CORRELATION OF TOTAL COUNT (WBC), NEUTROPHIL COUNT AND CRP TEST AMONG FEBRILE PATIENTS IN NAVIMUMBAL.

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Abstract

150 febrile patients included children (50), adult (50) and neonates (50) from outpatient departments and inpatients of private clinics and hospitals. Patients presented with fever and chills for more than 1 day to 3 days, throat infection, ear infection and cold and fever and only fever as the principal symptoms. After clinical examination all the patients were prescribed for Complete Blood Count (CBC) with differential count(DC) and C-reactive protein(CRP) tests, and in children below 14 years anti-Streptolysin O(ASO) tests (75) were prescribed.

Patients treated with antibiotics previously two weeks before the study period were not included. Qualitative and quantitative tests were performed on all patients' samples included in the study depending on the need/prescription by the physician or paediatrician. CBC, neutrophil count and CRP have been very useful indicators and significant in the diagnosis and treatment as well as follow-up of the febrile condition of the patients specially in patients suffering with bacterial infections. Even in patients with Dengue and malaria it gives a fair idea if there were leucocytosis or leukopenia, neutrophilia or neutropenia, thrombocytosis or thrombocytopenia.

CBC: Complete blood count, DC: Differential count; MP: malarial parasite, CRP: C-reactive protein, ASO: Anti-Streptolysin O.

Introduction:

Febrile illness indicates infection in active form. Fever is a non-specific immune response exhibited by the host's immune system whenever there is infection or inflammation. Clinical examinations with diagnostic investigations will give clear idea about the infection. If the infection is due to bacterial, viral, parasitic or fungal origin or also in response to inflammation by the hosts immune system for different type of antigens.

The complete blood count (CBC) is the most frequently requested blood test to understand the Haemoglobin percentage, total count, platelets cell counts and indices which give clear indication of infections and inflammation when levels are raised or decreased or within range. A major portion of the complete blood count is the measure of the concentration of white blood cells, red blood cells,

and platelets in the blood. The primary points of interest in the CBC are often whether a patient is anaemic, whether the white blood cell count shows evidence of infection and whether the platelets are at a level that may affect haemostasis. And increase or decrease in neutrophil count has lot of diagnostic value too. Neutrophils are phagocytic granulocytes that constitute an important component of the rapid "non-specific" immune defences. They, like other leucocytes, are derived from pluripotent stem cell progenitors in the bone marrow.¹ Appropriate investigation can lead to specific diagnoses, and general and specific management measures can reduce both mortality and morbidity and permit genetic counselling and antenatal diagnosis in some cases¹.

CBC with differential count has been a baseline test prescribed by the clinicians all over the world along with CRP, ASO test if the clinician suspects

Bacterial/Streptococcal infections specially in paediatric patients. There are many more parameters which are tested if the clinician could understand the course of the disease with classical symptoms like fever with chills, body ache, joint pains. Dengue and Malaria are prevalent in Mumbai and Navi Mumbai area especially during monsoon and post monsoon seasons.

The study included 150 patients with adult (50) and paediatric (50) and neonatal (50) population from private clinic and hospital, out patients and in patients from NaviMumbai. All the patients were suffering from febrile illness with at least one of these symptoms- throat pain, ear pain, cold, cough, and other general symptoms like malaise and headache. All the subjects were tested for CBC, DC, CRP &ASO test in 75 patients. Depending on the need, other investigations are done including ASO test, Malarial parasite detection, Dengue NS1 Ag, Dengue IgM, Widal test, Blood culture and sensitivity, other diagnostic tests including chest X-ray in case of lung infections.

CBC is done as a part of routine check-up, to know the haemoglobin count to check anaemia, to check the infection or inflammation and also to understand that how the medication or treatment (medication) is working (affecting) on patient's body etc.

CRP, White cell count and absolute neutrophil count are important inflammatory markers used in early detection of acute infections in children and neonates². CRP is acute phase protein in serum or plasma, rises during unspecified (non-specific) response to infections and non-infectious inflammatory processes including rheumatoid arthritis, cardiovascular diseases and also in peripheral vascular diseases. Increase in CRP values are non-specific and should not be interpreted without complete clinical history. CRP and CBC has been used routinely to diagnose the bacterial infections which are regularly requested by paediatricians and clinicians alike. C-reactive protein elevation is part of the acute phase response to acute and chronic inflammation. Commonly CRP is raised in bacterial infections & inflammation even though it's a non-specific marker. Total count and neutrophil count rises too in most bacterial infections. And also among bacterial infections of Group A Streptococcal infection which causes Strep throat, Pharyngitis, Laryngitis, Otitis media, other suppurative lesions. Asuppurative complications like Acute Rheumatic

fever and Acute Glomerulo-nephritis (by nephritogenic strains). In non-suppurative complications CRP is raised multiple times and CRP level may increase and even ASO titre may increase more than 100mg/dL to 1000mg/dL and beyond which depends on what stage of the complication, ASO and CRP test are done³.

WBC (leukocyte) count: $4.5-11.0 \times 10^9/L$. Neutrophil count: $3-7 \times 10^9/L$, CRP is usually measured in milligrams of CRP per litre of blood (mg/L). An anti-Streptolysin O titre greater than 166 Todd units (or >200 IU/mL) is considered a positive/mcL

Early diagnosis of dengue infection remains a challenge around the world in areas of limited resources, laboratory parameters like CRP, Neutrophil counts, platelets count may serve as predictive markers to promote early diagnosis. The objectives of one of the studies were to stratify the levels of C-reactive protein and Neutrophil counts in children with dengue fever and to determine the correlation of C-reactive protein and Neutrophil counts with the severity of dengue⁴. Whenever dengue is suspected in febrile patients, all these tests are done (haematological parameters) depending on the clinical suspicion and as dengue involves liver and other organs, Liver function tests show a clear biochemical indicator⁵.

Materials and methods:

150 patients are randomly selected who visit OPD and admitted as in-patients of tertiary care hospitals in Navi Mumbai. 50 patients were adult patients who were above 18 years of age and rest 50 were children who were less than 18 years of age and 50 patients were neonates.

5 ml blood was collected from all the patients (neonates :1-2 ml) and 2 ml is put into EDTA bulb and 3mL into plain/serum blood. EDTA bulb is rolled slowly to mix the anticoagulant and serum bulbs were allowed to clot up to 10 minutes before centrifugation at 3000 revolutions per minute(RPM) for 5 minutes.

Complete blood Count was performed by 3 part and 5-part differential methods in fully automated Hematology analysers and differential count was calculated by manual method by observing the peripheral smear which is stained by Leishman's staining technique.

ASO titre and CRP titre was performed by manual testing method for qualitative purposes and automated technique for further titration (quantitative method).

ASO latex agglutination is measured in IU/mL and CRP is measured in g/dL..or mg/L normal value for ASO test is considered as < 200IU/mL >200IU/ml is considered as positive and further titration(dilution) is done to know the exact titre., in CRP < 6mg/dL is considered normal.

Results:

Out of 150 patients tested for CBC, CRP and 75 ASO tests were performed. 24 patients showed ASO test positive among them 4 were adults 15 were children and 5 were neonates. Average of ASO titre was 400 IU/ml and highest was 1200IU/ml and lowest were negative that is less than 200IU/ml.

Total count among neonates ranged from 2300 being lowest, 9250 average and 18300 highest, which indicated there were definitely some infection or inflammation in the patients who showed above 11,000 total count. Neutrophil count showed 34 lowest, 55.16 average and 81 highest. And when CRP testing was done <6mg/dL was the lowest,8.32 was the average and 24 was the highest among neonates. When further investigation preceded bacterial isolates of *Staphylococcus aureus*, *Streptococcus* group A and other pyogenic bacteria were grown which clearly indicates the association of bacterial infection and increase in CRP in the tested patient's samples. 11 throat swab culture out of 25 yielded bacterial growth. Among 45 blood culture samples 18 pyogenic bacterial strains were isolated in same number of patients.

In children, lowest total count was 2900, 16700 was average and 18300 being highest which is almost similar in neonates too. In the neutrophil count,23 was lowest,82 was average and 85 was highest. That showed most patients were above average in their neutrophil counts. When CRP tests were done from the serum samples of the patients tested, 1.5mg/dl was lowest 27.17mg/dl was average and 85.2 mg/dl being the highest.

Among adult patients lowest total count was, 4100, and 16700 was average and 18100 was highest count. In neutrophil count 14 was lowest, 46 was average and 81 was highest. In CRP testing done 0.86mg/dl

was lowest,12.52 mg/dl was average and 59.25mg/dl was highest.

In children lowest total count was-2900, 16700 was average and highest was 18500. Neutrophils count showed different counts, 23 was lowest, 82 was average and 85 was highest. Here neutrophils were towards higher side when compared to adult patients.

Neonatal patients total count showed 2300 as the lowest count average being 9250 and highest being 18300. Among Neutrophils, lowest being 34, average was 55 and highest was 81. In CRP lowest was 0, average was 8.32 and highest was 24mg/dl.

Tables 1:

Age group	Results range	Total Count	Neutrophil count ()	CRP/ mg/dl
Neonates (n=50)	Lowest	2300	34	0
	Highest	18300	81	24
	Average	9250	55	8.32
Children (n=50)	Lowest	2900	23	1.5
	Highest	18500	85	85.2
	Average	16700	82	27.17
Adults (n=50)	Lowest	4100	14	0.86
	Highest	18100	81	59.25
	Average	16700	46	12.52

Statistical Analysis:

The data was entered into a MS-Excel worksheet and cleaned. Further analysis was performed using IBM SPSS Statistics (Version 21.0) Software. The results were presented using descriptive statistics such as Min, Max, Mean, SD, SEM and 95 % confidence interval for mean. Further the study variables compared among three study groups Adults, Children and Neonates using One Way ANOVA (F-Test). The correlation coefficient among study parameters were calculated using Karl Pearson's correlation coefficient. The level of significance was set at 55. All p-values less than 0.05 were treated as significant.

Analysis:

Table 2: Descriptive Statistics for Study Parameters

		N	Mean	SD	SEM	95% CI		Min	Max	F-stat	P-value
						Lower Bound	Upper Bound				
WBC Count	Adults	50	8312.00	3292.89	465.68	7376.17	9247.83	3600.00	18100.00	1.59	0.206, NS
	Children	50	9772.00	4160.95	588.45	8589.47	10954.53	2900.00	18300.00		
	Neonates	50	9250.20	4826.83	682.62	7878.43	10621.97	2300.00	18300.00		
	Total	150	9111.40	4157.98	339.50	8440.55	9782.25	2300.00	18300.00		
Neutrophils	Adults	50	55.30	13.42	1.90	51.49	59.11	14.00	86.00	0.34	0.707, NS
	Children	50	57.46	17.98	2.54	52.35	62.57	23.00	89.00		
	Neonates	50	55.30	12.98	1.84	51.61	58.99	34.00	81.00		
	Total	150	56.02	14.90	1.22	53.62	58.42	14.00	89.00		
CRP	Adults	50	19.94	40.20	5.68	8.52	31.37	0.85	264.00	6.19	0.003**
	Children	50	27.17	22.58	3.19	20.76	33.59	0.72	85.20		
	Neonates	50	8.28	8.62	1.22	5.83	10.73	0.00	24.00		
	Total	150	18.47	28.01	2.29	13.95	22.98	0.00	264.00		

****:** significant at 1% level of significant, **NS:** Not Significant

The above table shows descriptive statistics for the study parameters WBC count, Neutrophils & CRP among Adults, Children and Neonates. Further One Way ANOVA Analysis indicates no significant difference in the WBC Count and Neutrophils among Adults, Children and Neonates ($p = .206$). The study parameter CRP shows a significant difference among the Adults, Children and Neonates ($p .003$). The results were also shown in the means plot as below.

Means Plot for WBC Count

Means Plot for Neutrophils

Means Plot for CRP

Table 3: Correlations among study parameters

		Neutrophils	CRP
WBC Count	Pearson Correlation	.400**	.135
	Sig. (2-tailed)	.000	.101
	N	150	150
Neutrophils	Pearson Correlation		.181*
	Sig. (2-tailed)		.027
	N		150

****.** Correlation is significant at the 0.01 level (2-tailed).

*****. Correlation is significant at the 0.05 level (2-tailed).

The above table shows the Karl Pearson's correlation coefficient between study parameters WBC count, Neutrophils and CRP. The correlation coefficient between WBC count and Neutrophils ($r=0.400$) was significant ($p < .001$). The correlation coefficient between WBC count and CRP ($r = 0.135$) was not significant ($p = 0.101$). The correlation coefficient between Neutrophils and CRP ($r=0.181$) was found to be significant ($p=.027$). The results were also shown in the Scatter diagram below.

Table 4: Correlations among study parameters (For Adults)

		Neutrophils	CRP
WBC Count	Pearson Correlation	-.110	.173
	Sig. (2-tailed)	.448	.230
	N	50	50
Neutrophils	Pearson Correlation		-.004
	Sig. (2-tailed)		.977
	N		50

a. Group = Adults

The above table shows the Karl Pearson's correlation coefficient between study parameters WBC count, Neutrophils and CRP. The correlation coefficient between WBC count and Neutrophils ($r = -.110$) was not significant ($p = .448$). The correlation coefficient between WBC count and CRP ($r = .173$) was not significant ($p = .230$). The correlation coefficient between Neutrophils and CRP ($r = -0.004$) was found to be not significant ($p = .977$). The results were also shown in the Scatter diagram below.

Table 5: Correlations among study parameters (for children)

		Neutrophils	CRP
WBC Count	Pearson Correlation	.333*	-.157
	Sig. (2-tailed)	.018	.278
	N	50	50
Neutrophils	Pearson Correlation		.284*
	Sig. (2-tailed)		.046
	N		50

*. Correlation is significant at the 0.05 level (2-tailed).

a. Group = Children

The above table shows the Karl Pearson's correlation coefficient between study parameters WBC count,

Neutrophils and CRP. The correlation coefficient between WBC count and Neutrophils ($r = -.330$) was significant ($p < .018$). The correlation coefficient between WBC count and CRP ($r = -.157$) was not significant ($p = .278$). The correlation coefficient between Neutrophils and CRP ($r = 0.284$) was found to be significant ($p = .046$). The results were also shown in the Scatter diagram below.

Table 6: Correlations among study parameters (for Neonates)

		Neutrophils	CRP
WBC Count	Pearson Correlation	.860**	.872**
	Sig. (2-tailed)	.000	.000
	N	50	50
Neutrophils	Pearson Correlation		.869**
	Sig. (2-tailed)		.000
	N		50

**. Correlation is significant at the 0.01 level (2-tailed).

a. Group = Neonates

The above table shows the Karl Pearson's correlation coefficient between study parameters WBC count, Neutrophils and CRP. The correlation coefficient between WBC count and Neutrophils ($r = 0.860$) was significant ($p < .001$). The correlation coefficient between WBC count and CRP ($r = .872$) was significant ($p < .001$). The correlation coefficient between Neutrophils and CRP ($r = 0.869$) was found to be significant ($p < .001$). The results were also shown in the Scatter diagram below.

Discussion:

The neutrophil-lymphocyte count ratio has been identified as predictor of bacteraemia in medical emergencies⁶. One of the study showed that 'in patients with acute medical emergencies who are suspected of bacteraemia clinically, CRP concentrations although associated with bacteraemia, have limited role in bacteraemia prediction.

Study on effectiveness of WBC count and CRP to rule out diagnosis of appendicitis mentions that no patient with appendicitis had both a normal WBC count and CRP level⁷. As CRP, neutrophil count may serve as a predictive marker to promote early diagnosis and neutropenia for early predictor of severe dengue⁸. In bacterial infections with febrile illnesses with otitis media, acute tonsillitis CRP is one of the valuable tool in evaluating children who have been ill for more than 12 hours⁹. In the cases of *Legionella pneumophila* pneumonia had higher CRP levels than that of pneumonia of other aetiology and CRP test is cheap, readily available, may be a useful adjunctive procedure of community acquired pneumonia¹⁰. Monocyte and neutrophil direct counts with CRP plasma concentrations in patients with acute pancreatitis from Poland¹¹.

Conclusion:

CBC, Neutrophil count and CRP have been best indicators of bacterial infection among febrile patients in all age groups (Neonates, Children and adults). Increased total count(leucocytosis), Neutrophilia, raised CRP can be an indirect evidence of bacterial infection or inflammation. Results reveal that CBC, neutrophil count and CRP tests are must do tests in febrile patients although, ASO test, Culture & sensitivity tests, liver function tests can be added to cover viral infections too. Pyogenic bacterial infections causing throat infections especially in children these tests have best diagnostic value. ASO tests considered best for paediatricians point of view to diagnose post-Streptococcal infections and sequelae. (Acute rheumatic fever and acute glomerulonephritis). Even though culture and sensitivity (Gold standard) of affected areas (For eg.:

throat swab), blood culture in febrile patients should be added in case the patient suffers from blood stream infections which gives clinicians a clear evidence of the infection. Patients with low platelet count, neutropenia may indicate dengue or other viral infections too.

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