identifying patients with deep periprostheticjoint infection, with Sensitivity of 75%, specificity of 100%,

positive predictive value of 100%,negative predictive value of 90.9% and accuracy of 92.85%.

The periprosthetic tissue culture and sensitivity results showed that most patients in the infected group had infection with a low virulence specie of bacteria. The intraoperative cultures were positive for Coagulase negative staphylococcus(CoNS) for three patients, E.coli for three patients, MRSA(methicillin-resistant Staphylococcus aureus) for one patient, Pseudomonas aeruginosa for one patient, Klebsiellaspp for one patient, Staphylococcus aureus for one patient, Proteus vulgaris for one patient and Acinetobacterbaumanii for one patient (Table: 2 & 3).The most common organism grown in the periprosthetic culture was Coagulase negative staphylococcus and E.coli. The occurance of low virulence organism was related to long duration since the implantation of prostheses which was a mean of 54.5 months.

This study also revealed that neutrophils infiltration in periprosthtic tissue at a cut off value of more than five cells/HPF was highly indicative of infection, even when cultures of periprosthetic tissue was negative. The analysis demonstrated that histopathological examination of periprosthetictissue had Sensitivity of 75%, specificity of 100%, positive predictive value of 100%,negative predictive value of 90.9% and Accuracy of 92.85%. We concluded that HPE analysis of PMN infiltration in periprosthetic tissue is one of the most accurate methods to distinguish between aseptic and septic loosing of arthoplasty prostheses. Further analysis revealedcombined histopathological examination (HPE) of periprosthetic tissue and serum IL-6 had Sensitivity of 62.5%,

specificity of 100%, positive predictive value of 100%,negative predictive value of 86.95% and Accuracy of 89.28%.

The sensitivity and specificity for the serum total leucocyte count (TLC) and ESR was low. In this study we confirmed that both ESR and TLC are not useful in the diagnosis periprosthetic joint infection due to low diagnostic value (both having accuracy of 64%).

This study revealed CRP to be the most accurate, sensitive and specific marker with Sensitivity of 100%, specificity of 95%, likelihood ratio of 20, positive predictive value of 88.9% , negative predictive value of 100% and Accuracy of 96.42%. Both CRP and IL-6 are excellent screening markers to rule out deep infection of implant. Patients with either increased CRP or increased IL-6 levels identifies all patients with deep implant infection (sensitivity of 100%) or if both of CRP and IL-6 are elevated there is 100% specificity with 92.85% accuracy.

	CUT OFF	***SENSITIVITY***	***SPECIFICITY***	***PPV***	***NPV***	***AUC***	***ACCURACY***
IL-6	5.51 pg/mL	75%	75%	54.5%	88.2%	0.7531	75%
CRP	>10 mg/mL	100%	95%	88.9%	100%	0.9875	96.42%
ESR	>30 mm/hr	87.5%	55%	43.8%	91.7%	0.6906	64%
TLC	>10000 cells/ml	50%	70%	40%	77.8%	0.5719	64%
CRP/IL-6 EITHER ELEVATED	>10g/mL or pg/mL	100%	70%	57.1%	100%	0.85	78%
CRP & IL-6 BOTH ELEVATED	>10g/mL or pg/mL	75%	100%	100%	90.9%	0.875	92.85%

Table 18

CONCLUSION

This study clearly demonstrates the correlation between the inflammatory markers for the diagnosis of periprosthetic joint infection in patients undergoing revision arthroplasty. The important conclusions of our study are:

1. CRP was the most accurate marker of deep infection in revision arthroplasty (Periprosthetic Joint Infection) with Sensitivity of 100%, specificity of 95%, likelihood ratio of 20, positive predictive value of 88.9%, negative predictive value of 100% and Accuracy of 96.42%.
2. Interleukin-6(IL-6) as a new marker was found to be less accurate than CRP, with sensitivity of 75%, specificity of 75%, likelihood ratio of 3, positive predictive value of 54.5% , negative predictive value of 88.2% and Accuracy of 75%.
3. Combination of both CRP & IL-6 can be more useful in identifying patients with deep periprostheticjoint infection, with Sensitivity of 75%, specificity of 100%, positive predictive value of 100%,negative predictive value of 90.9% and accuracy of 92.85%.
4. Neutrophils infiltration in periprosthtic tissue at a cut off value of more than five cells/HPF was highly indicative of infection, even when cultures of periprosthetic tissue was negative.
5. Both ESR and TLC are not useful in the diagnosis periprosthetic joint infection due to low diagnostic value (both having accuracy of 64%).

LIMITATIONS OF THE STUDY

1. Small Sample Size:

   Since the sample size was small in this study, including cases of Periprosthetic joint infection, a bigger sample size would had given a better and clearer picture.

2. Synovial Fluid sample inadequate or unable to collect intraoperatively:

   In few cases the joint aspirate, either ultrasound guided or intraoperatively, yielded no joint fluid for analysis.

3. IL-6 assay is affected by diseases with acute inflammatory reaction:

   It is known that chronic inflammatory conditions do alter the IL-6 levels, it might be the reason for few false positive results related to elevation of IL-6 levels in non-infected patients, even though we had excluded patients on DMARD (Disease-modifying antirheumaticdrugs)

4. IL-6 assay is not readily available

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ANNEXURE

1. Information sheet in English
2. Information sheet translated to Tamil
3. Information sheet translated to Hindi
4. Proforma for data collection
5. Consent form
6. Excel data sheet
7. Institutional Review Board Acceptance Letter

ANNEXURE-I

Correlation of inflammatory markers of periprosthetic joint infection in revision arthroplasty.

INFORMATION SHEET

You are requested to participate in a study to see the relationship between various blood tests which are done to find out if there is infection around the artificial hip/knee joint. We hope to include about 25 patients from this hospital in this study.

We intend to explain to you about this study participation in a question answer format.

What is this study all about?

The diagnosis of infection around an artificial hip or knee joint is very challenging to the surgeon. There are blood tests to detect this infection, which include ESR, CRP, blood Total and differential counts, but these tests are not very accurate. In this study we will be looking at a new test called INTERLEUKIN-6(IL-6) and will find out about its accuracy as compared to the other mentioned tests.

***W*hat is the procedure if we participate?**

If you agree to participate in this study, there are different investigations which will be send:

a) Blood tests which will be send before surgery:

   i. ESR

   ii. CRP

   iii. TOTAL LEUCOCYTE COUNT

   iv. DIFFERENTIAL LEUCOCYTE COUNT

   v. INTERLEUKIN -6

b) Investigations which will be done during the surgery:

   I. CULTURE & SENSTIVITY OF TISSUE AROUND THE ARTIFICIAL JOINT X 3(3 SAMPLES WILL BE TAKEN)

   II. SYNOVIAL FLUID COUNT(FLUID IN THE JOINT SPACE WILL BE SEND FOR TOTAL COUNTS AND PERCENTAGE OF NEUTROPHILS)

   III. HISTOPATHOLOGIC FROZEN SECTION OF TISSUE AROUND THE ARTIFICIAL JOINT TO LOOK FOR NUMBER OF NEUTROPHILS PER HIGH POWER FIELD(MORE THAN 5 NEUTROPHILS IS INDICATIVE OF ARTIFICIAL JOINT INFECTION)

Once the tests are send, we will find out from the investigation which are send during the surgery if infection is present or not and we will correlate the blood tests results with the presence or absence of infection. Finally we will calculate the accuracy of the blood tests for infection, and will find which blood test is more accurate for diagnosis of infection.

Can you withdraw from this study?

Your participation in this study is entirely voluntary and you are free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What will happen if you develop any study related injury ?

We do not expect any injury during the study, but if there is any complication or problem due to study, these will be treated at this hospital.

What is the additional expenses occurring from this study?

There is no cost difference because of the investigations send during the study.

Will your personal details be kept confidential?

The result of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of result. However, the medical notes may be rereviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, please ask Dr. Elvis Benjamin (tel:9677341941), email elvisbenjamin@cmcvellore.ac.in

ANNEXURE-II

தகவல் அறிக்கை

அன்புடையீர், செயற்கை மூட்டு மாற்று அறுவை சிகிச்சையில் ஏற்படும் கிருமி தொற்று கண்டறிய பயன்படுத்தப்படும், பலவகை இரத்தப் பரிசோதனை முறைகளைப் பற்றி நடத்தப்படும் இந்த ஆய்வில். உங்களை பங்கேற்க அழைக்கிறோம். இந்த ஆய்வில் இந்த மருத்துவமனையில் இருந்து 25 நோயாளிகளை இணைக்க உள்ளோம். இதற்கான தேர்வு முறை விளக்கத்தை காண்போம்.

1. இந்த ஆய்வு எதைப்பற்றியது?

செயற்கை மூட்டு மாற்று அறுவை சிகிச்சையில் ஏற்படும் கிருமி தொற்றை கண்டறிவது மருத்துவருக்கு ஒரு சவாலான காரியம். இதை கண்டறிய இ. எஸ். ஆர், சி. ஆர். பி, வெள்ளை அணுக்களின் மொத்த மற்றும் தனித்தனி எண்ணிக்கை போன்ற இரத்த பரிசோதனைகள் உதவும், எனினும் இந்த பரிசோதனை முறைகள் துல்லியம் வாய்ந்தது அல்ல. இந்த ஆய்வில் நாங்கள் இண்டர்லுகின்-6 எனப்படும். இரத்த பரிசோதனையின் துல்லியத்தை மற்ற மேற்கண்ட பரிசோதனைகளோடு ஒப்பிட்டு கண்டறிய உள்ளோம்.

2. இந்த ஆய்வில் பங்கேற்றால் என்ன செய்யபடும்?

நீங்கள் இந்த ஆய்வில் பங்கேற்றால் கீழ்கண்ட பரிசோதனைகள் செய்யப்படும்.

3. இந்த ஆய்வில் இருந்து வெளியேற முடியுமா

இந்த ஆய்வில் உங்களின் பங்கேற்பு முழுக்க முழுக்க உங்களது சுதந்திரம். எப்பொழுது வேண்டுமானாலும் இந்த ஆய்வில்இருந்து நீங்கள் வெளியேறலாம். இதனால் உங்களது வழக்கமான சிகிச்சை பாதிக்காது.

4. இந்த ஆய்வினால் ஏதேனும் பக்க-விளைவுகள் உள்ளதா அல்லது ஏற்படுமா?

இந்த ஆய்வில் சார்ந்த பக்க விளைவோ, விபத்தோ ஏற்படாது. அப்படி ஏதேனும் விபத்து ஏற்பட்டால், அதை இந்த மருத்துவமனையில் சிகிச்சை அளித்து குணப்படுத்துவோம்.

5. இந்த ஆய்வில் ஏற்படும் கூடுதல் செலவு ஏதாவது உண்டா?

இந்த ஆய்வு உங்களுக்கு கூடுதல் செலவு கிடையாது.

6. இந்த ஆய்வின் முடிவுகளின் ரகசியம் காக்கப்படுமா?

இந்த ஆய்வின் முடிவுகள் மருத்துவ கட்டுரையாக மருத்துவ நாளிதழில்

அ) அறுவை சிகிச்சைக்கு முன்பு :

(i) இ. எஸ். ஆர்.
(ii) சி. ஆர். பி.
(iii) மொத்த வெள்ளை அணு எண்ணிக்கை
(iv) தனித்தனி வெள்ளை அணு எண்ணிக்கை
(v) கிளாடர்வுகின் – 6.

ஆ) அறுவை சிகிச்சையின் போது:

(i) செயற்கை மூட்டை சுற்றியுள்ள தசை பகுதியின் மாதிரியிலிருந்து செய்யப்படும் கல்ச்சர் மற்றும் சென்ஸிடிவிட்டி.

(ii) மூட்டு நீரின் மொத்த அணுக்கள் மற்றும் நியுட்ரோபில் எனப்படும் வெள்ளை அணுவின் எண்ணிக்கை.

(iii) செயற்கை மூட்டை சுற்றியுள்ள தசையின் திசுத்துயரியல் மற்றும் அதில் உள்ள நியுட்ரோபில் எண்ணிக்கை.

இந்த பரிசோதனைகள் மேற்கொண்ட பிறகு அறுவை சிகிச்சையின் போது மேற்கொள்ளபடும் பரிசோதனையில் இருந்து திரும்ப தொற்று உள்ளதா இல்லையா என கண்டறிந்து அதை இரத்தப்பரிசோதனை முலம் ஒப்பிட்டு, இரத்தப் பரிசோதனையின் நுணுக்கம் என்னவென்று அறியலாம்.

வெளியிடப்படும். ஆனால் அதில் தனி நபரின் பெயரோ அல்லது மற்ற குறிப்புகளோ வெளியிடப்படாது. இருப்பினும், இந்த ஆய்வு சார்ந்த ஆராய்ச்சியாளர்கள். உங்களின் மருத்துவ குறிப்புகளை அறிய வாய்ப்பு உண்டு.

மேலும் ஏதேனும் சந்தேகம் இருந்தால், மருத்துவர். எல்விஸ் பெஞ்சமினை தொடர்பு கொள்ளவும்.

கைபேசி. எண். – 9677349141
இமையில் – elvis benjamin@cmcvellore.ac.in

ANNEXURE-III

सूचना पत्र

आप से निवेदन है कि आप इस स्टडी में भाग लें, जिसमें खून जाँच के द्वारा आर्टिफीशल हिप और नी जोइंट में इंफेक्शन की जाँच की जा रही है। हम, 25 मरीजों को इस स्टडी में सम्मिलित करने की आशा करते हैं। हम आप को इस स्टडी के बारे में प्रश्नोत्तरी द्वारा, आप के योगदान को समझाएंगे।

यह स्टडी किस बारे में है?

हिप और घुटने जोड़ का इंफेक्शन की जाँच बहुत ही मुश्किल काम है खून जाँच के द्वारा हम इस इंफेक्शन की जाँच कर सकते हैं। यह खून जाँच ई.एस.आर, सी.आर.पी, टी.सी, डी.सी है। लेकिन यह खून जाँच पूर्ण रूप से सही नहीं होते। इस स्टडी में हम एक नए खून जाँच, इंटरलूकीन-6 (IL-6), का इस्तेमाल करके, यह जानने की कोशिश करेंगे कि यह जाँच, पुराने खून जाँचों की तुलना में कितना सही है।

इस स्टडी में भाग लेने का तरीका क्या है?

अगर आप इस स्टडी में भाग लेते हैं तो, आप को अलग-अलग खून जाँच करने होंगे।

a) खून जाँच जो ऑपरेशन से पहले भेजने हैं निम्नलिखित हैं :

i) ई . ई्स . आर
ii) सी . आर . पी
(iii) टी . सी .
(iv) डी . सी
(v) इंटरलूकीन - 6

b) खू जाँच जो ऑपरेशन के समय भेजने हैं, निम्नलिखित हैं :-

(i) कलचर और सेनसीविटि जाँच केलिए आर्टिफिशियल जोइंट के आस - पास का टिशू (3 सैंपल लेने होंगे)

(ii) जोइंट के अंदर का 'सायनोवियल फ्लूइड' (टी . सी और न्यूट्रोफिल संख्या केलिए)

(iii) 'हिस्टोपैथोलॉजिकल फ्रोसन सेक्शन' केलिए जोइंट के आस - पास का टिशू, जिसमें न्यूट्रोफिल की संख्या पर हाई पावर फील्ड में जाँचेंगें (5 से ज्यादा न्यूट्रोफिल आर्टिफिशियल जोइंट का द्योतक है)

फिर इस खून जाँच के द्वारा हम यह पता लगायेंगे के इंफेक्शन है या नहीं और इस को तुलना ऑपरेशन के दौरान किए गए जाँच से करेंगें | अतः हमें पता लगेगा की खून जाँच कितना सही से इंफेक्शन को दिखाता है और कौन सी खून जाँच सबसे सही और सस्ता है ।

क्या आप इस स्टडी से अपना नाम वापिस ले सकते हैं?

आप नाम वापिस लेने का निर्णय बिना किसी रोक-टोक के ले सकते हैं। इससे आपके इलाज में कोई रोकावट या परेशानी नहीं होगी।

अगर आप को इस स्टडी में शारीरिक चोट पहुंचती है, तो क्या हम ऐसी जानी या चोट की उम्मीद नहीं करते हैं। परन्तु अगर ऐसी कोई चोट हो जाती है तो इसका इलाज इस अस्पताल में हो जाएगा

इस स्टडी में क्या खर्चा आएगा?
इस स्टडी में किए जा रहे खून जाँच से कोई खर्चा नहीं लेंगे।

क्या आप की पहचान गुप्त रहेगी?
इस स्टडी को 'मेडीकल जर्नल' में छापा जाएगा, परन्तु आप की पहचान किसी भी पब्लीकेशन या प्रेसेनटेशन में नहीं बताई जाएगी। परन्तु इस स्टडी से जुड़े लोग, इस स्टडी को पढ़ सकते हैं, जिसके लिए आपकी अनुमति नहीं ली जाएगी।

इस विषय में और जानकारी के लिए, सम्पर्क करें डा० एलविस बेंजामिन (दूरभाष 9677341941). ई. मेल elvisbenjamin@cmcvellore.ac.in

ANNEXURE-IV

CLINICAL DATA FORM

STUDY TITLE:

Correlation of inflammatory markers of periprosthetic joint infection in revision arthroplasty.

NAME :

AGE:

SEX:(M/F)

OCCUPATION:

RELIGION:

ADDRESS:

DATE:

PHONE NUMBER:

EMAIL :

ANTHROPOMETRY DETAILS

WEIGHT:

HEIGHT:

ADMISSION DETAILS

DATE OF ADMISSION:

DATE OF DISCHARGE:

WARD:

PREVIOUS ARTHROPLASTY DETAILS

HIP OR KNEE ARTHROPLASTY:

DATE OF THE SURGERY:

DURATION SINCE SURGERY:

HOSPITAL WHERE SURGERY WAS DONE:

CHIEF COMPLAINS:

MEDICAL COMORBIDITIES:

PREOPERATIVE INVESTIGATION RESULTS

INTERLEUKIN-6:

TOTAL LEUCOCYTE COUNT:

E.S.R:

C.R.P:

SYNOVIAL FLUID ANALYSIS:

i) TLC
ii) PMN%

INTRAOPERATIVE INVESTIGATION RESULTS

S.NO ORGANISM SENSTIVITY

CULTURE :I)

II)

III)

HISTOPATHOLOGY: NUMBER OF NEUTROPHILS/HIGH POWER FIELD:
SINUS PRESENT OR ABSENT:

ANNEXURE-V

Department of Orthopaedics, Christian medical college vellore-4, Informed Consent Form

Informed Consent form to participate in a research study

Study Title: Correlation of inflammatory markers of periprosthetic joint infection in revision arthroplasty.

Study Number: _____

Subject's Initials: _____ **Subject's Name:** _____

Date of Birth / Age: _____

I _____

Declare that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]
I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]
I understand that the Sponsor of the clinical trial, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]
I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]
I agree to take part in the above study. [ ]

Name:
Signature or thumb print:
Date:__/__/__

Name of witness:
Relation to the participant:
Signature or thumb print:
Date : __/__/__

Signature of the Investigator:

Date: __/__/___Study Investigator's Name: _____

ANNEXURE-VI

s	name	unit	hosp	age	sex	doa	dod	dos	comor	prev	dur	pre	prel	under went	esr	crp	tlc	interleuki	sinus	cultures	num	organism	field1	field2	field3	histopath	sf	sf1	major	minor	no	status	postop
1	krishna kumc	2	043098g	50	1	1/10/2015	1/24/2015	25	1	2	30	3	5	2	9	3.48	13200	1.63	1	3	2	c n s?aboutfui	acinetobacter baumanii	nil	nil	1	0	0	2	2	1	1	1
2	mrtunjay ku	3	884297f	40	1	5/6/2015	6/1/2015	26	1	1	11	3	6	3	39	3.31	8900	5.51	1	0	0	nil	nil	nil	nil	1	40	41	2	2	0	1	2
3	saraswathan	3	355685f	75	2	5/17/2015	6/1/2015	16	2	2	0	3	3	6	35	188	25500	15.82	2	3	2	mrsa	e coli	nil	nil	18	235598	97	1	1	5	2	1
4	mohamad m	3	203425g	39	1	4/22/2015	5/11/2015	21	1	2	7	3	6	3	28	6.55	6300	3.05	1	1	4	e coli	pseudomonas aeruginosa	proteus vulgaris	klebsiella sp	1	200	85	2	2	2	1	1
5	srikanta mon	1	190866g	59	1	4/27/2015	5/8/2015	12	1	2	10	3	4	3	42	10.1	8800	5.55	1	0	0	nil	nil	nil	nil	0	10700	81	2	1	3	2	1
6	shushuma ra	3	21354g	56	2	5/6/2015	5/13/2015	8	1	2	13	3	5	2	120	11	7800	8.6	1	0	0	nil	nil	nil	nil	15	13630	95	2	1	4	2	1
7	marcus butle	3	126167g	47	1	5/6/2015	5/19/2015	14	2	2	144	3	7	3	28	3.8	10600	4.26	1	0	0	nil	nil	nil	nil	1	451.28	92	2	2	2	1	1
8	gopal das	2	191744g	64	1	4/4/2015	4/12/2015	9	1	2	11	3	3	5	54	13.4	8300	2.47	1	2	1	e coli	nil	nil	nil	15	13000	90	1	1	4	2	1
9	md abdul ma	3	146556d	50	1	4/4/2015	4/14/2015	11	1	1	48	2	2	2	2	5.59	9200	2.25	1	0	0	nil	nil	nil	nil	0	500	57	2	2	0	1	1
10	swarna prave	3	809853f	56	2	10/11/2014	10/31/2014	21	1	2	24	2	2	6	66	3.4	8800	1.76	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1
11	hameeda khc	2	016579g	55	2	11/4/2014	12/1/2014	28	1	2	51	3	3	2	25	3.48	7130	18.32	1	0	0	nil	nil	nil	nil	0	1	0	2	2	0	1	2
12	sibshankar d	3	110056g	48	1	3/21/2015	3/30/2015	9	1	2	108	2	2	2	3	3	7300	1.05	1	0	0	nil	nil	nil	nil	1	350	15	2	2	0	1	1
13	annammal s	2	35000ea	79	2	3/10/2015	4/23/2015	43	3	1	26	1	0	6	0	25.8	12900	15	2	2	1	staph aureus	nil	nil	nil	30	18000	98	1	1	3	2	1
14	chandra	3	766552d	69	2	3/8/2015	3/27/2015	20	9	1	28	2	2	2	46	4.79	11900	3.79	1	1	1	coagulaseneg staph	nil	nil	nil	0	800	90	2	2	2	1	1
15	prabhu s	3	061726g	30	1	2/7/2015	2/15/2015	9	7	2	3	3	4	3	5	3	8300	2.56	1	0	0	nil	nil	nil	nil	1	70	58	2	2	0	1	1
16	nabin das	1	154351g	32	1	2/5/2105	2/17/2015	12	1	2	84	3	4	3	1	3	12700	15	1	0	0	nil	nil	nil	nil	3	7179	53	2	2	1	1	1
17	rajendra kum	1	07575g	61	1	1/29/2015	2/16/2015	19	1	2	83	3	3	3	38	4.95	6300	2.64	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1
18	sauda	3	824070b	38	2	12/19/2014	1/17/2015	29	9	1	68	2	1	6	91	84.4	7100	15.04	1	0	0	nil	nil	nil	nil	5	1050	94	2	1	3	2	1
19	prabhavathi	3	377023f	67	2	12/17/2014	12/31/2014	15	2	2	144	1	0	1	23	4.54	8700	3.18	1	0	0	nil	nil	nil	nil	0	900	23	2	2	0	1	1
20	sefali mitra	1	900635c	66	2	11/24/2014	12/15/2014	22	2	1	60	1	0	6	73	31.3	11000	4.1	1	0	0	nil	nil	nil	nil	4	15200	84	2	1	3	2	2
21	humayoun ki	3	054113g	53	1	9/22/2014	10/7/2014	17	1	2	132	3	4	2	34	19.3	10000	8.07	1	1	1	coagulaseneg staph	nil	nil	nil	8	3600	92	2	1	4	2	1
22	seevanti sahu	1	032981g	64	2	9/18/2014	9/26/2014	11	2	2	24	3	4	3	57	5.31	6800	4.23	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1
23	dilip kumar p	3	463422b	54	1	9/14/2014	9/25/2014	12	9	1	180	2	2	2	42	4.28	22400	17.25	1	0	0	nil	nil	nil	nil	1	40	72	2	2	0	1	1
24	jiban krishna	2	047746g	32	1	9/12/2014	9/24/2014	13	9	2	30	3	5	3	26	11.3	5300	1.64	1	0	0	nil	nil	nil	nil	1	0	0	2	2	0	1	1
25	kn shilaja	3	842268c	34	2	8/25/2014	11/15/2014	81	9	1	94	2	1	3	66	6.48	11600	10.31	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	2
26	mashammac	3	028135g	41	1	8/24/2014	9/4/2014	11	1	2	26	3	6	3	72	3.44	8100	3.42	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1
27	sunil kumar g	3	879424f	48	1	8/17/2014	9/1/2014	15	2	2	12	3	7	3	72	3.44	8100	2.48	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1
28	lalit singh	3	865918f	21	1	8/16/2014	8/28/2014	13	1	2	75	2	2	2	30	3.44	9900	1.85	1	0	0	nil	nil	nil	nil	0	0	0	2	2	0	1	1

ANNEXURE-VII

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho, MS Ortho, DNB Ortho
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD., MNAMS., DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

April 20, 2015

Dr. Elvis Benjamin
PG Registrar
Department of Orthopaedics
Christian Medical College, Vellore 632 004

Sub: **Fluid Research Grant Project:**
Correlation of inflammatory markers of peri prosthetic joint infection in revision arthroplastry.
Dr. Elvis Benjamin, PG Registrar, Dr. Alfred Job Daniel, Dr. Thomas Mathai, Dr. Subin Babu, Orthopaedics, Dr. John Antony Jude Prakash, Microbiology, Dr. Pradeep Mathew Poonnoose, Dr. V.T.K. Titus, Dr. Vinoo Mathew Cherian, Dr. Anil Thomas Oommen, Orthopaedics, CMC, Vellore.

Ref: IRB Min No: 9267 [OBSERVE] dated 12.01.2015

Dear Dr. Elvis Benjamin,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Alfred Job Daniel, Orthopaedics, CMC, Vellore.

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 632 002.
Tel : 0416 - 2284294, 2284202 Fax : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA.

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Ref: IRB Min No. 9267 [OBSERVE] dated 12.01.2015

Dear Dr. Elvis Benjamin,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project entitled "Correlation of inflammatory markers of peri prosthetic joint infection in revision arthroplastry," on January 12th 2015.

The Committees reviewed the following documents:

1. IRB Application format
2. Curriculum Vitae' of Drs. Elvis Benjamin, Alfred Job Daniel, Thomas Mathai, Subin Babu, John Antony Jude Prakash, Pradeep Mathew Poonnoose, V.T.K. Titus, Vinoo Mathew Cherian, Anil Thomas Oommen
3. Informed consent form (English, Tamil, Hindi & Bengali)
4. Information Sheet (English, Tamil, Hindi & Bengali)
5. Inter Departmental Agreement & Clinical Data Form
6. No of documents 1 - 6

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on January 12th 2015 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Name	Qualification	Designation	Other Affiliations
Dr. Inian Samarasam	MS, FRCS, FRACS	Professor, Surgery, CMC	Internal, Clinician
Dr. Anand Zachariah	MBBS, PhD	Professor, Medicine, CMC	Internal, Clinician
Dr. Mathew Joseph	MBBS, MCH	Professor, Neurosurgery, CMC	Internal, Clinician
Dr. Niranjan Thomas	DCH, MD, DNB (Paediatries)	Professor, Neonatology, CMC	Internal, Clinician
Dr. Jacob John	MBBS, MD	Associate Professor, Community health	Internal, Clinician
Dr. Vivek Mathew	MD (Gen. Med.) D.M (Neuro) Dip. NB (Neuro)	Professor, Neurology, CMC	Internal, Clinician
Dr. Chandrasingh	MS, MCH, DMB	Professor, Urology, CMC.	Internal, Clinician
Dr. Anup Ramachandran	Ph. D	The Wellcome Trust Research Laboratory Gastrointestinal Sciences, CMC	Internal, Basic Medical Scientist
Dr. Simon Pavamani	MBBS, MD,	Professor, Radiotherapy, CMC.	Internal, Clinician
Dr. Visalakshi. J	MPH, PhD	Lecturer, Dept. of Biostatistics, CMC.	Internal, Statistician
Dr. T. Balamugesh	MBBS, MD(Int Med), DM, FCCP (USA)	Professor, Pulmonary Medicine, CMC	Internal, Clinician
Dr. B. J. Prashantham	MA(Counseling Psychology), MA(Theology), Dr. Min(Clinical Counselling)	Chairperson, Ethics Committee, IRB. Director, Christian Counseling Centre, Vellore	External, Social Scientist
Mrs. Pattabiraman	B. Sc, DSSA	Social Worker, Vellore	External, Lay Person
Dr. Denise H. Fleming	B. Sc (Hons), PhD	Honorary Professor, Clinical Pharmacology, CMC	Internal, Scientist & Pharmacologist

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Deputy Chairperson
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

Dr. Anuradha Rose	MBBS, MD	Assistant Professor, Community Health	Internal, Clinician
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgical Nursing	Internal, Nurse
Mr. C. Sampath	BSc, BL	Legal Expert, Vellore	External, Legal Expert
Rev. Joseph Devaraj	B. Sc, BD	Chaplaincy Department, CMC	Internal, Social Scientist
Dr. Nihal Thomas	MD, MNAMS, DNB(Endo), FRACP(Endo) FRCP(Edin) FRCP (Glasg)	Professor & Head, Endocrinology. Additional Vice Principal (Research), Deputy Chairperson, IRB, Member Secretary (Ethics Committee), IRB	Internal, Clinician

We approve the project to be conducted as presented.

The Institutional Ethics Committee expects to be informed about the progress of the project, any **adverse events** occurring in the course of the project, any **amendments in the protocol and the patient information / informed consent**. On completion of the study you are expected to submit a copy of the **final report**. Respective forms can be downloaded from the following link: http://172.16.11.136/Research/IRB_Polices.html in the CMC Intranet and in the CMC website link address: http://www.cmch-vellore.edu/static/research/index.html.

Fluid Grant Allocation:

A sum of 50,000/- (Rupees Fifty Thousand only) will be granted for 1year.

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS.,DNB(Endo),FRACP(Endo),FRCP(Edin) FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
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Christian Medical College, Vellore - 632 002

Cc: Dr. Alfred Job Daniel, Orthopaedics, CMC, Vellore.

IRB Min No: 9267 [OBSERVE] dated 12.01.2015

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