Assessment of Interleukin-6 and Other Inflammatory Markers in the Diagnosis of Egyptian Patients with Periprosthetic Joint Infection

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The orthopedic community continues to struggle for accurate diagnosis of periprosthetic joint infection (PJI) as it is a devastating complication after total joint arthroplasty. There is no universally accepted diagnostic test that is absolute or reliable for detection of PJI. Recent research has raised doubt regarding the utility of various inflammatory markers in diagnosis. The aim of study is to evaluate the diagnostic value of interleukin- 6 (IL-6) and other inflammatory markers; C- reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell count (WBC) in the diagnosis of PJI. The study group included 40 patients (21 males, 19 females) admitted for surgical intervention after knee or hip arthroplasties. Patients were subjected to careful history taking, thorough clinical examination and preoperative laboratory investigations including serum IL-6, CRP, WBC and ESR. Peri-implant tissue specimens were subjected to microbiological culture and histopathological examination. The mean age of the studied patients was (58.4 year) (range, 38-72years). Intraoperative cultures and histopathological examination revealed that 11 patients had been infected (PJI), and 29 patients were aseptic failure of the prosthesis. Four presumed markers of infection were tested preoperatively: ESR; CRP; WBC; and IL-6. Results showed that ESR (p=0.0001), CRP (P=0.004), WBC (0.0001), and IL-6 (P=0.0001) were significantly higher in patients with septic revision arthroplasty than those with aseptic failure of the prosthesis both among patients with hip arthroplasty and those with knee arthroplasty. Serum IL-6 (> 10.4pg/ml) reportedly had a sensitivity (100%), a specificity (90.9%), a PPV (79%), a NPV (100%), and accuracy (92.5%). In conclusions, the present study demonstrated that IL-6 was the most accurate laboratory marker for diagnosing PJI when compared to ESR, CRP, and WBC. We also found that IL-6 above 10.4 pg/ml and CRP level above 18 mg/L identified all patients with PJI and the combination of CRP+ IL-6 was an excellent screening test to identify all such patients (sensitivity 100%, NPV 100%).

Periprosthetic joint infection (PJI) after total joint arthroplasty constitutes a major diagnostic and therapeutic challenge (Buttaro *et al.,* 2010; Miyamae *et al.,* 2012). PJI occurs in 1-3% of total joint arthroplasty patients and it is associated with a large economic burden to the healthcare system, a high rate of dissatisfaction (loss of function), and mortality rate of up to 7% (Phillips *et al.,* 2006; Kurtz *et al.,* 2008; Jämsen *et al.,* 2010; Poultsides *et al.,* 2010). In addition, PJI is one of the most common indications for revision hip and knee arthroplasties in which distinction between septic and aseptic failure is critical (Ong *et al.,* 2009; Buttaro *et al.,* 2010; Wetters *et al.,* 2012).

Despite the availability of numerous diagnostic modalities to detect microbial infection, PJI remains often undetected (Kurtz *et al.,* 2010). In addition, clinical signs such as fever or the presence of sinus tract may often be absent; therefore such signs are insensitive in PJI diagnosis (Berbari *et al.,* 2010). Several studies have demonstrated limited accuracy of conventional culture methods for diagnosing PJI, particularly low-grade infections resulting in false negative results (Tunney *et al.,* 1999; Kobayashi *et al.,* 2008). Intraoperative analysis of frozen sections is a tedious

procedure, and is not a universally accepted method (Buttaro *et al.,* 2010).

Several inflammatory markers including serum WBC, CRP, ESR, IL-6, procalcitonin, and TNFα have been used for diagnosing suspected PJI, (Kalore *et al.,* 2011). IL-6 is a cytokine produced by activated macrophages, monocytes and T cells in the context of inflammatory response; it also induces production of acute phase proteins including CRP. It may be a valuable marker of infection after major surgery, however, the use of IL-6 as a diagnostic marker is still under investigation and it is not used routinely (Writz *et al.,* 2000; Shah *et al.,* 2009; Kalore *et al.,* 2011).

The aim of the present study was to validate the usefulness of IL-6 and other inflammatory markers (CRP, ESR, WBC count) in combination with other established tests such as microbiological culture in diagnosing PJI.

Subjects, Materials and Methods

This is a prospective study including 40 patients with hip or knee replacement admitted to Mansoura University Hospital for operative interference over a period of 2 years. Informed consent was signed by all included patients. The study group included 21 males and 19 females with a mean age of 58.4 year, range, 38-72 years. There were 26 cases with hip prosthesis (total or hemi-arthroplasties) and 14 cases with knee prosthesis.

Four types of intervention were performed; lavage without changing the implant (n=12), one stage implant revision (n=5), two-stage implant revision (n=21), and prosthesis removal (n=2).

Exclusion criteria, patients with chronic inflammatory conditions (e.g. rheumatoid arthritis), patients with malignancy, or patients with previous antibiotic treatment prior to surgery were not enrolled in the study.

Blood samples were withdrawn from patients within 2 hours before surgery under complete aseptic conditions into 2 separate tubes; one tube was allowed to clot for 30 min. then centrifuged at 3,000 RPM for 15 min., and serum was then divided into two aliquots. One serum aliquot was tested immediately for CRP, and the other aliquot was stored at -80°C until IL-6 analysis. The second tube contained sodium citrate and was used for ESR and WBC estimation.

Intraoperative tissue samples with the most obvious inflammatory changes according to the surgeon's judgment were collected for histopathological evaluation (formalin preserved, paraffin embedded sections), and for conventional microbiologic culture. Samples were packed into a sterile surgical container and immediately sent to the laboratory within one hour .

The white blood cell (WBC) count was determined in the hospital clinical laboratory by hematology analyzer (Sysmex KX21, Roche, USA). Serum CRP level measurement was tested by the semi-quantitative latex agglutination test (Omega Diagnostics kits, UK) according to manufacturer's guide. ESR was determined using the Westergren method. The rate of sedimentation of erythrocytes is measured in a 1:5 dilution of 3.2% sodium citrate solution to whole blood. Blood is drawn up in a column and allowed to sit undisturbed for one hour. The sedimentation is read as the millimeter distance from the top of the column to the meniscus of the erythrocyte sediment (Sood, 2009). IL-6 levels were measured by enzyme immunoassay (EIA) for the in vitro quantitative measurement of human IL-6 in serum (Boster Biological Technology Co., LTD; human IL-6 ELISA kit).

Peri-implant tissue specimens were homogenized in 3 ml brain heart infusion broth (Bio-Rad) for one min. The homogenate was inoculated onto aerobic blood agar, chocolate agar, MacConckey agar (Oxoid) and anaerobic blood agar. Aerobic and anaerobic agar plates were incubated at 35-37^{o}C in 5-7% CO2 aerobically, anaerobically for 2-4 and 7 days respectively. The isolated organisms were identified by standard bacteriologic methods (Roberts, 2007).

The study patients were allocated into 2 groups; infected and aseptic. The definitive diagnosis of infection (PJI) was determined if at least one of the following was present: (1) visible purulence surrounding the prosthesis; (2) acute inflammation on histopathologic examination of permanent tissue sections (defined by the presence of five or more polymorphnuclear leukocytes per high-power field); (3) a sinus tract communicating to the implant; and (4) growth of bacteria on culture. Aseptic failure was defined as implant failure not meeting these criteria (Piper *et al.,* 2010).

Statistical Analysis

Data obtained from the present study was analyzed using SPSS version 17. Continuous data were expressed in the form of mean ± SD while categorical data were expressed in the form of count and percent. Comparison of continuous data was performed by student t test while categorical data was done using Chi-square test. Correlation between variables was investigated by Pearson's correlation coefficient. *P* value less than 0.05 was considered statistically significant. Cut-off values, sensitivity, specificity, PPV, NPV and test accuracy were calculated according to Receiver Operating Characteristic (ROC) curve analysis.

Results

The study group included 40 patients; 11(27.5%) were proved to be infected (PJI) (had a positive culture and histopathological evidence of infection), and 29 (72.5%) experienced aseptic failure of the prosthesis (negative for all diagnostic criteria mentioned above) (Table 1).

Table 1. Demographic data for the study population

	Infected (n=11)	Aseptic (n=29)	*P* value
Age (years)	59.6 ± 6.1	57.9 ± 7.8	NS
Gender			
male	4 (36.4%)	r17 (58.6%)	NS
Female	7 (63.6%)	12 (41.4%)	
Joint type			
Knee	4 (36.4%)	10 (34.5%)	NS
hip	7 (63.6%)	19 (65.5%)	

P >0.05 is not significant (NS)

The isolated bacteria included Gram positive cocci; *Staphylococcus aureus* (n=5; 45.4%), Coagulase-negative *Staphylococci* (*CoNS*) (n=3; 27.3%), *Enterococci* (n=1; 9.1%), and Gram negative bacilli; *Escherichia coli* (n=1; 9.1%), and *Pseudomonas aeuroginosa* (n=1; 9.1%) (Table 2).

Table 2. index procedure and bacterial species in infected patients

Previous procedures	Organisms	Number of patients
Knee arthroplasty	*Staphylococcus aureus*	2
	*CoNS**	1
	Pseudomonas aeuroginosa	1
Hip arthroplasty	*Staphylococcus aureus*	3
	*CoNS**	2
	Enterococci	1
	Escherichia coli	1

*CoNS= Coagulase-negative *Staphylococci*

We found in patients with PJI significantly higher levels of preoperative ESR, WBC count, serum CRP, and IL-6 than in aseptic group (table 3). In addition, such inflammatory markers were still observed to be significantly higher among patients who had hip arthroplasty and those who had knee arthroplasty than aseptic group (Table 3).

Table 3. Analysis of Inflammatory markers in patients with infected and aseptic revision arthroplasty

Inflammatory marker	Knee arthroplasty			Hip arthroplasty			Hip and knee arthroplasties		
	Infected (n=4)	*Aseptic (n=10)*	*P value*	*Infected (n=7)*	*Aseptic (n=19)*	*P value*	*Infected (n=11)*	*Aseptic (n=29)*	*P value*
ESR *(mm/hour)* (mean±SD)	102.0± 68.9	15.2± 17.5	0.002	68.5± 59.4	10.0± 10.3	0.04	85.9± 33.9	28.3± 20.5	0.0001
WBC *(cell x 10^9/L)* mean±SD	13.5± 2.8	7.9± 1.8	0.001	11.6± 3.0	7.6± 1.8	0.0003	12.3± 2.9	7.7± 1.7	0.0001
CRP *(mg/L)* mean±SD	111.2± 16.5	30.3± 17.5	0.0001	71.4± 33.3	27.2± 22.2	0.0006	80.7± 61.9	11.7± 13.1	0.004
IL-6 *(pg/ml)* mean±SD	86.6± 32.4	6.0± 3.1	0.016	52.1± 30.2	5.8± 3.1	0.006	64.6± 34.1	5.9± 3.1	0.0001

ESR= erythrocyte sedimentation rate; CRP= C-reactive protein; WBC= white blood cell count; IL-6= interleukin 6

P<0.05 is significant.

Using the values identified by ROC analysis, we demonstrated that both CRP and IL-6 had the highest sensitivity (100%), and negative predictive value (100%). IL-6 had a higher specificity (90.9% vs. 86.2%), and accuracy (92.5% vs. 87.5%) than CRP. The combination of CRP and IL-6 showed the highest sensitivity of (100%), specificity of (99%), and accuracy of (97.5%) (Table 4).

Table 4. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of inflammatory markers

Test	Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
ESR *(mm/hour)*	45.0	81.8	82.8	64.3	92.3	82.5
CRP *(mg/L)*	18.0	100.0	86.2	68.8	100.0	87.5
WBC (cell/109/L)	9.2	90.9	75.9	58.8	95.6	80.0
IL-6 *(pg/mL)*	10.4	100.0	90.9	79.0	100.0	92.5
IL-6 + CRP		100.0	99.0	92.0	100.0	97.5

ESR= erythrocyte sedimentation rate; CRP= C-reactive protein; WBC= white blood cell count; IL-6= interleukin 6

Discussion

Diagnosis of PJI remains a real challenge to the orthopedic community as there is no single laboratory test that can accurately detect PJI prior to revision arthroplasty (Virolainen *et al.,* 2002). Primary standard laboratory analytical tools namely: ESR, WBC count, CRP that are used to determine the presence of PJI, are not consistently reliable (Shih et al., 1987). Hence, there is a universally recognized need to identify new and reliable markers of inflammation that can aid in the rapid diagnosis of PJI (Drago *et al.,* 2011).

In the present study, intraoperative cultures revealed growth of Gram-positive and -negative bacteria. Berbari *et al.* (1998) and Di Cesar *et al.* (2005) reported that Staphylococci (*Staphylococcus aureus* and Coagulase-negative *Staphylococcus species*) were the most common organisms associated with PJI. Although intraoperative cultures have been considered the gold standard for PJI diagnosis, nearly 10% of the cases with infections may be culture-negative; conventional culture results may be delayed for 2-5 days and contamination of samples may cause false positive results (Bauer *et al.,* 2006).

In this study, we evaluated the utility of preoperative ESR, WBC count, serum CRP, and IL-6 as markers for PJI. Data obtained indicated clear differences in such markers levels between patients with PJI and those with aseptic failure, in patients with hip arthroplasty and those with knee arthroplasty.

In the current study, the determination of ESR parameters was of little value in the diagnosis of PJI since its sensitivity and specificity were low in comparison to other tests used such as IL-6 and as stated elsewhere (Bottner *et al.,* 2007; Kalore *et al.,* 2011). In contrast, other studies suggested that ESR is an important marker for the diagnosis of low grade infection; however they did not evaluate ESR in patients without deep implant infection (Sanzen & Sundberg, 1997). Also, Spangehl *et al.,* (1999) suggested that a combination of ESR and CRP would have the highest diagnostic accuracy.

Studies evaluating the role of WBC count in diagnosing PJI found inconsistent results. Although some authors have concluded that WBC count is a useful test, as its mean levels were significantly higher in patients with infection compared with aseptic failure (Virolainen *et al.,* 2002). Others reported that serum WBC count as a diagnostic marker for PJI lacks sensitivity and specificity and therefore of little value in diagnosing PJI *(Bottner et al.2007;* Kalore et al., 2011*).* A cut-off value of 11.000 cells/µl for WBC count provided a sensitivity of 20%, a specificity of 96%, a PPV of 54%, and a NPV of 85% (Spangehl *et al.,* 1999). In addition, a retrospective study showed that a leucocytic count of more than 27,800 cells/µl carries higher sensitivity and specificity (Bedair *et al.,* 2011).

In the present study, CRP testing showed a high sensitivity (100%) and specificity (86.2%). Sanzen & Sundberg (1997) and Spangehl *et al.* (1999) have reported that CRP is the most accurate diagnostic marker for infection after joint replacement. However, such test can give false positive values in acute postoperative settings as the CRP levels can remain elevated for up to 3 weeks after surgery (Kalore *et al.,* 2011).

In our study, serum IL-6 test showed the highest sensitivity (100%), specificity (90.9%), PPV (79%), NPV (100%), and accuracy (92.5%) as compared to the other studied markers. This in accordance to Di Cesare *et al.* (2005) who carried out a study with 58 patients with revision hip and knee arthroplasties and showed that sensitivity, specificity, PPV, NPV, and accuracy of IL-6 to be 100, 95,89, 100, and 97% respectively.

Although the sensitivity of IL-6 was similar to CRP, its specificity, and accuracy were higher than CRP. The present study demonstrated that IL-6 was the most accurate laboratory marker for diagnosing PJI when compared to other inflammatory markers studied. The advantage of using IL-6 as a diagnostic marker is that it exhibits a more rapid increase and quicker return-to-normal values as opposed to CRP or ESR which levels are generally still elevated up to 3 weeks after surgery. Such results suggest that IL-6 levels may be a superior indicator of postoperative inflammatory response; furthermore, IL-6 levels can be used to monitor the infected patient's response to treatment (Wirtz *et al.,* 2000; Bottner *et al.,* 2007; Kalore *et al.,* 2011).

When we combined two inflammatory markers such as IL-6 and CRP with cut-off values above 10.4 pg/ml and 18 mg/L, respectively; we were able to identify in this study all patients with PJI. In addition, CRP and IL-6 were reported to be excellent screening markers to rule out deep infection of the implant (Bottner *et al.,* 2007).

Limitations of our study, is the relatively small number of study patients and although intra-operative tissue samples were collected with the most obvious inflammatory changes according to the surgeon's judgment, there is a possibility that the area chosen did not sustain microorganisms.

Data obtained in this study suggest that the use of IL-6 either alone or in combination with CRP may be accurate diagnostic markers of PJI after hip or knee arthroplasties. Although the use of other inflammatory markers (WBC count, ESR, CRP) may also be of value in helping to diagnose PJI, however, they are of low specificity in particularly for detection of early infection as they remain elevated up to 2 weeks after surgery (Buttaro *et al.,* 2010). Therefore, IL-6 was found to be a good marker for inflammation, as it distinguished between patients with infection and those with aseptic failure of the prosthesis and the combined use of IL-6 and CRP provided an excellent diagnostic tool to rule out PJI after knee or hip arthroplasties. In conclusion, the addition of IL-6 to the standard work-up of patients with suspected PJI could increase diagnostic certainty and generate an improved patient management. Further studies are needed to confirm our findings in a larger cohort, to serially monitor the level of IL-6 and other markers after surgery.

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