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Research Article

# Stat3 as Potential Biomarker in Chronic Myeloid Leukemia Patients

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## Abstract

The search for non-invasive tools for cancer diagnosis and management is extremely important. STAT3 shows potential as a biomarker since it is present in the serum of healthy individuals. This study aimed at assessing the relationship between STAT3 level and BCR-ABL transcript percent in chronic myeloid leukemia (CML) patients and its prognostic significance. Thirty CML patients in hematological remission were divided into 2 groups, 15 patients each. In group I, BCR-ABL transcript percent is less than 10% while in group II it is more than 10%. Fifteen healthy subjects of matched age and sex were included as controls. Complete blood count, quantitative BCR-ABL by RT-PCR and STAT 3 level by western blot were done for CML patients. Patients were reevaluated 3 months later for BCR-ABL transcript percent and STAT 3 level. Statistically significant increase in STAT3 level was present in both CML groups as compared to controls being higher in group II than group 1. There was a positive correlation between BCR-ABL transcript percent and STAT3 level in the first and the follow up samples ($P < 0.05$) of both groups. STAT 3 level declined in the follow up sample to near control level in group I while its level remained high in group II. This denoted that STAT3 can be used as a biomarker in CML patients in parallel with BCR-ABL percent especially in borderline cases. However, further studies are still needed to confirm this finding

**Keywords:** BCR-ABL; STAT3; Chronic Myeloid Leukemia; Imatinib.

## Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm with an incidence of 1 to 2 cases per 100.000 adults. Thirty percent of patients are 60 years old or older [1]. CML is divided into three phases based on clinical characteristics. The initial chronic phase is often asymptomatic or the patients may experience mild anemia or splenomegaly [2].

The pathogenesis of CML involves fusion of ABL gene on chromosome 9 with the BCR gene on chromosome 22 [3]. This translocation results in expression of the protein tyrosine kinase *BCR-ABL1* [4]. Imatinib mesylate have improved CML treatment by inhibiting Bcr-Abl tyrosine-kinase activity (TKI); however, response failure occurs in about 25% of cases due to the presence of point mutations in the BCR-ABL kinase domain [4] or due to BCR–ABL independent mechanisms [2]. The presence of additional genetic and/or epigenetic defects cause the disease to progress to an accelerated phase and ultimately into a blastic phase over a period of several years [2].

The targets for BCR-ABL include Ras, phosphatidylinositol-3 kinase/Akt, Jak/Stat signaling pathways [5,6,7,8], interleukin-3 [9] and focal adhesion kinase and associated proteins [10,11]. Previous studies have shown that BCR-ABL activates STAT3 via the MEK pathway [12].

The human genome encodes seven STATs [13]. STAT3 governs signal transduction in hematopoiesis and myeloid cell differentiation. It up-regulates the expression of genes associated with cell survival and proliferation [14,15,16]. Persistent STAT3 activation has been detected in a variety of hematopoietic malignancies and solid tumors [14,15,17]. Enforced expression of BCR-ABL in primitive embryonic stem cells retains their primitive morphology and inhibits their differentiation due to persistent STAT3 activation [18]. Resistance of CML cells cultured in conditioned media to imatinib –may be explained by the anti-apoptotic effects of increased, aberrant STAT3 activation [19]. Data lend support to involvement of STAT3 activation in CML progression and transition to blast crisis phase [20].

Activation of STAT3 by extrinsic or intrinsic mechanisms was identified by Eiring et al as a critical signaling mode in BCR-ABL1 kinase independent TKI resistance. STAT3 inhibitor BP-5-087 (1.0 mM) restored sensitivity of CML progenitor cells, including leukemic stem cells to TKI in vitro [21].

## Aim of the Work

This study aimed at correlating STAT 3 level to BCR-ABL transcript percent in CML patients in hematological remission and to assess its prognostic value.

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## Patients and Methods

This study was conducted on 30 CML patients in hematological remission following imatinib 400 mg/day. They were selected from the Hematology outpatient clinic of Alexandria Main University Hospital during March 2011 to October 2012. Using BCR-ABL transcript percent of 10% as cut off level, the patients were divided into 2 groups. Group I consisted of 15 CML patients in whom the percent of BCR-ABL transcript level is less than 10%. Eight patients were males and 7 patients were females with a mean age of 48.13±4.9 years. Group II consisted of 15 CML patients in whom the percent of BCR-ABL level is more than 10%. Eight patients were females and 7 patients were males with a mean age of 53.6±5.6 years. Compliance of patients to medication was ensured in all patients. All patients were followed up thoroughly for further 3 months. Diagnosis and follow up of these patients were done according to European Leukemia guidelines [22]. Fifteen healthy subjects of matched age and sex were included as control group (group III). Patients with hepatic or renal failure, concomitant chronic illness and pregnant females were excluded from the study. Written consent was taken from all patients. The study was approved by the local Ethics Committee

## Methods

All patients were subjected to thorough history taking and clinical examination, routine investigations including complete blood count [23] and BCR-ABL transcript percent by quantitative RT-PCR [24]. Detection of Human STAT3 by Western Blot [25] was done to all patients and controls. CBC, BCR-ABL transcript percent and STAT 3 level were reevaluated after 3 months.

**Measurement of STAT 3 level by western blot technique** (25)

The Western blotting procedure relies upon three key elements: the separation of protein mixtures by size using gel electrophoresis; the efficient transfer of separated proteins to a solid support; and the specific detection of a target protein by appropriately matched antibodies. Once detected, the target protein will be visualized as a band on a blotting membrane. We used Human/Mouse/Rat STAT3 Antibody (R&D systems, USA). (Monoclonal Mouse IgG, Clone # 232209, Catalog Number: MAB17992B)

### Statistical Analysis

Data were analyzed using statistical software SPSS version 16. Mean and standard deviation were used to describe the scale data while percent was used to describe the categorical data. Analysis of numeric data was done using t test to compare 2 means and ANOVA test to compare more than 2 means followed by least significant difference for each two independent groups of cases. Categorical data were analyzed using chi square test. Comparison between the results of STAT 3 before and after treatment in group 1 and 2 at 3 months and 6 months was done using paired t test. Correlation between variables was done by the Pearson coefficient. P value equals to or less than 0.05 was considered to be significant.

## Results

Age, Sex and Sokal score risk of the studied groups at diagnosis are shown in table 1. Correlations between STAT3 level and BCR-ABL transcript percent in group 1 at 3 and 6 months are shown in figures 1 and 2. Comparison between the same parameters in group 2 is shown in figures 3 and 4. There was a positive correlation between the BCR-ABL transcript percent and STAT3 level in the first sample

| Parameter | Group I (n=15) | Group II (n=15) | Group III (Control, n=15) | Test of significance p value |
|---|---|---|---|---|
| **Age (years)** | 49.13±50.13 | 53.6±5.60 | 50.13±6.33 | **F**= 2.04 p=0.086 |
| **Sex** Female | 7 (46.7%) | 8 (53.3%) | 7 (46.7%) | $X^2$=0.178 |
| Male | 8 (53.3%) | 7 (46.7%) | 8 (53.3%) | p=0.915 |
| **Sokal score** | | | | |
| High | 0 (0%) | 8 (53.3%) | | $X^2$=18.33* p=0.03 |
| Intermediate | 5 (33.3%) | 7(46.7%) | | |
| Low | 10 (66.7%) | 0 (0.0%) | | |
| **STAT 3 at 3 months** (RGB unit/mgprotein) | 24.4± 7.51 | 41.8 ± 6.646 | 12.8 ± 3.895 | **F**=82.834* P=0.0001 |

Table (1): Clinical data of the studied groups at diagnosis
p value is significant if < 0.05; LSD: (I, II)*, (II, III)*, (I, III)*

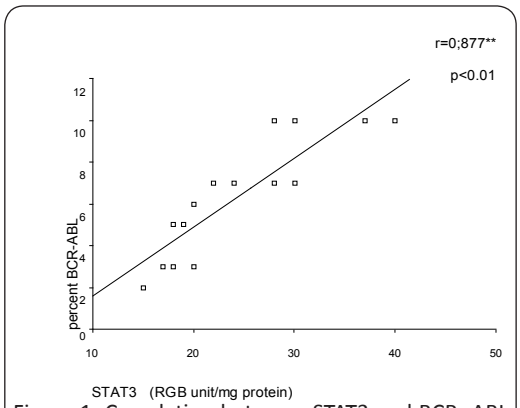


Figure 1: Correlation between STAT3 and BCR- ABL transcript percent in group l in the first sample

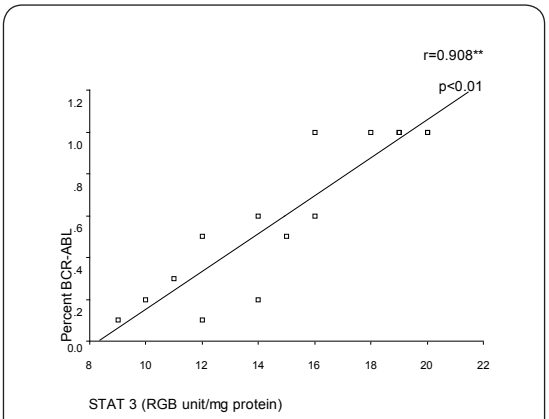


Figure 2: Correlation between STAT3 and BCR- ABL transcript percent in group l in the second sample

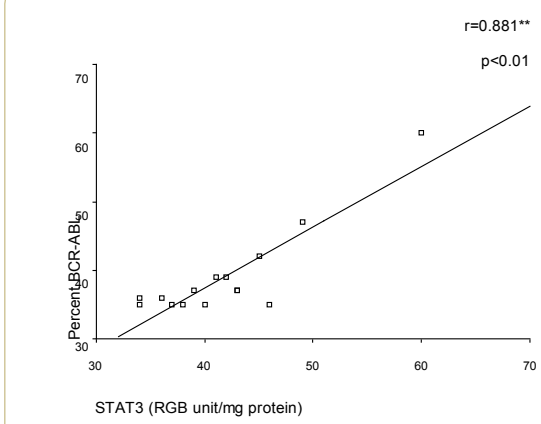


Figure 3: Correlation between STAT3 and BCR- ABL transcript percent in group 2in the first sample

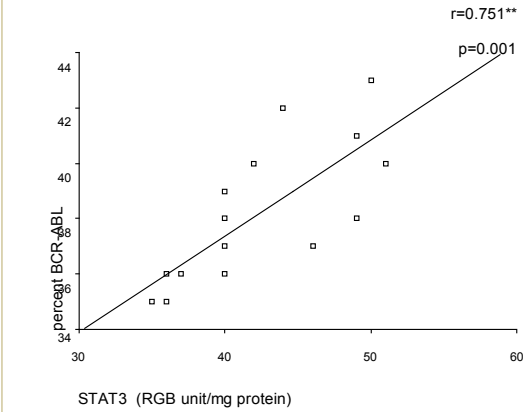


Figure 4: Correlation between STAT 3 and BCR- ABL transcript percent in group 2in the second sample

| Parameter | STAT3 (RGB unit/mg protein) | | Paired t test |
|---|---|---|---|
| | At 3 months | At 6 months | p value |
| **Group 1** | 24.4± 7.51 | 15±3.68 | 4.567*<br>0.00 |
| **Group 2** | 41.8±6.65 | 42.33±5.499 | 0.307<br>0.76 |
| **t test**<br>**p value** | 0.947*<br>0.00 | 3.692*<br>0.00 | |

Table 2: STAT3 level at 3 and 6 months postimatinib treatment in group 1 and 2
P value is significant if < 0.05

and the follow up sample (P <0.05) of both groups. Comparison between the results of STAT 3 before and after treatment in group 1 and 2 as well as comparison between STAT3 level in both groups at 3 months and at 6 months is shown in table 2. After 3 months follow up, STAT 3 level reduced to near control level in group I, in which BCR-ABL transcript percent is less than 10% while it remained high in group II in whom BCR-ABL transcript percent is more than 10%.

## Discussion

Signal transducer and activator of transcription 3 (STAT3) is a member of the STAT protein family. STAT3 acts as transcription activator [26]. Cells with constitutive STAT activation inhibits apoptosis by upregulating the expression of anti-apoptotic proteins Bcl-xL, Bcl-2 and Mcl-1 and promote malignant cell proliferation by upregulating the cyclin D1 and c-Myc expression. c-Myc is a key transcription factor that promotes cell cycle progression by inhibiting the expression of p21. The combined effects of deregulated cell cycle progression and reduced apoptosis results in uncontrolled cell proliferation and malignant cell transformation characteristic of CML pathogenesis [27].

Using the 10% percent BCR-ABL transcript level as cut off value, we divided our CML patients into 2 groups: group I had BCR-ABL transcript percent less than 10% while group II had BCR-ABL transcript percent more than 10%. All our patients were in hematological remission at the time of the study. STAT3 level was significantly increased in both CML groups compared to its level in the control group, being higher in those with BCR-ABL higher than 10% (group II). Reassessment of the same parameters 3 months later revealed decrease STAT 3 level to near controls level in CML patients of group I while its level was still elevated in group II where BCR-ABL is higher than 10%. Furthermore, comparing STAT3 level between both groups showed statistically significant decrease in group 1 than group II at 3 months and at 6 months after initiating imatinib therapy.

The level of STAT3 was higher in resistant CML cases than in responsive cases. STAT3 expression was increased in advanced stages of CML. Imatinib treatment was found to suppress the expression of STAT3 in bone marrow cells, which suggest the beneficial use of STAT3 as an indicator to follow the clinical course and the treatment response [28].

Anergy is acquired unresponsiveness of immune effector cells against tumor antigens. It results from aberrant tyrosine phosphorylation of critical activation molecules including STAT3 [29]. The decrease in STAT3 level after treatment in group I may be speculated to be responsible for break in the immunotolerance and enable anti-tumor immune responses [30].

BCR-ABL transcript percent is more sensitive than cytogenetic response to monitor response to imatinib therapy [31]. Achievement of BCR-ABL1 levels ≤10% at 3 months of imatinib treatment significantly correlated with achievement of major molecular response at 12 months [32]. These findings reinforce the importance of identifying patients with suboptimal response early in TKI treatment [17].

The search for non-invasive tools for diagnosis and follow up of cancer is extremely important. There was a significant positive correlation between STAT3 and BCR- ABL transcript percent in both groups of CML patients in the first and the second sample. Coppo et al [12] demonstrated a dramatic dose-dependent decrease of STAT3 protein level in parallel with a decrease in BCR–ABL Tyr phosphorylation with imitanib treatment. Gaiger et al [33] reported increased constitutive STAT3 level that correlates with the increase in intracellular levels of BCR–ABL during transition to blast crisis. These results provide strong evidence that BCR–ABL may regulate STAT3 protein level.

Targeting STAT3 signaling may provide the potential for selective tumor-cell killing by eliminating tumor cells with minimal effects on normal cells due to the increased dependence of the former on activated STATs. Moreover, STAT-signaling inhibition could increase the efficacy of conventional treatment modalities (chemotherapy, radiotherapy) [34].

From this study we concluded that STAT3 can be used as a potential biomarker and as a prognostic factor in CML patients treated with imatinib in parallel with BCR-ABL transcript percent especially in borderline percent cases. However, further studies are still needed to confirm this finding.

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