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Evaluation of Leukergy Test As a Diagnostic and Prognostic Tool in Bone Infections.

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ABSTRACT

Background: The prevalent haematological parameters indicating infections of bone and joint are of poor sensitivity and specificity. The early diagnosis of infections of bone and joint are of supreme significance in better clinical outcome. So there is a need for other haematological tests with good predictive values of such conditions. Leukocytes tend to aggregate in the peripheral blood of patients with inflammatory conditions is referred as 'Leukergy'. Studies have shown that this phenomenon can be used to evaluate the infectious and inflammatory conditions. **Objective:** To assess leukergy as a diagnostic modality in bone and joint infections and to evaluate its prognostic value. **Methods:** Longitudinal, prospective, analytical study conducted from November 2009 to November 2011 in the department of orthopaedics, Yenepoya medical college hospital, Mangalore, India, among 30 proven cases of osteomyelitis, septic arthritis and patients with infections related to implant surgeries. Routine haematological investigations such as TLC, ESR, and CRP along with leukergy tests which were done on Day zero, Day three, Day seven & 21st day to see effect of treatment. **Results:** Only 17 patients had positive leukergy test at the initial stage. In those with positive leukergy, it correlated with ESR, TLC and CRP. There was statistically significant decrease in the haematological parameters other than ESR with treatment. **Conclusion:** Leukergy can only be used for diagnostic modality in conjunction with other haematological parameters as a negative test cannot rule out inflammatory bone condition. An initial positive leukergy test can subsequently be used as a tool for prognostic evaluation of bone and joint infections.

Keywords: Leukergy; bone and joint infections; erythrocyte sedimentation rate; total leukocyte count; C-reactive protein.

INTRODUCTION

Bone infections can be difficult to treat especially in areas of relatively poor vascularity, often requiring prolonged use of antimicrobial therapy in association with surgical drainage and debridement. Effective initiation of therapy depends on prompt diagnosis. If the obvious signs of inflammation are present, the diagnosis can be made easily. But low-grade and occult infections will be more difficult.[1] Although it may be expected that erythrocyte sedimentation rate (ESR) will be elevated in osteomyelitis and septic arthritis, it neither sensitive nor specific. Other laboratory investigations using total and differential leukocytes count (TLC and DLC), C-reactive proteins (CRP) levels shows wide variations.[2-6]

The aggregation of leukocytes in the peripheral blood of patients with inflammatory conditions is referred as 'Leukergy'and was first described by Fleck.[6] It has been shown to occur in several inflammatory diseases including pancreatitis and burns. In 1993, Otremski et al. has described it as a possible simple laboratory test to diagnose OM.1It was found to be as sensitive as ESR in a series of 26 patients with known OM or septic arthritis, but has not been reported on since.

This study was conducted to assess leukergy as a diagnostic modality in bone and joint infections because of poor sensitivity and specificity of other tests like ESR, TLC, CRP, blood cultures, radiological investigations. To our knowledge ours is the only study from recent past to evaluate use of

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leukergy phenomenon in infections of bone and joint. Certainly, this is the first study evaluating leukergy as prognostic indicator in bone and joint infections.

MATERIALS AND METHODS

This was a longitudinal, prospective, analytical study conducted from November 2009 to November 2011 in the department of orthopaedics, Yenepoya Medical College Hospital, Mangalore, India.

Thirty patients diagnosed with osteomyelitis, septic arthritis and other infections related to implant surgeries were included in the study. Patients with secondary infections complicating bone carcinoma, chronic inflammatory bone and joint conditions such as osteoarthritis, rheumatological conditions were excluded from the study. Written informed consent was taken and approval was obtained from institutional review committee Yenepoya medical university.

Detailed histories as well as physical examination of all patients were recorded. All patients were subjected to routine haematological investigations such as TLC, ESR, and CRP along with leukergy tests which were done on Day zero, Day three, Day seven & 21st day to see effect of treatment. All the 30 patients were treated with empirical antibacterials along with the necessary surgery, other than the 2 patients with failed back syndrome, who were treated conservatively. Culture & sensitivity reporting was done and antibacterials was changed as per the sensitivity report.

Samples were sent for ESR on day 0, 3, 7 and 21. CRP and WBC counts were done using automatic analyser and automatic cell counter on the same days.

Leukergy test was done by taking 1.6ml of venous blood and mixing with 0.4ml of 3.8% sodium citrate. A thick venous blood smear was made by allowing several drops of blood to slip across a slide slanted at 45° by gravitational force. The blood was allowed to dry at room temperature. The slide was then cooled to below -10°C keeping inside a deep freezer for 5-10 minutes. Erythrocyte haemolysis of slide was achieved by repeated freezing and thawing of the blood sample and fixation was performed with methanol, followed by staining with Leishman stain on each slide for 10 minutes and then washed by gentle flow of water and dried. The slides were then examined first under low power to see the pattern of distribution of leucocytes in clump and then under oil immersion. The cells occurring in clumps were counted in relation to those remained not clumped. Agglomeration was considered to be positive when at least three leukocytes were in close proximity, the distance between their nuclei being less than the diameter of one cell, when the proximal, middle and distal parts of the slide is seen under magnification to avoid misinterpretation.[1,6]

The total of 300 cells was counted in each of the slide. Percentage of clumpedcells of leukergy were then determined, using the below formula and graded accordingly [Table 1].

$$\text{Leukergy\%} = \frac{\text{Number of clump cells x100 (Aggregated 3 or more at distance <1cell diameter)}}{300}$$

Table 1: Leukergy Grading according to percentage of aggregates observed

Leukergy percentage	Grade
0-10	1
11-19	2
20-34	3
>34	4

Collected data were entered and analysed using SPSS software 16.0. Both descriptive and inferential statistics were measured.

RESULTS

Out of total of 30 cases selected in the study, seventeen cases of osteomyelitis, nine cases were of septicarthritis two cases each of infected implant and failed back syndrome (FBS). Out of thirty cases seven were females and twenty three males. The mean age of study group was 57.4 years. Table 2 shows clinical details of study group.

The organisms that were detected by blood culture were S.aureus (57%), Streptococcus (32%), Klebsialla, Pseudomonas and S.epidermis (3.5%each) with S.aureus in one and Strepotococcus in septic arthritis. The antibacterial treatment were started on day zero and changed in midcourse depending on the culture & sensitivity report.

Table 2: Clinical details of 30 patients with 28 patients with bacteriolagically proven sepsis with respective treatment.

Type of infection	Site	Organism	Treatment
Septic arthritis (n=9)	Elbow(2) Ankle(2) Knee(3) Shoulder(1) Hip(1)	Klebsiella(1) Streptococcus (1) Streptococcus Streptococcus Streptococcus Streptococcus	Drainage Drainage Drainage Drainage Drainage
Osteomyelitis (n=17)	Calcaneum(3) Fibula(1) Foot(3) Tibia(7) Femur(3)	S.aureus (3) S.aureus (1) S.epidermis (1) S.aureus (5) Streptococcus(2) S.aureus	Curettage Curettage Ray excision Curettage Amputation Curettage
Infected implant (n=2)	Radius plate(1) Femoral nail(1)	S.aureus S.aureus	Implant removal Implant removal
Failed back syndrome (n=2)	L5-S1 L5-S1		Conservative Conservative

The leukergy test was carried along with ESR, CRP and TLC on day zero, three, seven and twenty one and compared with each other [Table 3] Leukergy grading was positive in only 17 cases in study group on day zero. In patients with positive leukergy, it correlated well with ESR. Leukergy correlated well with CRP and was statistically significant both in the initial period, during treatment and after treatment. (p =0.003, [Table 3]). Grade 3 leukergy corresponded clinically and haematologically in 5 patients only during day 0. [Table 4] tabulates the comparison between the leukergy and clinical grading at different intervals in study group.

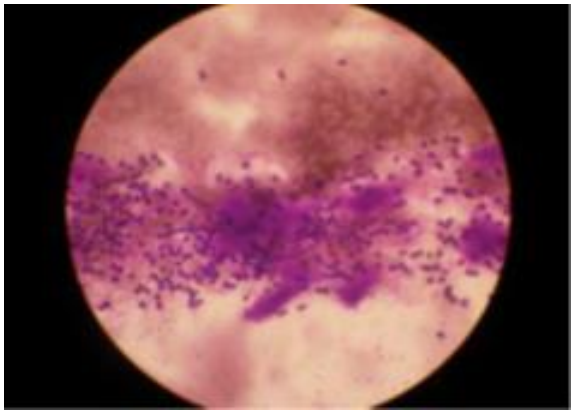

Figure 1: Demonstration of leukergy - grade 3 in peripheral smear of patient with bone infection under low power (X10)

Table 3: Tabulation of means of different haematological parameters at different intervals.

Haematological procedures	Day 0	Day 3	Day 7	Day 21
ESR (mm/hr)	111.28	84.09	43.75	23.769
CRP (mg/L)	2.21	1.68	1.08	0.75
TLC (cells per mm3)	11800	9786.7	7776.7	5500
Leukergy (grade)	2	2	1	0

Table 4: Comparison of leukergy grading and clinical grading at different intervals in study group.

	Day 0	Day 3	Day 7	Day 21
Leukergy	2	2	1	0
Clinical grading	Grade 3– Severe local pain and pus formation associated with systemic signs.	Grade 2 – Moderate local pain with pus formation and no systemic signs.	Grade 1 – Mild local pain without pus formation and no systemic signs.	Grade 0 – No local or systemic signs of disease.

DISCUSSION

In a study done by Otremski et.al. where sample size of 26 patients with proven bone or joint infection, leukergy was found to be positive in 25 of them. They concluded that leukergy test was more accurate than ESR and white cell count. The test detected severity of the infection and reactivation of the septic process better than any other haematological tests1. Meera Sharma and et.al, evaluated leukergy test in 25 children of age group 5-12 years diagnosed with septic arthritis.[5] 25 cases of proved septic arthritis on bacteriological examination after arthrocentesis and/or arthrotomy/biopsy were evaluated. Here all the patients were treated with antibiotics. The values of leukergy was found to be most sensitive in all the disease groups (100% on Day zero and 36% on day 21) compared to other haematological parameters following treatment. Leukergy correlated well with CRP compared to TLC and ESR and both TLC and ESR failed to show any relation to the effect of treatment. They further stressed the significance of leukergy in prognosis of disease.[5-7]

In our study we were not able to prove leukergy to be better than all other haematological parameters, as proved by previous studies as the initial leukergy was positive only in 17 cases out of 30. But like the study done by Meera Sharma et.al., leukergy is proved to be better indicator than TLC.[5] Changes in ESR at different stages were statistically insignificant. CRP and TLC values declined significantly after treatment ($p<0.05$) so does leukergy grading (change from initial 2 to 0 after three weeks of treatment). CRP is a more sensitive and accurate reflection of acute phase response than ESR and TLC. The half life of CRP is also constant and CRP returns to normal more quickly than ESR and TLC in response to therapy. Whereas TLC reaches peak after 3 days of inflammatory insult onset and ESR reaches after 24- 48 hours.[8-9] In grade III leukergy responded with TLC while other two indicators did not respond. Thus CRP is a better indicator in comparison with the other haematological parameters like TLC and ESR, but quantitative estimation of CRP is much more expensive and manual method in which only qualitative estimation is possible does give the only the presence or absence of infection and not the grading of infection.

CONCLUSION

Leukergy can only be used for diagnostic modality in conjunction with other haematological parameters as a negative test cannot rule out inflammatory bone condition. An initial positive leukergy test can subsequently be used as a tool for prognostic evaluation of bone and joint infections. CRP is a better haematological marker than leukergy, TLC and ESR especially in the early diagnosis of the inflammation and infection. Further study with increased sample size of

statistical significance is required to further prove the diagnostic and prognostic value of leukergy.

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