



Baseline Tryptase Levels Correlate with Baseline Basophil Levels in Chronic Myeloid Leukemia- Chronic Phase

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AM, MN and UKN designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors SS and MN managed the analyses of the study and performed the statistical analysis. Author AM managed literature search while author RC helped to provide the sample population and final proof reading of draft. All authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Aims: To study whether there is any correlation between baseline blood basophil count and serum tryptase levels in newly diagnosed chronic phase chronic myeloid leukemia (CML-CP) patients.

Settings and Design: 40 newly diagnosed CML-CP patients were enrolled from Medical Oncology Hematology OPD based on their baseline BCR-ABL status (done in department of Biochemistry).

Methods and Materials: Serum tryptase level was measured using Sandwich ELISA and peripheral blood basophil count was estimated using automated cell counter & peripheral blood film examination. BCR-ABL quantification was done using real time PCR after conversion of RNA (extracted from whole blood) to cDNA.

Statistical Analysis Used: SPSS Version 23.

Results: Baseline peripheral blood basophil levels showed a significant correlation with baseline serum tryptase levels ($p < 0.01$) and tryptase level also correlated with EUTOS score, which has basophil count as one of the parameters. This may signify that serum tryptase levels can be a surrogate marker of the basophil compartment in CML-CP.

Conclusions: Based on findings of the present study and other studies available in literature, serum tryptase can be utilised as a surrogate marker of the basophil compartment in CML-CP.

Keywords: Basophil marker; serum tryptase; novel biomarker; serine protease.

ABBREVIATIONS

CML-CP : Chronic Myeloid Leukemia Chronic Phase
BCR-ABL : Breakpoint cluster region-Abelson murine leukemia
TKI : Tyrosine Kinase Inhibitor
EUTOS : European treatment and Outcome Score
CD : Clusters of Differentiation
MAKK : Mitogen Activated Protein Kinase
MEKK : Mitogen-activated Protein kinase/ Extracellular Signal- related Kinase
VEGF : Vascular Endothelial Growth Factor
MDS : Myelodysplastic Syndrome
AML : Acute Myeloid Leukemia
LTC4 : Leukotriene C4

1. INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal hematopoietic cancer caused by acquired genetic defect of translocation of chromosome 21 on chromosome 9. This cancer accounts for 15% of all leukemias with predominance in males than females and increase incidence with increase in age. A tri-phasic or bi-phasic disease, patients are often diagnosed incidentally, usually in chronic phase [1]. CML is a paradigm for targeted drug treatment with TKI's taking over the older drugs. With induction of TKI in treatment of CML, there has been an improvement in even free survival of patients [2].

Prognostic risk scores in CML-CP patients, namely the Sokal, Hasford, and EUTOS scores, incorporate one or more parameters including age, baseline blood basophil count, eosinophil count, spleen size, blast count, etc. [3,4]. These scores help to establish level of risk, i.e. will patient advance further in disease or will they have a better survival prognosis. With use of hazard scores and early diagnosis and treatment, progression of disease has been stalled and prognosis have improved [2-4]. Despite these advances, relapses and resistance to treatment are still an ongoing problem.

Along with this, the hazard scores are not always reliable as most of these scores rely on peripheral blood blast and basophil percentages

which may be affected by inter-observer variability and other factors [5]. Basophilia is a very important marker in CML diagnosis and treatment monitoring. It has also been used in EUTOS scoring to hazard score patients. As its levels increases mostly in chronic myeloid leukemia, it is often considered as an important pinnacle of diagnosis [2-5]. Basophil levels heavily depend on recognition of its morphology and its impedance count and can also be identified on flow cytometer by its cell surface markers such as CD123, CCR3 (C-C Motif Chemokine Receptor 3), or CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells). However, overlap with other leukocytes and improper gating can cause a wrong measurement which why it is important if there can be a marker which can be a significant representative of basophil compartment. Thus, tryptase, a serine protease has been investigated as a marker to replace basophil count [6].

Tryptase is a serine protease enzyme mostly secreted by mast cells and is a marker of CD34 and has been widely used as a specific marker to identify mast cells in mastocytosis and other hematologic disorders. It has also been studied as a marker of angiogenesis and tumor cell proliferation. Tryptase activates many pathways such as MEKK/MAKK and increase cell proliferation while activating VEGF receptors and increase calcium levels leading to increase in tumor angiogenesis. Because of these factors,

tryptase is being continuously studied in various hematological and solid tumors as a target for cancer therapy [7].

Although secreted mostly by mast cells, Tryptase is shown to be also secreted by granules of basophils [7]. Although mature basophils do not secrete tryptase, immature basophils have been shown to release tryptase in many hematological disorders [8].

These observations lead one to wonder if tryptase can be correlated with basophils levels at baseline and can be a predictor of disease progression in CML like conditions where basophil compartment is a part of disease diagnosis and monitoring. In this short study, we estimated baseline serum tryptase levels in newly diagnosed CML-CP patients, and correlated it with their baseline basophil counts.

2. METHODS AND MATERIALS

2.1 Study Design

Cross-sectional study.

2.2 Study Population

Newly diagnosed CML-CP patients attending Hematology OPD at AIIMS, Rishikesh, after obtaining informed consent (and with approval by institutional ethics committee, AIIMS, Rishikesh).

2.3 Sample Size

Total 40 patients (calculated using G-power) of age ≥ 12 years, who were *BCR-ABL* positive, and not yet started on TKI or any other cytoreductive therapy for CML.

2.4 Excluded Study Population

Patients with accelerated phase or blast phase CML; age <12 years; patients started on TKI treatment prior to study; and CML-CP patients receiving treatment other than TKIs were excluded from present study.

2.5 *BCR-ABL* Estimation

Quantitative Real Time PCR (Biorad CFX96).

2.6 Basophil Count

Peripheral blood basophil count using impedance based cell count (Sysmex XN-1000 hematology analyzer).

2.7 Tryptase Levels

Sandwich ELISA based (Genebio Elisa reader).

2.8 Statistical Analysis

All parameters underwent normality testing after which it was categorised into parametric & non-parametric. Tryptase was correlated against blood basophil count using Pearson's and Spearman's correlation in one tail test.

3. RESULTS

Median age group was found to be 36 years (IQ 25-52 years), with slight male predominance. Mean blood basophil count was 6.26% ± 2.5 at baseline, normal basophil count in healthy individuals being 0-1%. Mean serum tryptase level was 16.63 ng/ml ± 2.4 ng/ml. Reference interval of serum tryptase level was estimated to be 5-11 ng/ml. Thus the levels were found to be increased in CML-CP patients in our study. Details are given in Table 1.

In our study, serum tryptase levels were found to be higher in patients with higher blood basophil counts, and a significant positive correlation was observed between baseline peripheral blood basophil counts and baseline serum tryptase ($p < 0.01$) (Fig. 1). This suggests that in CML, tryptase may be secreted by basophils rather than mast cells, unlike other cancers. Since basophils are included in calculation of prognostic risk scores in CML, with this result, it can also be investigated whether tryptase can be used as a marker of treatment response. When tryptase levels were correlated with baseline EUTOS score, it showed a significant positive correlation ($p < 0.05$) (Fig. 2). This suggests that serum tryptase can be considered to be a surrogate marker of the basophil compartment in CML.

4. DISCUSSION

With advances in treatment of CML with TKI agents and improved survival rates, it is pertinent that the prognostic scores are re-evaluated [1,4-5]. Various markers have been studied to replace the basophil count for prognostication in CML [9]. Considering inter-observer variability in reporting blood basophil percentage, plus variations in blood samples drawn at different time points in the same patient, serum tryptase could be incorporated as a surrogate marker for accurate estimation of blood basophils in CML-CP.

Table 1. Descriptive statistics of newly diagnosed CML patients

Parameter	Min	Max	Mean	Median	Std. Deviation
1. Age	14	78	39.64	36.00	16.70
2. Basophils (%)	2	14	6.26	6.00	2.49
3. Baseline BCR-ABL ISMMR (%)	0.02	125.07	36.72	34.39	28.48
4. Tryptase levels (ng/ml)	13.20	22.90	16.63	16.06	0.378

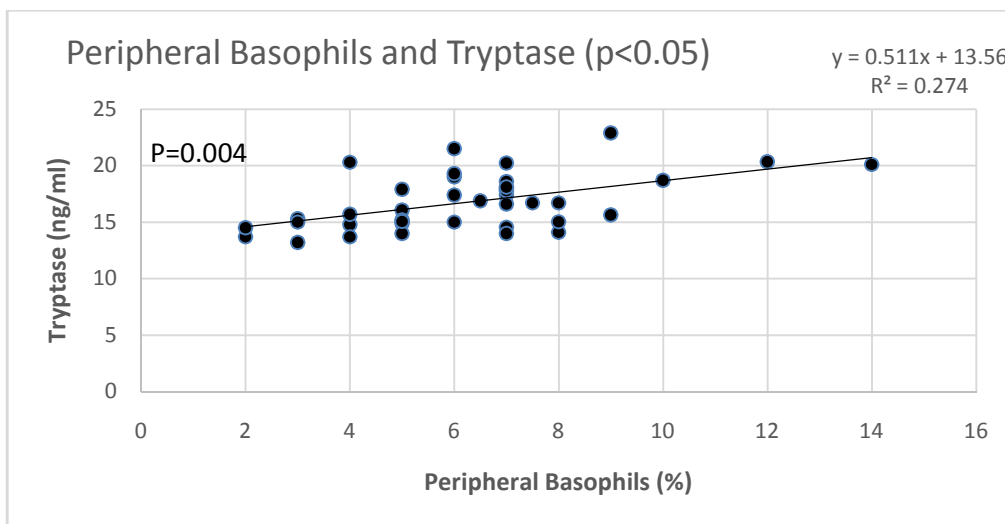


Fig. 1. As it has been theorized that tryptase is secreted by immature basophils in CML-CP, a correlation was seen between tryptase and basophil count. There is a significant positive correlation between serum tryptase levels and basophil count (p=0.004)

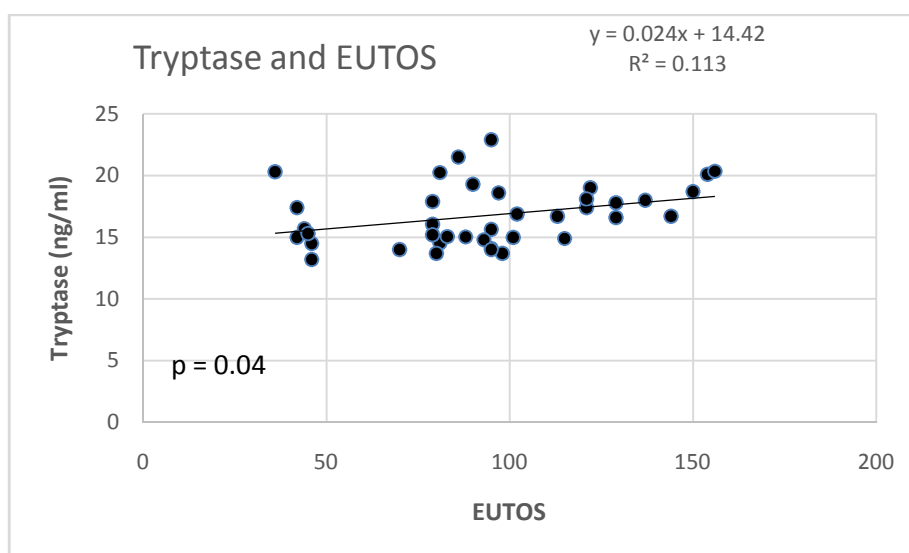


Fig. 2. EUTOS score utilizes basophil count as one of its parameters to calculate the risk of progression in CML-CP patients, making it imminent to see any correlation between tryptase and EUTOS score. There is a significant correlation between EUTOS score and serum tryptase levels (p=0.04)

Unlike most cancers, in CML tryptase has been shown to be released by immature basophils rather than mature basophils or mast cells.

Samorapoompichit et al. found immature basophil like cells which were shown to react with monoclonal anti-tryptase antibody [8]. It has

been observed on electron microscopy that basophil granules express tryptase in patients of CML. These granules are a typical ultrastructural feature of basophils. This could be because the expression of tryptase has been increased due to transformation of myeloid precursors [8].

Lately many biomarkers have been studied as a representation of basophil count. These include histamine, LTC₄, monoclonal antibodies J175-174 etc. Histamine however is difficult to be measured and it is often released in times of inflammation. LTC₄, is allergen specific and may not be increased in CML. Immunostaining with J175-7D4 has been found to be basophil-specific in human skin, nasal polyps, and oesophagus. However, J175-7D4 does not stain mature mast cells or eosinophils but stain eosinophil precursors [9].

Tryptase, a mast cell enzyme, is a widely studied marker of allergic response [8]. Lately, many studies are looking at tryptase as a therapeutic target due to its role in tumor angiogenesis and proliferation [7]. In CML, it has been noticed that tryptase is released from leukocytic basophil granules rather than mast cells, thus making it a possible marker of basophil compartment [5,8]. Samorapoompichit et al. found that unlike leukocytic basophils, mature basophils did not secrete much tryptase [8]. This showed that in CML, tryptase levels maybe correlated with leukocytic basophil count.

Sperr et al. found that basophil levels were significantly correlated with tryptase level. There were cases where tryptase levels were more than that of estimated basophils and the enzyme levels were found to be more sensitive in predicting treatment when compared with total basophil compartment [5]. Valent *et al* noticed that conditions like myelodysplastic syndrome, idiopathic myelofibrosis, CML and AML, tryptase levels have been found to be elevated and correlated with total basophil count. In line with this notion, it was observed that in CML, where basophilia is a characteristic feature, serum tryptase levels were found to be almost always elevated [9].

In our study, mean baseline blood basophil count was 6.29% and mean serum tryptase was 16.63 ng/ml and both showed a significant correlation ($p=0.004$) with each other. Also, there were three patients who had very high levels of tryptase (>18.00 ng/ml) but had a relatively small basophil compartment ($\sim 4\%$) consistent with observations by Ghalaut et al. [10], and Sperr et

al. [5]. When correlated with EUTOS score, a hazard score of disease progression which uses basophils as a parameter, tryptase levels had a significant correlation ($p<0.05$). This shows that serum tryptase levels could be utilised as a sensitive marker of basophil count. Sperr et al had estimated Tryptase mRNA levels in the leukocytes and found a significant correlation, suggesting tryptase's role as a vital biomarker of leukocytic basophil compartment [5].

5. CONCLUSION

Based on findings of the present study and in light of other studies in literature, the authors conclude that serum tryptase is a potential surrogate marker of the basophil compartment in chronic phase chronic myeloid leukemia. However, further studies with larger sample size are essential to incorporate tryptase in CML prognostic scores.

CONSENT

Signed written informed consent were obtained from study patients.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s) AIIMS/IEC/18/89.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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