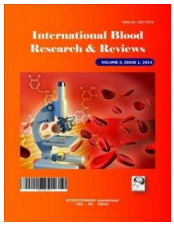

International Blood Research & Reviews
4(1): 1-7, 2015, Article no.IBRR.17737
ISSN: 2321–7219

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Level of Pentraxin-3 in Patients with Acute Leukemia in Septicemia and Its Prognostic Value

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Authors' contributions

This work was carried out in collaboration between all authors. Author AE designed the study and contributed in writing protocol and manuscript. Authors HN and M. Eldefrawy contributed in writing manuscript. Author M. Elbordeny did the laboratory work and contributed in writing manuscript. Author HM collected samples & data and contributed in writing manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IBRR/2015/17737
Editor(s):
(1) Tadeusz Robak, Medical University of Lodz, Copernicus Memorial Hospital, Poland.
Reviewers:
(1) Golam Hafiz, Department of Pediatric Hematology and Oncology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.
(2) Shigeki Taga, Department of Obstetrics and Gynecology, Japanese Red Cross Okayama Hospital, Japan.
Complete Peer review History: http://sciencedomain.org/review-history/10215

Original Research Article

Received 25th March 2015
Accepted 24th June 2015
Published 16th July 2015

ABSTRACT

Introduction: In acute leukemia, sepsis is potentially fatal. Pentraxin3 is a protein rapidly produced in response to primary inflammatory signals. It shows high levels in sepsis, specially associated with vascular and end-organ damage.
Aim of the Work: To measure the level of PTX3 in sepsis in patients with acute leukemia and correlate its level to higher risk of complications compared with CRP.
Study Design: Prospective study.
Place and Duration of the Study: Department of hematology, Alexandria main university hospital, from April 2012 to August 2013.
Methods: The study included 60 patients, they had routine workup for leukemia. Serum CRP and plasma PTX3 levels were measured with ELISA on days 1, 2, 3 of febrile neutropenia after chemotherapy.
Results: Male to female ratio 1:1, age ranged from 18 to 62 years (median of 40 yrs). 41 patients

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suffered from acute myeloid leukemia, and 19 from acute lymphoblastic leukemia. High PTX3 levels on the 1st day of sepsis have been a strong indicator for development of complications (septic shock and mortality) (*P*=.001) compared to CRP (*P*=.032). High PTX3 level has been associated with coagulation impairment (*P*=.001). PTX3 showed sensitivity of 100% and specificity of 70% for prediction of bad prognosis, whereas CRP showed sensitivity of 88.5% and specificity of 60.5%.

Conclusion: PTX3 is highly recommended in diagnosis of sepsis in patients with acute leukemia during neutropenia and it shows high sensitivity and specificity in prediction of bad prognosis (septic shock, coagulation impairment and mortality) in comparison with CRP.

Keywords: Sepsis; febrile neutropenia; PTX3; CRP.

1. INTRODUCTION

Sepsis is a potentially fatal condition characterized by an inflammatory state called SIRS orsystemic inflammatory response syndrome which is induced by an infection. It is considered one of the most common causes of complications and mortality during neutropenia after chemotherapy in acute leukemia patients. [1,2].

Early detection of infection as well as early prediction of bad prognosis using serological markers can aid in early intervention and treatment of infectious condition and thus improvement of outcome of acute leukemia treatment and reduction of mortality during bone marrow aplasia [3].

Pentraxin 3 (PTX3) is a member of the pentraxin superfamily. It is a protein which is encoded by the PTX3 gene in humans. The PTX3 protein is composed of 381 amino acids, of 40,165 Da molecular weight. It has a carboxy-terminal (203 amino acid long) pentraxin domain and an amino-terminal (178 amino acid long) domain unrelated to other known proteins [4].

PTX3 is released by several types of cells, particularly by mononuclear phagocytes, dendritic cells (DCs), fibroblasts and endothelial cells in response to inflammatory signals (e.g. Toll-like receptor (TLR) engagement, TNFα, IL-1β). It then binds to the complement component C1q, the extracellular matrix component TNFα induced protein 6 and selected microorganisms. PTX3 activates the classical pathway of complement activation and facilitates pathogen recognition by macrophages and DCs. In addition to its pro-inflammatory activity, PTX3 also has been shown to play a role in protecting against severe inflammatory reactions [5-9].

PTX3 concentration in healthy individuals is lower than 2 ng/ml and increases rapidly in response to inflammation and infection [10,11]. It is expressed in a variety of cells at inflammatory sites and also stored in neutrophil-specific granules. The stored PTX3 in neutrophils is released into the extracellular space and localizes to neutrophil extracellular traps (NETs) In septic patients, specially associated with vascular damage, the circulating PTX3 concentration increases to characteristically high levels [12-15]. C-reactive protein (CRP) belongs to the group of short pentraxins. Because of its origin and induction by proinflammatory cytokines and bacterial products, PTX3 level is used to diagnose sepsis more rapidly than CRP. Also the plasma PTX3 level shows a good correlation with mortality and dysfunction of several organ systems [16-19].

2. METHODOLOGY

The study is a prospective observational analytical study, it took place at the Haematology Department in Alexandria University Main Hospital between 2012 and 2013. The study has included 60 patients with sepsis and acute leukemia patients after receiving chemotherapy, during severe neutropenia (ANC< 500).

All patients diagnosed with acute leukemia based on complete blood count, bone marrow examination and flow cytometry [20-21] will be subjected to the following during the period of neutropenia: Thorough history taking by checking all the complaints that may imply a source of infection, Thorough daily clinical examination during the period of neutropenia, Daily complete blood picture with differential blood count [20,21]. Complete sepsis work up for neutropenic fever including blood culture from peripheral lines as well as from central lines, sputum culture, urine analysis, chest radiograph, swab from any evident infected sites [22,23]. Measurement of quantitative C-reactive protein level in the serum [24] and measurement of pentraxin 3 level in

plasma measured with a sandwich-type ELISA [25,26]. (Both are measured from day 1 to day 3 afterthe onset of fever with a cut-off point of 3 mg/l and 2 ng/dl respectively), Hepatic and renal function tests [27].

3. RESULTS AND DISCUSSION

Table 1 shows the demographic data of the patients. In patients with AML, M1 was found in 22%, M2 was found in 9.7%, M4 was found in 29.2% and M5 was found in 39% of patients. Most of the studied patients were AML M5.

Table 1. Demographic data of the 60 patients

Table 1 shows characters of studied cases		
Gender		
Males	28	(46.7%)
Females	32	(53.3%)
Mean age (years)		39.2±7.22
Range (years)		18-62
Type of leukemia		
AML	41	(68.3%)
B- ALL	15	(25%)
T- ALL	4	(6.7%)

There has been 16 patients developed septic shock and 12 died from 60 patients. Eight patients suffered from severe sepsis and multiorgan failure on days 6, 7, 9, 10 and 11. Two patient developed severe chest infection and intubated, died after mechanical ventilation. Two patient developed sudden arrest on day 6 & 9. Four other patients developed septic shock, but survived after treatment of sepsis and shock. For other 44 patients, correction of sepsis by antibiotics & antifungal drugs was successful.

Table 2 shows the Distribution of the studied patients regarding pentraxin 3 and CRP levels on the three days of the study. There was positive correlation between PTX3 and CRP on the three days as shown in Table 3.

In cases with severe sepsis who developed septic shock or death, PTX3 showed high levels from the 1st day, whereas CRP showed less sensitivity. Relation between bad and good prognosis regarding PTX3 and CRP level on day 1 is shown in table 4. Fig. 1 and Tables 5 and 6 show ROC curve, area under the curve and curve coordinates respectively, to represent the sensitivity and specificity of CRP and pentaraxin 3 on day 1 in cases with bad prognosis.

Table 2. Distribution of the studied patients regarding pentraxin 3 and CRP levels on the three days of the study

Pentraxin 3 (PTX3) (ng/ dl)			
Day 1 (Mean)	5.32±4.91		
Median	3.44		
Day 2 (Mean)	9.2±6.34	.003*	
Median	7.0		
Day 3 (Mean)	8.88±6.11	.020*	.22
Median	6.2		
C-reactive protein (CRP) (mg/L)			
Day 1 (Mean)	100.54±33.1		
Median	101		
Day 2 (Mean)	147.8±66.6	.0001*	
Median	140		
Day 3 (Mean)	169.33±92.42	.0001*	.107
Median	150		

There was positive correlation between PTX3 level on day 1 and INR with P value =.001. Blood culture were positive in 12 cases (20% of cases), while 48 cases (80%) were negative for organisms after 7 days of incubation. In 26 patients there was evidence of lower respiratory tract infection either by clinical examination or by chest radiographs, in other 14 patients upper respiratory tract infection was present. Cannula site infection in 4 patients, gastrointestinal infection in 12 patients and urinary tract infection in 10 patients were present. The evidence of fungal pneumonia and/or fungal oropharyngitis was present in 10 patients.

Table 3. Correlations between the PTX3 at different days and CRP

		PTX3 day 1	PTX3 day 2	PTX3 day 3
CRP day 1	Pearson Correlation	.531		
	P	.003*		
CRP day 2	Pearson Correlation		.450	
	P		.013*	
CRP day 3	Pearson Correlation			.525
	P			.003*

4. DISCUSSION

There was statistically positive correlation between PTX3 and CRP on the three days of the study. In agreement with our results, Vänskä et al. [28] held a similar study in Kuopio University which evaluated pentraxin 3 as a marker for complications of neutropenic fever in 100 hematologic patients receiving intensive chemotherapy in comparison with CRP.

High PTX3 was showed to be associated with mortality in severe sepsis and bacteremic patients [15,29,30] CRP is a widely-used short pentraxin. As an inflammatory marker, it has a limited specificity and poor diagnostic value [31,32]. In this study, there was a separate analysis for the 16 patients who developed complications (septic shock or mortality), and comparison of plasma PTX 3 level and serum CRP level on day1 revealed that: Ten patients had plasma PTX3 level above 10 ng/ml on the 1st, 2nd and 3rd day. For the same patients, CRP levels were high in a rising manner along the 1st, 2nd and 3rd day to reach the peak on the last one.

Table 4. Relation between bad and good prognosis regarding PTX3 and CRP level on day 1

PTX3	Bad prognosis	Good prognosis
Mean	8.22	4.26
S.D.	1.39	2.01
T		3.65
P		.001*
CRP	**Bad prognosis**	**Good prognosis**
Mean	125.6	92.6
S.D.	22.6	30.2
T		.98
P		.032

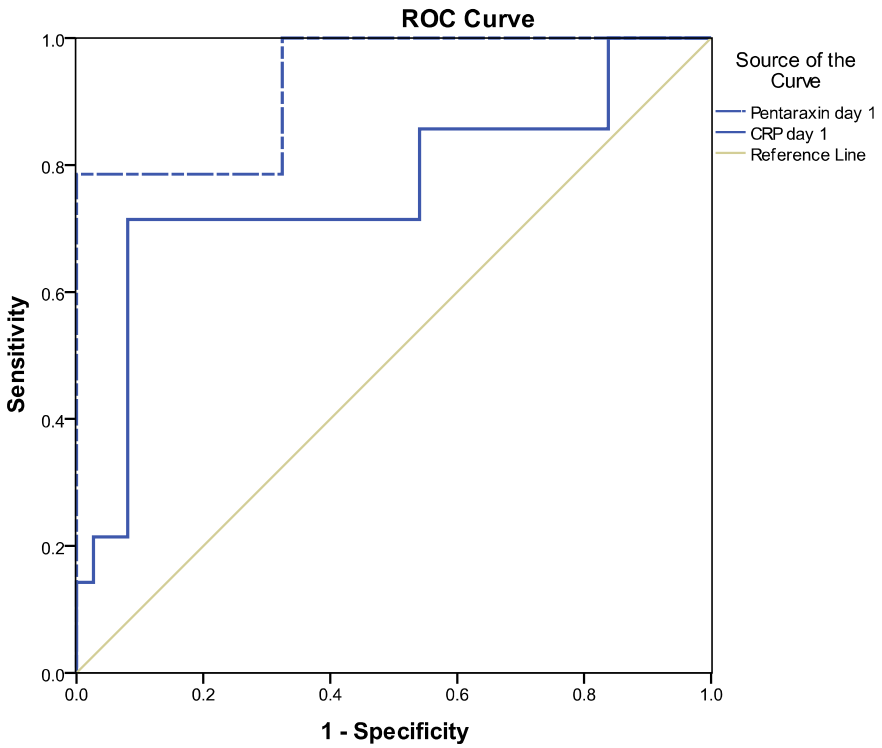

Fig. 1. ROC curve to determine the sensitivity and specificity of CRP and PTX3 on day 1 in cases with bad prognosis (septic shock and death)

Table 5. Area under the curve

Test result variable(s)	Area	Std. error(a)	Asymptotic sig.(*P*)	Asymptotic 95% confidence interval	
				Lower bound	Upper bound
Pentaraxin day 1	0.906	0.071	.001*	0.813	1.016
CRP day 1	0.789	0.107	.022	0.574	0.987

Table 6. Coordinates of the curve

Test result variable(s)	Positive if greater than or equal to(a)	Sensitivity %	Specificity %
Pentraxin 3 day 1	3.3500	100.0	70.0
CRP day 1	89.00	88.5	60.5

So, Peak levels of pentraxin 3 were obtained from the first day unlike CRP. Six patients had pentraxin3 level > 5 ng/ml on the first day and > 10 ng/ml on the 2nd day. In either case CRP attained arising manner reaching the maximum on the third day.

From the results mentioned above, high PTX3 on day one can predict bad prognosis with a strong statistical significance. This was agreed by, Vänskä et al. [28] who said that high PTX3 level on day 0 was associated with the development of septic shock and its level was constantly high in non-survivors. Another study at The Tampere University hospital of Finland in 2011 included 132 patients affected by bacteremia. Measuring of PTX3 level revealed that the maximum PTX3 values on days 1-4 were markedly higher in non-survivors compared with survivors [29]. Coppadoro [33] said that PTX3 remained significantly higher in nonsurvivors than in survivors over the first 5 days of sepsis, and that septic shock patients had higher PTX3 levels than patients with severe sepsis on day one. He also said that early persisting elevation of plasma pentraxin 3 is associated with coagulation impairment in severe sepsis and septic shock.

PTX3 can up-regulate tissue factor in activated monocytes, so an association between PTX3 and clotting activity in sepsis may be involved in development of DIC in these conditions [34]. In this study, 22 cases were complicated by coagulation impairment in the form of prolonged INR and/or PTT. There was statistically positive correlation between PTX3 level on day one and INR value. It is worth saying that the coagulation defect in these cases may be affected by impairment in liver functions in some of them due to severe sepsis and Multiorgan failure. However, in some cases impairment of liver functions occurred without concurrent coagulation defect.

A number of cases [16] showed decrease in the levels of both CRP and pentraxin 3 level after the first (12 cases) or second day (4 cases), all of them showed improvement of fever within hours after treatment which indicated a good response to antibiotic therapy. This proves that early interventions and appropriate antimicrobial treatment can be lifesaving. This requires early diagnosis and risk determination which can be provided by the use of pentraxin 3 which is highly sensitive.

Respiratory tract infection was the most common cause of infection during sepsis. With increased incidence of lower than upper respiratory tract infection. Rodrigues et al. [35] said that chest infection is the most common source of infection in neutropenic fever. The evidence of fungal infection was determined in 10 patients either clinically (oropharyngeal) or by chest CT. Four of them were complicated by severe sepsis and septic shock and showed very high levels of Pentraxin 3 (>10 ng/ml). It is worth mentioning that these cases were treated from septic shock.

A study in mice made by Gaziano et al. [36] showed that PTX3 induced a curative response in mice with invasive aspergillosis either alone or in combination with antifungal agents. Prophylactic PTX3, either locally or systemically, was effective but it did not show direct activity on fungal cells. Therefore, the effect of PTX3 appears to rely on its ability to increase protective T-helper1-dependent resistance. So, the results showed the following: 1) complete resistance to infection and reinfection in mice treated with PTX3 alone. 2) the protective effect of PTX3 was found similar or superior to that observed with liposomal or deoxycholate amphotericin B, respectively. 3) protection was associated with accelerated recovery of phagocytes and T-helper-1 lymphocytes in lung. 4) PTX3 potentiated the therapeutic effect of suboptimal doses of antifungal drugs. These data suggest the potential therapeutic use of PTX3 either alone or in combination to antifungal therapy in *Aspergillus fumigatus* infections [36].

5. CONCLUSION

PTX3 is a highly sensitive marker for detection of sepsis with high prognostic value. High PTX3 levels indicate septic shock, coagulation impairment and multiple organ failure with high sensitivity and specificity. It is recommended to perform further studies to confirm the therapeutic value of PTX3 in treatment of fungal infection and production of drugs approved for human use.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this results.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Research was agreed by the ethical committee on March 2012 (IRB 0101978)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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