Research article

# Additional cytogenetic abnormalities in resource constraint countries; an additional burden on Chronic Myeloid Leukemia patients ?

**Nida Anwar, Naveena Fatima, Sana Khurram, Tahir Shamsi**

National Institute Of Blood Diseases And Bone Marrow Transplantation, Karachi Pakistan

**Correspondence:**

**Dr. Nida Anwar**

**Email:**

**drnidairfan@yahoo.com**

**https://doi.org/10.38106/LMRJ.2021.3. 1-03**

Received: date: 16-03-2021

Accepted: date 05-04-2021

Published: date: 30-04-2021

**Abstract: The objective of this study was to analyze the additional cytogenetic abnormalities at baseline in chronic myeloid (CML) leukemia patients, compare its characteristics with patients having normal karyotype and to identify the rationale of performing cytogenetics in treatment naive CML patients. A case control study was conducted, 18 cases and 36 controls were recruited from 2010-2018. Controls were diagnosed CML patients without additional cytogenetic abnormalities. SPSS was used to analyze the data, chi-square and independent sample t- test were applied to observe the association. Kaplan -Meier was used to observe the survival outcomes. At follow up, after the initiation of treatment, there was no differences in cases and controls with respect to the hemoglobin, total leucocyte and platelet count. Molecular response at 06 month was similar between two groups while at 12 months there was a significant difference, where controls were found to have higher response rate. Survival outcomes were also found comparable in cases and controls. Our findings reflect negligible difference in clinical and molecular responses between cases and controls in CML patients. Thus, performing cytogenetics at baseline might not be helpful to predict progression of disease and treatment outcome.**

**Keywords: Additional cytogenetic abnormalities, case-control, Chronic myeloid leukemia, Pakistan**

## Introduction

Chronic Myeloid Leukemia (CML) is a myeloproliferative neoplasm distinguished by the existence of Philadelphia chromosome (Ph) resulting from a translocation between chromosome 9 and 22, i.e. t(9;22)(q34;q11) [1]. The translocation causes the formation of a chimeric oncogene, breakpoint cluster region-Abelson leukemia virus oncogene (BCR-ABL) encoding the p210BCR-ABL[2]. Regardless of the distinguished cytogenetic and molecular features harbored by CML; the patients have a diverse clinical presentation, treatment responses, and survival [3]. Moreover, the heterogeneous characteristics of the disease are also evident at cytogenetic and molecular levels [4,5]. Depending upon the different breakpoints of the BCR gene, most CML cases possess a fusion oncogene comprising either the b3a2 or b2a2 transcripts[5]. Moreover, 5% - 10% of patients have variant translocations in which at least a third chromosome is involved in the rearrangement[6] addressed as additional cytogenetic abnormalities (ACA). The presence of these abnormalities is responsible for the prediction of adverse prognosis, disease progression, poor overall survival, and treatment outcome with conventional therapy being reported widely in the blast and accelerated phase as compared to the chronic phase[7]. The most common ACAs

include trisomy[8], a second Ph chromosome, isochromosome (17)(q10), 1der(22) which are considered as "major route changes," . However, the infrequent chromosomal aberrations such as trisomy 21, t(3;12), t(4;6), t(2;16), and t(1;21) are designated as minor ACAs[8]. The major route abnormalities have auxiliary negative prognosis as compared to minor route abnormalities[9] and previous studies have reported a negative prognostic impact on treatment response and survival of patients particularly in patients who are treated by first-line tyrosine kinase inhibitors (TKI). However, the findings are conflicting and ambiguous [7,8,10] and it might be due to the heterogeneous collection of cytogenetic abnormalities[14]. However, according to the ELN recommendation 2013, it was proposed that it is not essential to perform cytogenetics at baseline. On contrary, it is also emphasized to conduct cytogenetic analysis until the achievement of complete cytogenetic response (CCyR) and major molecular response(MMR)[11,12]. European society of medical oncology (ESMO) guidelines also suggest performing the cytogenetics at 3 and 6 months and every 06 months subsequently until the achievement of complete cytogenetic response[13].

Majority of studies in the literature have discussed significance of assessment of cytogenetics during the treatment to rule out progression of the disease and to initiate a dose adjustment of TKI. The existing guidelines reflect the international literature while local data is diminished and in fact none of the guidelines has been developed for developing countries so far. In this context, we aimed to conduct a case control study in which we recruited patients with additional cytogenetic abnormalities as cases and compared the clinical and molecular responses with control group in order to identify the rationale of conducting cytogenetic at baseline which is costly and putting more burden on patients when it is a prerequisite to perform BCR-ABL by PCR at baseline as per the ELN recommendations.

## Materials and Methods

This study was approved by Institutional Review Board (IRB) in March 2018 and the data was recruited from the patients who visited the National Institute of Blood Diseases and Bone Marrow Transplant Karachi Pakistan during May 2010- September 2018. In this case control study, 18 CML cases with ACA were recruited retrospectively and for each case, 02 age, sex, and Sokal score matched controls without ACA were enrolled. Baseline cytogenetic analysis was performed overnight, 24-hrs unstimulated, and 72-hrs stimulated bone marrow cultures using standard procedures. The GTG (G-bands via trypsin using Giemsa) banding technique was applied, karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN) 2013, karyogram was made using Metasystem®. BCR-ABL1 by real-time quantitative PCR (RT-qPCR) was done by QIAGEN kits on Rotor-Gene Q 5plex HRM instrument with 72-tubes rotor, performed on peripheral blood and bone marrow. Response to treatment was assessed according to ELN recommendations 201312. Informed consent was obtained from all the participants included in the study. Statistical Package for Social Sciences (SPSS) version 23.0 was used to analyze the data. Descriptive and inferential statistics including chi-square and independent t-test was applied to observe the association. P-value $\leq 0.05$ was considered significant. Statistically significant differences in complete hematological response (CHR) at 03 months and molecular response at 06, 12, and at the end of study between cases and control were assessed by chi-square test. Differences in hemoglobin (Hb), total leucocyte count (TLC), and platelet counts at baseline and at the end of the study between cases and controls were assessed by independent t-test. Progression-Free Survival (PFS) and Overall Survival (OS) were estimated by the Kaplan–Meier method. Progression-free survival was calculated from the first dose of TKI to the first documentation of disease progression into accelerated or blast phase and OS was calculated from the first dose of TKI to the date of death or last follow-up.

## Results

Fifty-four participants were included in the study. Of these, 18 were diagnosed cases of CML having ACA, and 36 were taken as matched controls. Baseline hemoglobin (P-value 0.293), TLC (P-value 0.607), and platelet counts (P-value 0.698) were the same in both groups and were found to be non-significant. The complete hematological response was assessed at 03 months post-treatment and it was found that control group was greater in number achieving CHR than cases (P-value 0.016). However, at the study's end, the normalization of Hb (P-value 0.076), TLC (P-value 0.292), and platelet counts (P-value 0.655) were same in both groups.Fifteen cases were evaluable for molecular response and survival outcome analysis for which 30 best matched controls were selected. It was found that achievement of molecular response at 06 months was similar in both groups. However, at 12 months controls were greater in number than cases in molecular response achievement. At the study's end as per ELN recommendations, both the groups had a similar molecular responses. (Table 01). Estimated PFS and OS for cases and controls were 80% and 96 % and 87% and 90% respectively. (Table 02, Figure 01 & 02). Treatment response and survival of ACA cases along with compare and contrast with international studies is depicted in Table 03. [1,8,7,14-19]

**Table : 01 Molecular Response Between Cases And Controls**

| | Molecular Response Achieve (n) | Molecular Response Not achieved (n) | P-Value |
|---|---|---|---|
| at 06 months | | | |
| Cases | 06 | 09 | 0.399 |
| Control | 16 | 14 | |
| 12 months | | | |
| Cases | 8 | 07 | 0.032 |
| Control | 25 | 05 | |
| at the study's end | | | |
| Cases | 08 | 07 | 0.111 |
| Control | 23 | 07 | |

**Table : 02 Progression Free Survival and Overall Survival of Cases and Controls**

| | Groups | Total number of cases and control | Number of Events | PFS /OS (%) | 95% Confidence Interval | | Log Rank (Mantel-Cox) P-value |
|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | |
| Progression Free Survival (PFS) | Cases | 15 | 03 | 80 | 906.224 | 1443.376 | 0.066 |
| | Control | 30 | 01 | 96 | 3305.826 | 3772.174 | |
| Overall Survival (OS) | Cases | 15 | 02 | 87 | 1029.933 | 1489.622 | 0.597 |
| | Control | 30 | 03 | 90 | 2912.171 | 3685.132 | |

**Table: 03 Treatment response and survival of ACA cases: Compare and contrast with international studies[1,7,8,14-19].**

| Author, Country | Year of publish | Hematological Response | Molecular Response | Progression Free Survival | Overall Survival |
|---|---|---|---|---|---|
| Present Study Anwar et al. | 2019 | 3months: significant Overall: non significant | Overall: Non significant 12 months: significant | Non significant | Non significant |
| Chandran et al India[14] | 2019 | Non significant | Non significant | Non significant | Non significant |
| Safaei et al Iran[15] | 2018 | Not assessed | significant | Not assessed | significant |
| Millot, et al France [16] | 2017 | Not assessed | Non significant | Non significant | Non significant |
| Alhuraiji et al USA [7] | 2017 | Non significant | Non significant | Non significant | Non significant |
| Savasoglu et al Turkey [17] | 2016 | Not assessed | Non significant | Not assessed | Non significant |
| Crisan et al Romania[18] | 2015 | Not assessed | 10/11 achieved CCyR | Non significant | Non significant |
| Aissata et al Cote d'Ivoire[19] | 2013 | 59% patients achieved CHR | Major Cytogenetic response in 52% patients , MMR in 3% patients | Not assessed | Not assessed |
| Luatti et al Italy [8] | 2012 | Non significant | 12 Non significant Overall significant | Non significant | Non significant |
| Hsiao et al Taiwan[1] | 2011 | Non significant † | Not assessed | significant | Non significant † |

*Non significant: results in patients with and without ACAs were same, † evaluated in patients in chronic phase only*

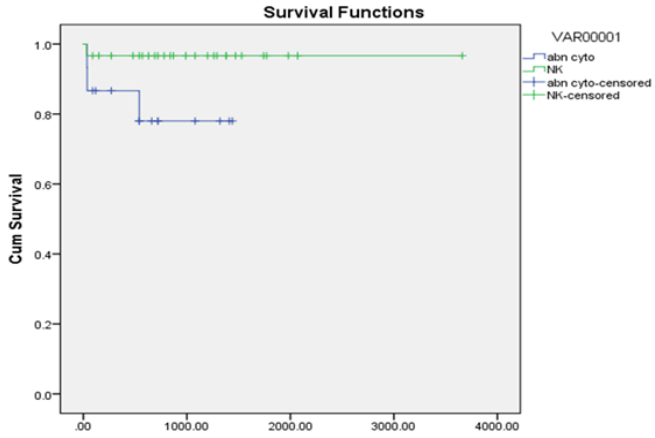


*Figure: 01 Progression Free Survival in Cases and Controls*
*NK= Normal Karyotypr*
*abn cyto= Abnormal cytogenetics*

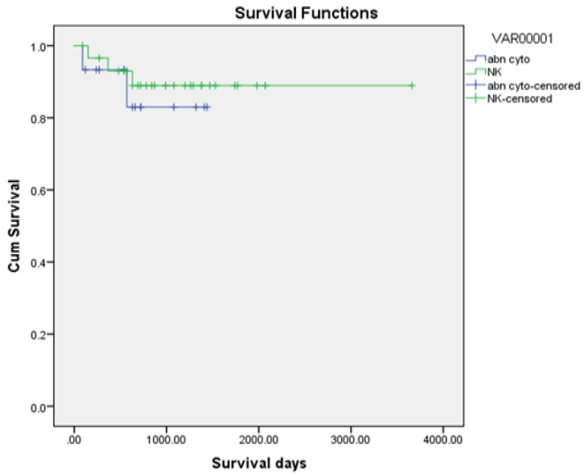


*Figure: 02 Overall survival in Cases and Controls*
*NK= Normal Karyotype*
*abn cyto= Abnormal cytogenetics*

## DISCUSSION:

Additional chromosomal abnormalities in CML at baseline and during treatment is a well-known phenomenon. There are some suggested mechanisms but the exact pathogenesis and underlying biology remain unclear and the adverse impact conferred by its presence has been controversial in the literature19,20. In our study we investigated the difference in post-treatment hematological and molecular response in patients with and without ACA in order to omit cytogenetic analysis at baseline at least in old age and non-affordable patients. ELN recommendations consider the presence of ACA at diagnosis as a warning feature requiring close monitoring12 particularly the major-route abnormalities16 and this is in concordance with previous studies reporting low response rate and overall inferior survival of patients compared to those without ACA7,8, 10. On the contrary some studies reported that ACA at diagnosis could not be considered an adverse prognostic factor in the chronic phase under first-line TKI treatment and the type of abnormality at baseline have a minimal role on the outcome, although the highest risk abnormalities (i.e., abnormalities in chromosomes 3 and i17q) are rarely if ever detected at diagnosis7, 16.

In our study when we assessed hematological response, the normalization of Hb, TLC, and platelets were identical in cases and controls but majority of patients in control group achieved CHR at 03 months as compared to cases. However, Alhuraji et al found similar median time of achievement of CHR in patients with and without ACAs7. Other regional and international studies also did not find significant difference in CHR between cases and controls as depicted in Table :021, 7,8, 14. One of the reasons in our cases for not achieving early CHR could be the TLC outlier readings of 02 of the patients. One of them was non compliant and other patients had complex karyotype at baseline as some studies have reported an adverse outcome of patients having complex karyotype9. In molecular response assessment it was found that patients who were having ACA had similar overall response as that of control in the study. At 06 months, the attainment of response was similar between cases and controls whereas another study reported a higher response rate at 06 months in patients without ACA7. Also the reverse pattern of response was observed at 12 months and we found higher rate of response at 12 months in controls whereas the study by Alhuraji et al7 reported no significant difference between the two groups. Studies were done in France, USA, Romania, Turkey, Italy, and India also reported non significant difference in molecular response amongst patients with and without ACA7,8,14,16-18. Molecular response at the end of the study was also similar in patients with and without ACA in our study which is in concordance with the findings reported in literature7. Overall, there was no difference in OS and PFS in the current study between the cases and controls and it is also in

concordance with other studies7,8,14,16-18 . It is reported in the literature that major route abnormalities are associated with early progression to advanced phase and a German study stated that PFS and OS were shorter than in patients with standard t (9; 22)9, 18. However, in our study patients who were progressed to advanced phase had minor, major, and complex ACA. In our cases, out of 15, 02 patients died and they were detected with minor and complex ACA at diagnosis. Hence the association of having major route abnormality and mortality that has been discussed in literature is unlike in our study and it is supported by another study done in Turkey in which they did not find statistical correlation between patients with major and complex ACA and without ACA17. In this study they also investigated the association of BCR-ABL kinase domain (KD) mutations and ACA and didn't find a significant association17.

In our study, we didn't identify ACA in patients during treatment. One of the studies emphasized that the detection of ACAs during the TKI treatment is a warning sign of disease progression and intermittent cytogenetic monitoring is mandatory as it is considered to be a form of treatment failure18. However some other studies did not describe the significant contribution of cytogenetic analysis in patients with molecular failures and also its impact on the course of the disease12.

Cytogenetics as compared to RT-qPCR have low sensitivity due to the low number of analyzed metaphases, the need for a bone marrow aspiration makes it a painful procedure for the patients11,20 particularly in the old age group. The option to exclude routine cytogenetic monitoring may not only prevent uncertain classifications complicating the analysis of response assessment11 but may also reduce the additional burden on non affordable patients in terms of cost when it is a prerequisite to get BCR-ABL done for disease diagnosis and monitoring. Thus it is still a matter of debate and we cannot predict the treatment outcome solely on the basis of presence of ACA and it could be questionable whether cytogenetic evaluation at diagnosis carries value If regular molecular monitoring is available. Cytogenetic assessment could however be worth performing in instances where a molecular warning or failure response is obtained.

Limitations of our study were that we didn't look for TKI mutation analysis and its correlation with ACA, hence the outcome in these patients could not be predicted in our study, also the sample size is less to conclude such findings assertively although we reduced this by adding controls studies with larger sample size are needed.

## CONCLUSION:

Patients with additional cytogenetic abnormalities in our cohort showed similar hematological and molecular responses as controls. In developing countries with limited resources, financial constraints and scarce medical facilities, omission of cytogenetic analysis at baseline when molecular level tests are available may be considered except in instance where molecular warning or failure occurred. However, further prospective studies with a large sample size are needed in this regard.

Acknowledgement: None

Conflict of interests: None

Funding : None

*REFERENCES:*

1. *Hsiao, H. H., Liu, Y. C., Tsai, H. J., Hsu, J. F., Yang, W. C., Chang, C. S., & Lin, S. F. (2011). Additional chromosome abnormalities in chronic myeloid leukemia. The Kaohsiung journal of medical sciences, 27(2), 49-54.*
2. *Aplan, P. D. (2006). Causes of oncogenic chromosomal translocation. Trends in Genetics, 22(1), 46-55.*
3. *5Pane, F., Intrieri, M., Quintarelli, C., Izzo, B., Muccioli, G. C., & Salvatore, F. (2002). BCR/ABL genes and leukemic phenotype: from molecular mechanisms to clinical correlations. Oncogene, 21(56), 8652-8667.*
4. *Reid, A. G., Huntly, B. J., Grace, C., Green, A. R., & Nacheva, E. P. (2003). Survival implications of molecular heterogeneity in variant Philadelphia-positive chronic myeloid leukaemia. British journal of haematology, 121(3), 419-427.*
5. *Deininger, M. W., Goldman, J. M., & Melo, J. V. (2000). The molecular biology of chronic myeloid leukemia. Blood, The Journal of the American Society of Hematology, 96(10), 3343-3356.*
6. *Reddy, K. S., & Sulcova, V. (2000). A FISH study of variant Philadelphia rearrangements. Cancer genetics and cytogenetics, 118(2), 121-131.*

7. *Alhuraiji, A., Kantarjian, H., Boddu, P., Ravandi, F., Borthakur, G., DiNardo, C., ... & Cortes, J. (2018). Prognostic significance of additional chromosomal abnormalities at the time of diagnosis in patients with chronic myeloid leukemia treated with frontline tyrosine kinase inhibitors. American journal of hematology, 93(1), 84-90.*

8. *Luatti, S., Castagnetti, F., Marzocchi, G., Baldazzi, C., Gugliotta, G., Iacobucci, I., ... & Testoni, N. (2012). Additional chromosomal abnormalities in Philadelphia-positive clone: adverse prognostic influence on frontline imatinib therapy: a GIMEMA Working Party on CML analysis. Blood, 120(4), 761-767.*
9. *Fabarius, A., Leitner, A., Hochhaus, A., Müller, M. C., Hanfstein, B., Haferlach, C., ... & Hehlmann, R. (2011). Impact of additional cytogenetic aberrations at diagnosis on prognosis of CML: long-term observation of 1151 patients from the randomized CML Study IV. Blood, 118(26), 6760-6768.*
10. *Cortes, J. E., Talpaz, M., Giles, F., O'Brien, S., Rios, M. B., Shan, J., ... & Kantarjian, H. M. (2003). Prognostic significance of cytogenetic clonal evolution in patients with chronic myelogenous leukemia on imatinib mesylate therapy. Blood, 101(10), 3794-3800.*
11. *Geelen, I. G., Thielen, N., Janssen, J. J., Hoogendoorn, M., Roosma, T. J., Valk, P. J., ... & Westerweel, P. E. (2018). Omitting cytogenetic assessment from routine treatment response monitoring in chronic myeloid leukemia is safe. European journal of haematology, 100(4), 367-371.*
12. *Baccarani, M., Deininger, M. W., Rosti, G., Hochhaus, A., Soverini, S., Apperley, J. F., ... & Hehlmann, R. (2013). European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. Blood, 122(6), 872-884.*
13. *Hochhaus, A., Saussele, S., Rosti, G., Mahon, F. X., Janssen, J. J., Hjorth-Hansen, H., ... & Buske, C. (2017). Chronic myeloid leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Annals of Oncology, 28, iv41-iv51.*
14. *Krishna Chandran, R., Geetha, N., Sakthivel, K. M., Suresh Kumar, R., Jagathnath Krishna, K. M. N., & Sreedharan, H. (2019). Impact of additional chromosomal aberrations on the disease progression of chronic myelogenous leukemia. Frontiers in oncology, 9, 88.*
15. *Safaei, A., Monabati, A., Safavi, M., Atashabparvar, A., & Hosseini, M. (2018). Additional cytogenetic aberrations in chronic myeloid leukemia: a single-center experience in the Middle East. Blood research, 53(1), 49.*
16. *Millot, F., Dupraz, C., Guilhot, J., Suttorp, M., Brizard, F., Leblanc, T., ... & Guilhot, F. (2017). Additional cytogenetic abnormalities and variant t (9; 22) at the diagnosis of childhood chronic myeloid leukemia: The experience of the I nternational R egistry for C hronic M yeloid L eukemia in C hildren and A dolescents. Cancer, 123(18), 3609-3616.*
17. *Savasoglu, K., Payzin, K. B., Ozdemirkiran, F., Subasioglu, A., & Yilmaz, A. F. (2017). The effect of the additional cytogenetic abnormalities on major molecular response and BCR-ABL kinase domain mutations in long-term follow-up chronic myeloid leukemia patients, a cross sectional study. Leukemia & lymphoma, 58(8), 1958-1962.*
18. *Crisan, A. M., Coriu, D., Arion, C., Colita, A., & Jardan, C. (2015). The impact of additional cytogenetic abnormalities at diagnosis and during therapy with tyrosine kinase inhibitors in Chronic Myeloid Leukaemia. Journal of medicine and life, 8(4), 502.*
19. *Aïssata, T. D., Sawadogo, D., Nanho, C., Kouakou, B., Emeuraude, N. D., Roméo, A., ... & Sanogo, I. (2013). Imatinib mesylate effectiveness in chronic myeloid leukemia with additional cytogenetic abnormalities at diagnosis among Black Africans. Advances in hematology, 2013.*
20. *Verma, D., Kantarjian, H., Shan, J., O'Brien, S., Estrov, Z., Garcia-Manero, G., ... & Cortes, J. (2010). Survival outcomes for clonal evolution in chronic myeloid leukemia patients on second generation tyrosine kinase inhibitor therapy. Cancer: Interdisciplinary International Journal of the American Cancer Society, 116(11), 2673-2681.*