

CRP for diagnosis and IL-6 for monitoring of infection in lower limb fracture patients

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Abstract

Background: The focus of this study was to assess the role of IL-6, ESR, CRP and WBC count as markers of infection in patients with lower limb fractures.

Materials & Methods: 137 patients with lower limb fractures presenting within 24 hrs after injury and requiring operative intervention were included in our study. Serum IL-6, ESR, CRP and WBC count were measured pre-operatively on admission, on postoperative day 2 and on day of suture removal. An additional sample was also taken from patients who showed signs of infection post-operatively. The trends of these markers were measured and their co-relation with each other was analysed. Mann-Whitney U test was used to identify significant difference between patients with and without infection.

Results: The follow-up 37 patients was lost post-operatively and so study subjects were reduced to 100. 16 out of 100 patients developed wound infection post-operatively. The minimum, maximum and mean values of IL-6, CRP, ESR and WBC count were calculated in all patients. There was significant difference in post-op day 2 serum CRP levels ($p=0.002$) between infected and non-infected patients however, no such significant difference was seen in IL-6, ESR and WBC count. Significant difference was observed in serum ESR levels on day of suture removal ($p=0.004$) between infected and non-infected patients but no such significant difference was seen between IL-6, WBC count and CRP. IL-6 levels returned to normal values quicker as compared to other markers after control of infection on day of suture removal.

Conclusions: CRP is an excellent marker for early diagnosis of infection whereas, IL-6 is a better marker to monitor the course of infection in patients with lower limb fractures.

Keywords: IL-6, CRP, fractures, infection.

Introduction

Infection is a disastrous complication seen in orthopaedic surgeries. In the present era, there are very few parameters which are useful for diagnosis of infection following trauma and surgery. Most of the parameters in current use like fever, ESR (Erythrocyte sedimentation rate), white blood cell count (WBC) are not very specific for acute bacterial infection and are also elevated in inflammation due to other causes. Also, these infected patients are subjected to long duration of antibiotic therapy as most of the currently used serological markers of infection remain elevated for a prolonged duration even after control of infection. Hence, we need to identify new markers which can help in early diagnosis of infection even before it becomes clinically evident and also to monitor the course of infection which is necessary to determine the effectiveness of the ongoing treatment for control the infection. ESR has a slow response to inflammatory reaction, its levels rise keep rising upto five to seven days post-operatively and gradually decreases over a period of few months and sometimes upto one year after surgery [1]. C-reactive protein (CRP) is one of the acute-phase response proteins produced by hepatocytes and is usually found in concentrations of 0.3 to 1.7 mg/l [2]. Production CRP is

induced by cytokines in response to infection, inflammation and neoplasm and its level in serum rises after an operative procedure reaching peak levels two to three days after surgery and falls down to normal levels approximately three weeks post-operatively [3]. Interleukin-6 (IL-6) is a multifunctional cytokine which is a regulator of both acute and chronic inflammation and is also a primary stimulator of most of acute phase proteins including CRP. It has been recently investigated as a marker of infection in peri-prosthetic joint infections and has proved to be a better marker of infection because of its early rise and fall in serum of infected patients as compared to other serological markers. [4,5], A variety of inflammatory mediators are also elevated in response to trauma [6,7], as well as surgical procedures. [8,9], Hence it is difficult to identify whether the rise in these inflammatory markers is due to the normal physiological response of the body or is it because of infection. Hence, we conducted this study to assess the role of IL-6, ESR, CRP and leucocyte count as markers of infection in lower limb fracture patients.

Material & Method

A total of 137 consecutive patients between 1st October 2015 and 1st September 2017, with both open and closed lower limb fractures who required operative intervention were enrolled in this study. Patients excluded from our study were those whose fractures were treated conservatively, patients having fractures other than lower limb fractures which required operative intervention and patients who had associated head injuries. Patients showing signs of infection elsewhere in the body which were clinically detectable such as pneumonia and urinary tract infections were also excluded from our study.

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Ethical committee approval was obtained for this study. An informed consent was taken from all patients. Blood samples were collected from patients on day of admission, post-operative day 2 and the day of suture removal which was variable, from post-operative day 10 to post-operative day 16, because patients used to present for suture removal on OPD basis as per their convenience. Additional blood samples for CRP, ESR, WBC, IL-6 were also collected from patients if they showed any signs of infection (fever, purulent discharge from suture line, positive culture reports, elevated serological markers) post-operatively before suture removal. Due to cost constraints, we did not collect an additional sample on post-operative day 7 (half way between surgery and suture removal). Pre-operative antibiotic prophylaxis as per routine protocol at our institute with cefuroxime 750 mg 1 hour pre-operatively, 8 hrs after surgery and 16 hrs after surgery for closed fractures and a combination of cefuroxime 1.5 gm twice a day, amikacin 750 mg once a day and metronidazole 100 ml thrice a day for a total duration of 5 days for compound fractures was given to all patients. IL-6 levels >10pg/ml, CRP levels >6mg/l, ESR >20mm/hr, WBC count >11000/cu.mm were considered as a diagnostic threshold beyond which these values were presumed to be positive for infection. The ESR and WBC count was determined using automated Beckman Coulter LH-750 analyzer. For CRP, approximately 3ml of blood samples were collected in a plain bulb from each patient, centrifuged for 10 mins at room temperature and then processed using fully automatic analyzer, RX-imola by immunoturbidimetric assay. For IL-6 the blood samples were centrifuged for 10 mins, serum was separated and stored at -80°C. These samples were thawed at room temperature and processed using Diaclone IL-6 ELISA kits. This was done to have adequate number of samples for processing the IL-6 ELISA kits and also to reduce the cost of the study. Data analysis was performed using SPSS (Statistical package for social sciences) version 20.0 software. To find the significant difference between infected and non-infected patients for each test value (IL-6, ESR, CRP, WBC) Mann-Whitney U tests were used. P-value < 0.05 was considered as significant.

Results

The follow up of 37 patients was lost post-operatively and so the study subjects were reduced to 100 patients. 16 out of 100 patients with lower limb fractures developed wound infection post-operatively. The minimum, maximum and mean values of IL-6, ESR, CRP and WBC of infected and non-infected patients are shown in the table 1. In 16 infected patients blood samples were collected on day of appearance of signs infection and sent for the above mentioned investigations. Out of these 16 patients 100% patients had IL-6 and CRP values beyond the diagnostic threshold level for infection, whereas for ESR, 93.75% patients and for WBC, 62.5% patients had values beyond the threshold level. On the day of suture removal 31.25% patients had IL-6

values below the threshold level for infection, whereas for ESR 18.75%, for CRP 12.5% and for WBC 87.5% patients had values below the threshold level. There was no significant difference in the measurement of IL-6 level ($p=0.858$), ESR ($p=0.371$), CRP ($p=0.183$), WBC ($p=0.746$) pre-operatively between infected and non-infected patients using Mann-Whitney U test. There was significant difference between serum CRP levels ($p=0.002$) on post-op day 2 samples between infected and non-infected patients; however no significant difference was seen between infected and non-infected patients on post-op day 2 samples between IL-6, ESR, WBC. Significant difference was observed in the measurement of serum ESR levels on day of suture removal ($p=0.004$) between infected and non-infected patients but no significant difference was seen between IL-6, CRP and WBC.

Discussion

Trauma is one of the major reasons for death among the population worldwide. Mortality occurring early after injury is usually due to "first hit" including severe organ damage, head injury, hypoxia or hypovolemia. Massive injury leads to activation of immune system. This early inflammatory immune response has been defined as systemic inflammatory response syndrome (SIRS). "Second hits" such as surgeries and infections can further augment the immune response and is co-related with high morbidity and mortality in the latter times after trauma. Thus, identifying markers which can detect infection in borderline stages before it becomes clinically evident in trauma victims can help us to aggressively treat the infection and eventually reduce their post-operative complications and duration of hospital stay. Many diagnostic tests are used for the diagnosis of infection such as ESR, CRP, procalcitonin, IL-6, tumor necrosis factor, Interleukin-8 (IL-8) but their usefulness in diagnosis of infection has been controversial. Culture from wound and intra-operative specimens can be considered an ideal marker for diagnosis of infection but its use is limited because of a number of reasons such as contamination and difficulty in collecting adequate sample. The objective of our study was to identify a series of inflammatory markers which can help in early and accurate diagnosis infection in trauma patients. In 84 non-infected patients, we observed large elevations in IL-6 (mean IL-6-247.8 pg/ml) and CRP (mean CRP-49.72 mg/l), values as compared to ESR (mean ESR-29.42 mm/hr) and WBC (mean WBC-11159.52/cu.mm) values which were just marginally raised. This suggests that IL-6 and CRP are more sensitive indicators of tissue injury. Sixteen out of 100 patients showed signs of infection post-operatively. Out of these patients, 11 patients had discharge from suture line, clinical signs of infection with a positive culture report. Three patients had discharge from suture line with clinical signs of infection but negative culture reports. One patient showed clinical signs of infection but no discharge from suture line and hence, sample for culture

	IL6(pg/ml)				CRP(mg/l)				ESR(mm/hr)				WBC(/cu.mm)			
	Day of admission	Post-op day 2	Day of suspicion of infection	Suture Removal	Day of admission	Post-op day 2	Day of suspicion of infection	Suture Removal	Day of admission	Post-op day 2	Day of suspicion of infection	Suture Removal	Day of admission	Post-op day 2	Day of suspicion of infection	Suture Removal
16 Infected patients																
Min	10.82	41.4	24.32	0	6.89	30	27.41	2.8	5	12	16	11	5600	6500	2700	4500
Max	1519.54	1393.64	737.27	144.09	216.7	769.24	386	50.91	102	122	112	107	24800	19000	22000	12800
Average	345.22125	425.0169	255.4263	45.18375	67.74813	218.1756	160.6531	19.93313	34.8125	71.3125	79.375	60.4375	11700	12456.25	12156.25	7831.25
SD	449.661107	337.268	204.558	47.75846	57.24425	172.9081	108.6182	12.59667	28.55922	34.56919	24.79751	33.81709	4740.464	3775.883	5507.204	2529.221
84 Non-infected patients																
Min	0	25.45		0	1	0.99		2	0	4		2	3500	3100		3800
Max	2014.1	1883.6		1590.9	272.4	411		137.9	99	131		150	30200	18600		13500
Average	247.8	429.7		85.0	49.72798	112.0213		24.94405	29.42857	65.16667		35.59524	11159.52	10732.14		7350
SD	296.9	376.4		195.5	54.2691	81.97787		23.93644	25.05526	35.90813		30.39464	3923.397	3034.586		2164.695
p-value	0.858	0.811		0.717	0.183	0.002		0.789	0.371	0.504		0.004	0.746	0.076		0.745
Mann Whitney U test						Significant						Significant				

Table : Comparison of values of IL-6, CRP, ESR and WBC count in infected and non-infected patients

could not be sent. One patient developed discharge from suture line along with wound dehiscence and a positive culture report which was treated by vacuum assisted closure dressings followed by secondary closure which eventually resulted in complete resolution of infection. All infected patients were treated with broad spectrum antibiotics initially followed by culture specific antibiotics. All 16 patients responded well to treatment which resulted in complete resolution with small healed wounds at suture removal. IL-6 concentration in serum was elevated beyond threshold values in all 16 patients who showed positive signs of infection postoperatively, whereas the IL-6 concentrations in 5 out of these 16 patients had returned to baseline levels after control of infection, on the day of suture removal. When compared to other markers, IL-6 levels remained elevated during the onset of infection and had returned to normal level more rapidly. Thus, the return of IL-6 to normal levels was much quicker as compared to ESR, CRP, WBC indicating that it is a better marker to monitor the course of infection to treatment. The assessment of positive response to treatment of infection can be crucial in critically ill patients and those with liver and renal disorders who cannot undergo long duration of antibiotic therapy. The association of rise in IL-6 concentration 12-48 hours before onset of major complications and with presence of infection has also been mentioned.[10,11], However, from our study we observed that IL-6 levels are markedly elevated after surgery but it is a poor marker for early diagnosis of infection as there was no statistical significant difference between infected and non-infected patients on post-operative day 2 samples between infected and non-infected patients. Our findings were in contrast to a study conducted by Douraiswami B, et al.[12] which found that IL-6 levels were significantly increased in infected patients after open fractures. IL-6 being the major stimulator of CRP, other cytokines can also stimulate the production of acute phase reactants[13] and hence it is not necessary that IL-6 levels will always co-relate with CRP levels in serum. Persistently

high levels of CRP beyond 3 days after surgery has been observed in patients with post-operative infections [14,15,16]. CRP is a widely used marker for diagnosis on infection in post-operative orthopaedic patients and multiple studies have mentioned its effectiveness in post-operative complications[17,18]. Even in our study CRP has proved to be a valuable marker of identifying infection in early post-operative period as there was significant difference between the levels of CRP in early post-operative period between infected and non-infected patients($p=0.002$) using Mann-Whitney U test and also CRP levels were elevated in all 16 infected patients on the day of appearance of signs of infection. Talebi-Taher M et al.[19] concluded from his study that ESR is a better negative predictor than a positive predictor of infection. We also observed similar findings in our study as there was significant difference between serum ESR levels between infected and non-infected patients on day of suture removal($p=0.004$) using Mann-Whitney U test. Certain limitations were observed in our study. Ideally, we should have calculated the IL-6 levels of these patients every alternate day from the day of sustaining injury till the day of suture removal and compared them with levels of ESR, CRP and WBC to find the exact rise and fall of these parameters in response to infection. But this was not possible because of the high cost of IL-6 investigation. We didn't collect an additional sample in non-infected between post-op day 2 and day of suture removal to compare it with the additional sample taken post-operatively in infected patients as the day of appearance of signs of infection varied in each patient.

Conclusions

We conclude that CRP is a better marker for early diagnosis of infection of lower limb fracture patients as compared to IL-6, ESR, WBC. However, IL-6 is a better marker to monitor the course of infection because of its early return to baseline levels as compared to ESR, CRP and WBC.

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