

## Serum Levels of Angiopoietin-2 and C-Reactive Protein in Chronic Myeloid Leukaemia Patients: Relation to Different Phases of the Disease

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**ABSTRACT:** Serum levels of Angiopoietin-2 (Ang-2) and C-reactive protein (CRP) were measured in 50 patients with chronic myeloid leukemia (CML) (30 patients in chronic phase (group A) and 20 patients in advanced phase (group B)) and 15 healthy age and sex matched subjects as a control group, to investigate their relation to different phases of the disease. Serum levels of both Ang-2 and CRP were significantly higher ( $p < 0.05$ ) in patients group compared to controls, and in advanced stage compared to chronic phase. Furthermore a significant positive correlation was detected between Ang-2 and CRP in the whole patients group which could support the hypothesis that CRP might play a role in modulating angiogenesis. The present data suggest that both Ang-2 and CRP could play a role in the leukemic process. Understanding their roles may help in follow-up care and in designing new therapeutic strategies for CML. Furthermore, the role of CRP in modulating angiogenesis should not be underestimated.

### INTRODUCTION

Chronic myeloid leukaemia (CML) is a clonal disease that results from an acquired genetic change in a pluripotential stem cell.<sup>(1)</sup> It is characterized by granulocytic leucocytosis, granulocytic immaturity, basophilia, anaemia, intense marrow granulocytic hyperplasia and splenomegaly.<sup>(2)</sup> It is a polyphasic disease which progresses from its chronic phase to an accelerated phase where the leukaemia is more difficult to control and additional chromosomal abnormalities appear, followed by a progressive increase in blast cells in blood and marrow termed the blastic phase or blast crisis.<sup>(3)</sup> Angiopoietin-2 (Ang-2) is a member of

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the angiopoietin family; plays an important role in angiogenesis during the development and growth of human cancers.<sup>(4)</sup> The system involving Ang-2 and its receptor Tie-2, appears to play an important role not only in tumor angiogenesis, but also in the biology of haematological and non-haematological malignancies.<sup>(5)</sup>

Ang-2 modulates angiogenesis in a cooperative manner with another important angiogenic factor, vascular endothelial growth factor (VEGF) <sup>(4)</sup>. In the presence of VEGF, Ang-2 collaborates at the front of invading vascular sprouts, serving as an initial angiogenic signal.<sup>(6)</sup>

C-reactive protein (CRP) is a plasma protein, produced mainly by the liver <sup>(7)</sup> and by adipocytes.<sup>(8)</sup> under the control of interleukin-6 (IL-6). It is an acute phase reactant expressed during active inflammation.<sup>(9)</sup> CRP has been shown to promote production of the pro-angiogenic molecules such as endothelin-1 (ET-1) and IL-6 in human saphenous vein

endothelial cells.<sup>(10)</sup> with a possible role in angiogenesis.<sup>(11)</sup>

The aim of the present work was to study the serum level of Ang-2 and CRP in chronic myeloid leukemia patients, and their relation to different phases of the disease, also to investigate the angiogenic properties of CRP and to find out if there is any correlation between these two parameters.

#### **PATIENTS AND METHODS**

The present study included 65 subjects divided as follows: 15 apparently healthy age- and sex- matched individuals as a control group, 50 patients with CML (referred to hematology department, Medical Research Institute, Alexandria University, Egypt for routine follow up), 20 males (40%) and 30 females (60%). The patients were subdivided into two groups: Group A: 30 patients in chronic phase, their ages ranged from 17 to 70 years with a median age of 41 years, and Group B: 20 patients in advanced phase (accelerated phase and blastic crisis), their

ages ranged from 22 to 70 years with a median age of 42.50 years.

A written informed consent was obtained from all patients and Controls before enrollment in the study.

All patients were subjected to the following:

- Full history taking and thorough clinical examination with special consideration to the size of the spleen.
- Routine laboratory investigations including complete blood picture as well as renal and liver function tests.
- Imaging studies including CT scans and/or MRI were done whenever indicated.
- Measurement of CRP serum level using turbidimetric immunoassay.<sup>(12)</sup>
- Measurement of Ang-2 serum level using enzyme linked immunosorbant assay.<sup>(13)</sup>

The diagnosis of CML was done by:

- \* Standard morphology of peripheral blood and bone marrow films confirmed by PCR analysis of bcr/abl gene.<sup>(14)</sup>
- \* Immunophenotyping (for blast cells)

using a comprehensive panel of monoclonal antibodies (mAbs) against myeloid and lymphoid associated antigens as proposed by the European group for the characterization of leukaemia (EGCL) group.<sup>(15)</sup> was done to cases in blastic crisis.

#### **Statistical methods:**

Statistical analysis was done using SPSS program" version 17"(Statistical Package of social sciences, Chicago, USA)

#### **RESULTS**

Table 1 shows some important haematological findings in group A and group B. There was a statistically significant difference between the two groups as regards haemoglobin concentration, platelets count and bone marrow blast percentage.

Patients group showed a statistically significant ( $p < 0.001$ ) higher median level of serum Ang-2 and CRP when compared to control group (Figure1).

A statistically significant higher median serum level of both Ang-2 and CRP was

observed when comparing patients group to control group and when comparing group A to group B (table 2).

#### **Spleen size and serum levels of Ang-2 and CRP in patients:**

When subdividing all CML patients according to spleen size it was found that, in the chronic phase, 16 patients showed mild (53.3%), 8 patients showed moderate (26.7%) and 6 patients showed huge splenomegaly (20%). In the accelerated and crisis phases, 6 patients showed moderate (30%) and 14 patients showed huge splenomegaly (70%).

Median Ang-2 and CRP serum levels showed statistically significant difference among patients with different spleen size (table 3).

#### **Correlations between serum levels of Ang-2 and CRP in patients:**

Figure (2) showed a statistically significant positive correlation between serum levels of Ang-2 and CRP in all patients group ( $r=0.804$ ,  $p<0.001$ ). Figure

(3 a& b) shows statistically significant positive correlation between bone marrow blast percentage and serum levels of both Ang-2 ( $r=0.630$ ,  $p<0.001$ ) and CRP ( $r=0.512$ ,  $p<0.001$ ); respectively.

#### **DISCUSSION**

Angiogenesis is critical for the clinical progression of haematopoietic malignancies and depends on angiogenic factors.<sup>(16)</sup> Thus, angiogenic factors and angiogenesis clearly play a significant role in the course and disease process of some leukemias.<sup>(17)</sup> New evidence suggests more complicated roles for Ang-2 in angiogenesis, in both physiologic processes and invasive phenotypes of cancer cells during progression of human cancers.<sup>(5)</sup>

The present study included 50 patients with CML; 30 patients in chronic phase and 20 patients in advanced phase and 15 apparently healthy individuals were included in this study as controls.

The present study revealed a statistically significant higher Ang-2 serum level in CML patients compared to controls.

This finding is in accordance with the study of Quartarone *et al.*<sup>(5)</sup> who reported that in CML patients the serum level of Ang-2 was significantly higher than in controls.

Ang-2 modulates angiogenesis in a cooperative manner with the important angiogenic factor, vascular endothelial growth factor (VEGF).<sup>(5)</sup> Aguayo *et al.*<sup>(17)</sup> observed a significant increase in the number of blood vessels in CML. The increased vascularity was associated with a significant increase in VEGF.

In the present work, serum Ang-2 level was significantly higher in CML patients in advanced stage (group B) than in patients in chronic phase (group A). This finding coincides with the results of yerstovesk *et al.*<sup>(14)</sup> which /revealed that the expression of VEGF protein was more pronounced in the accelerated phase. They concluded that the expression of VEGF is dependant on the CML stage which could be of clinical importance in deciding the timing of therapy. In addition, Quartarone *et al.*<sup>(5)</sup>

found that in patients with multiple myeloma serum Ang-2 level was significantly increased with advanced stages of the disease, from stage I to stage II. They concluded that abnormal serum levels of Ang-2 were present in some haematological malignancies. These markers may play a role in the pathophysiology of these conditions and their progression. .

Regarding the spleen size, the present study showed that, patients with moderate and huge splenomegaly showed significantly higher serum Ang-2 level when compared to patients with mild splenomegaly ( $p < 0.001$ ). This finding is in accordance with the work of Liu *et al.*<sup>(18)</sup> who found significant associations between plasma VEGF and enlarged spleens. In contrast, yerstovesk *et al.*<sup>(14)</sup> reported that the level of VEGF expression correlated inversely with the degree of splenomegaly.

In the present study a statistically

significant positive correlation was detected between serum Ang-2 level and blast percentages in the bone marrow. These findings are in accordance with the findings of Liu et al.<sup>(18)</sup> who reported significant association between plasma VEGF and blast percentage in chronic myeloid leukaemia.

As regard C- reactive protein, a statistically significant higher level was found in CML patients compared to controls, and in patients with advanced stage compared to those with chronic phase. These results are in accordance with the study of Akanni et al.<sup>(19)</sup> who showed that CRP level was significantly higher in the CML patients when compared to the controls. They attributed this to the rise in plasma concentration of interleukin-6 which is produced predominantly by the macrophages.<sup>(7)</sup> Also, Humlová et al.<sup>(20)</sup> reported increased serum levels of CRP in newly diagnosed CML patients. In addition, Pavlu et al <sup>(21)</sup> conducted a study on optimizing patient selection for

myeloablative allogeneic hematopoietic cell transplantation (HCT) in CML patients in chronic phase. They reported elevated preconditioning CRP levels and detected that there was no association between these elevated preconditioning CRP levels and the occurrence of infection.

Concerning spleen size, patients with moderate and huge splenomegaly had statistically significant higher serum levels of CRP compared to patients with mild splenomegaly.

In the present work a statistically significant positive correlation was detected between serum levels of Ang-2 and CRP. This finding goes hand in hand with the study of Turu et al <sup>(11)</sup> which showed that CRP is strongly angiogenic both *in vitro* and *in vivo*, and activates the expression of some important pro-angiogenic genes in endothelial cells. In addition, Vilaa et al <sup>(22)</sup> demonstrated a relationship between markers of inflammation (fibrinogen & CRP) and angiogenesis (VEGF) in patients

with congestive heart failure (CHF). This relationship supports the findings of the present work, since Ang-2 modulates angiogenesis in a cooperative manner with the important angiogenic factor, VEGF.<sup>(5)</sup>

In conclusion, the present study revealed that both CRP and Ang-2 could play a significant role in the leukemic process. Both CRP and Ang-2 serum levels could be useful factors in determining disease progression or monitor the effectiveness of treatment in the chronic myeloid leukaemic patients; also,

as a part of follow-up care to check for recurrence. Newly diagnosed patients with CML should be screened for CRP and Ang-2 as baseline investigations before the commencement of treatment to enable the efficient monitoring of the effect of therapy in these patients.

Further studies aiming to explore the detailed angiogenic profile and angiogenic role of Ang-2 and CRP in CML may help in developing new therapeutic strategies for this myeloproliferative disorder.

**Table (1): The difference in hematological analysis between the two studied groups (A and B)**

	Group A	Group B	P
<b>Hb concentration (gm/dl)</b>			
<b>Median</b>	10	6.8	
<b>Min-Max</b>	6.9-13	4.8-11.7	<0.001*
<b>Platelets count(x10<sup>9</sup>/l)</b>			
<b>Median</b>	470	139	
<b>Min-Max</b>	245 – 850	17 – 1152	<0.001*
<b>Bone marrow Blast (%)</b>			
<b>Median</b>	6	39	
<b>Min-Max</b>	3-10	6-90	<0.001*

\*Statistically significant at p<0.05.

**Table 2: Median serum level of Ang-2(pg/ml) and CRP (mg/ml) in the control and patients groups (A & B).**

	Control (n=15)	Group A (n=30)	Group B (n = 20)	P	P1	P2	P3
<b>Ang-2</b>							
<b>Median</b>	1720	3907.5	7951	<0.001*	<0.001*	<0.001*	<0.001*
<b>Min-Max</b>	1450-2060	2400-7000	4282.85-13000				
<b>CRP</b>							
<b>Median</b>	1.5	6.85	18.95	<0.001*	<0.001*	<0.001*	<0.001*
<b>Min-Max</b>	0.9-2.20	1.4-19	1.1-32.5				

P: Statistical significance between all groups.

P1: Statistical significance between group B and control

P2: Statistical significance between group A and control.

P3: Statistical significance between group A and group B.

\*: Statistically significant at  $p < 0.05$ .

**Table 3 : The relation between the median serum level of both (angiopoietin-2 and CRP) with spleen size in CML patients**

	Mild (n=16)	Moderate (n=14)	Huge (n = 20)	P	P1	P2
<b>Ag-2</b>						
<b>Median</b>	3700	5050	7000	0.000*	0.006*	0.000*
<b>Range</b>	2400-6000	2999-13.000	3366.1-12000			
<b>CRP</b>						
<b>Median</b>	5.3	9.4	13.5	0.000*	0.034*	0.000*
<b>Range</b>	1.4-9.5	1.1-32	3.5-32.5			

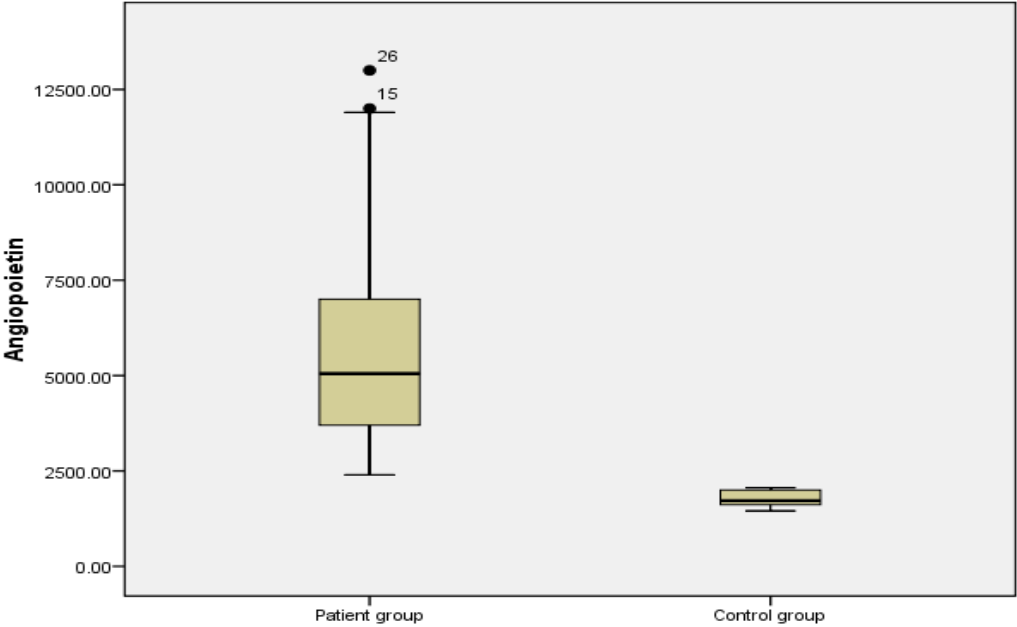
P: Statistical significance between 3 groups.

P1: Statistical significance between mild and moderate splenomegaly.

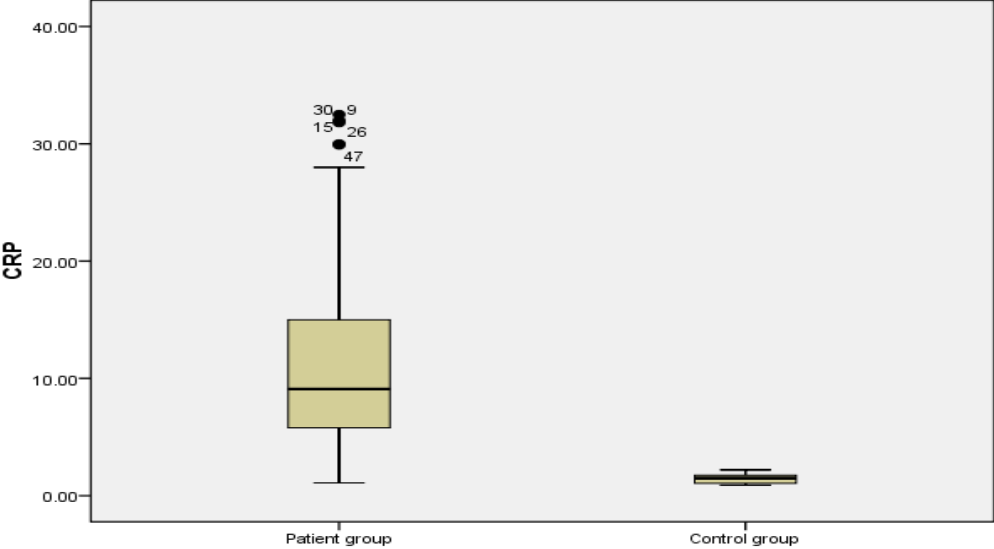
P2: Statistical significance between mild and huge splenomegaly.

\*: Statistically significant at  $p < 0.05$





(a)



(b)

Figure (1): Comparison between patients and control groups regarding serum levels of (a) Ang-2 (pg/ml) and (b) CRP (mg/ml).

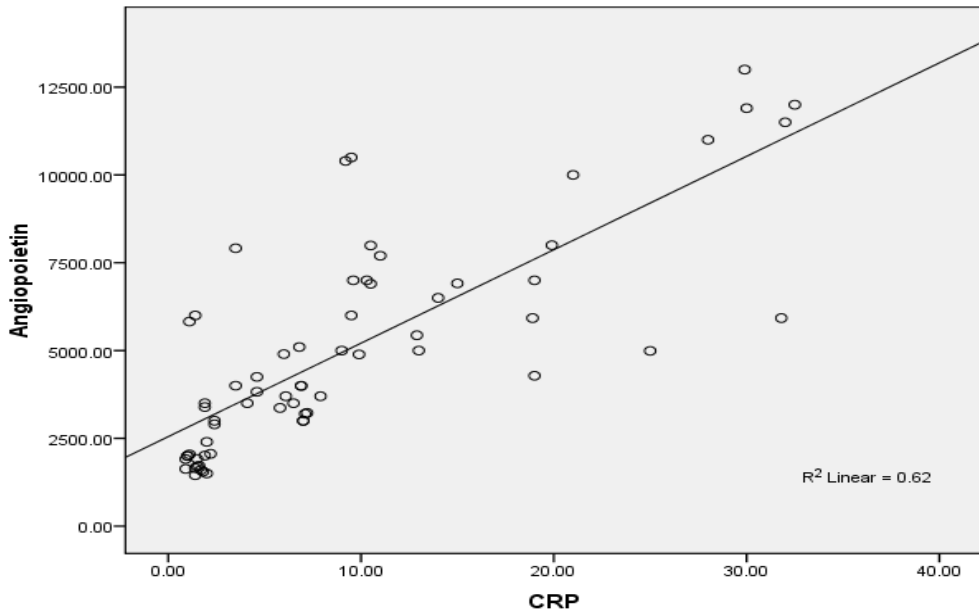
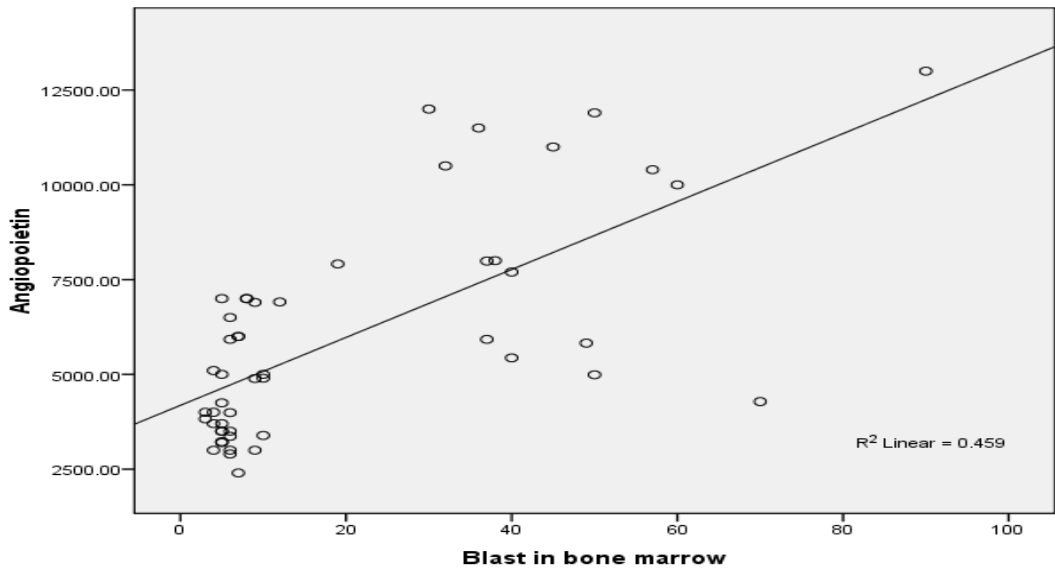
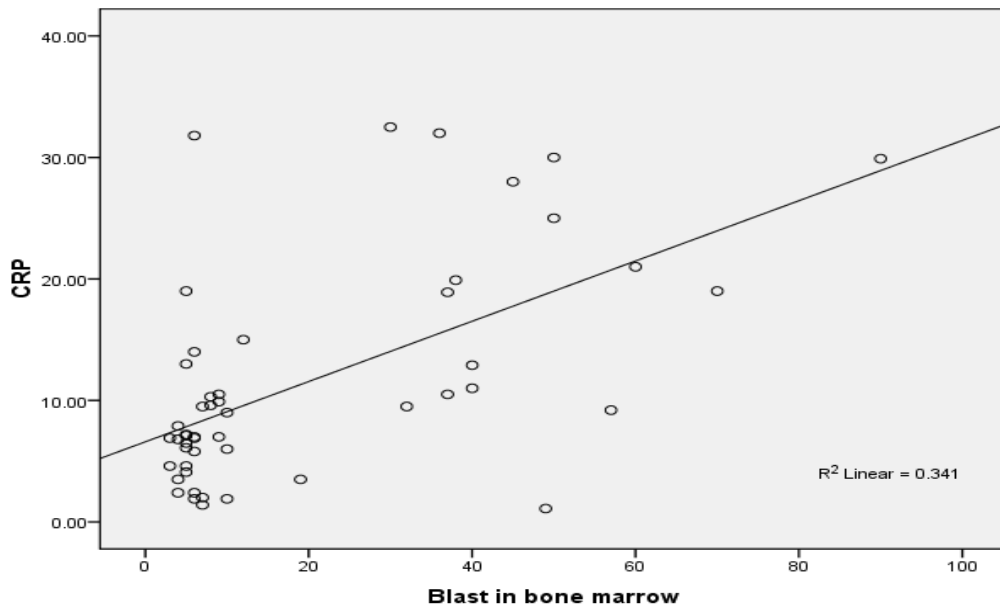


Figure (2): Correlation between serum levels of Ang-2(pg/ml) and CRP (mg/ml) in CML patients.



(a)



(b)

**Figure (3): Correlation between the percentages of bone marrow blasts and serum levels of (a) Ang-2(pg/ml) and (b) CRP (mg/ml) in, CML patients.**

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