Original Article / Özgün Araştırma

# Molecular Response Assessment in Patients with Chronic Myeloid Leukemia; Clinicopathological Retrospective Research

## *Kronik Miyeloid Lösemili Hastalarda Moleküler Yanıt Değerlendirmesi: Klinikopatolojik Retrospektif Bir Araştırma*

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**Keywords**
Chronic myeloid leukemia, major molecular response, molecular follow-up, prognosis

**Anahtar Kelimeler**
Kronik miyeloid lösemi, majör moleküler yanıt, moleküler takip, prognoz

**Received/Geliş Tarihi** : 13.08.2021
**Accepted/Kabul Tarihi** : 23.09.2022

doi:10.4274/meandros.galenos.2022.63497

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## Abstract

**Objective:** Chronic myeloid leukemia (CML) is a stem cell disease caused by clonal increase of precursor cells. In the studies conducted, it is stated that follow-up of patients has positive effects on the prognosis. In this study, it is aimed to review the molecular response assessment used in the follow-up of CML patients by sharing the clinical-pathology experience and to review the literature.
**Materials and Methods:** Seventy-six cases who underwent bone marrow biopsy samples assessment in Adnan Menderes University Faculty of Medicine Department of Pathology in 2018-2019 and clinically diagnosed as myeloproliferative neoplasia/CML, followed by *BCR-ABL* analysis at the 3rd, 6th and 9th months.
**Results:** Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase. Major molecular response (MMR) was observed in 31 patients in the 3rd month (40.8%), 42 patients in the 6th month (55.3%) and 51 patients (67.1%) in the 9th month. The mean follow-up period of the patients was 20.5 months. During this period, uneventful survival was observed in 65 patients according to ELN criteria, death in 5 patients (6.6%) and relapse in 7 patients (7.9%). While the MMR observed in the early period was observed to be related to the patient's life span ($p \leq 0.05$), it was not associated with relapse ($p \geq 0.05$).
**Conclusions:** Achieving the MMR is important for prognosis. The importance of molecular monitoring, which is a more sensitive method for evaluating treatment effectiveness and monitoring the response, is increasing.

## Öz

**Amaç:** Kronik miyeloid lösemi (KML), öncü hücrelerin klonal artışından kaynaklanan bir kök hücre hastalığıdır. Yapılan çalışmalarda hastaların takibinin prognoz üzerinde olumlu etkileri olduğu belirtilmektedir. Bu çalışmada klinik ve patoloji deneyimi paylaşılarak KML hastalarının takibinde kullanılan moleküler yanıt değerlendirmesinin gözden geçirilmesi ve literatürün gözden geçirilmesi amaçlanmıştır.
**Gereç ve Yöntemler:** 2018-2019 yıllarında Adnan Menderes Üniversitesi Tıp Fakültesi Tıbbi Patoloji Anabilim Dalı'nda kemik iliği biyopsi örneği değerlendirmesi yapılan ve klinik olarak miyeloproliferatif neoplazi/KML tanısı alan, ardından 3., 6. ve 9. aylarda *BCR-ABL* analizi yapılan 76 olgunun retrospektif olarak dosya taraması yapıldı.
**Bulgular:** Olgularımızın 71'i (%93,4) kronik, 4'ü akselere (%5,3) ve 1'i (%1,3) blastik fazdaydı. Otuz bir hastada (%40,8) 3. ayda, 42 hastada (%55,3) 6. ayda ve 51 hastada (%67,1) 9. ayda majör moleküler yanıt görüldü. Hastaların ortalama takip süresi 20,5

aydı. Bu dönemde European Leukemia Network kriterlerine göre 65 hastada sorunsuz sağkalım, 5 hastada (%6,6) ölüm ve 7 hastada (%7,9) nüks görüldü. Erken dönemde gözlenen majör moleküler yanıt hastanın yaşam süresi ile ilişkili iken ($p \leq 0,05$), nüks ile ilişkili değildi ($p \geq 0,05$).

**Sonuç:** Majör moleküler yanıt prognoz değerlendirilmesinde önemlidir. Majör moleküler yanıtın alınamadığı durumlarda ek kromozomal mutasyonlar veya direnç oluşabilir. Bu durumlarda tedavi değişiklikleri yapılarak hastanın takibine devam edilmesi gerekir. Tedavi etkinliğinin değerlendirilmesinde ve yanıtın izlenmesinde daha duyarlı bir yöntem olan moleküler izlemenin önemi giderek artmaktadır.

## Introduction

Chronic myeloid leukemia (CML) is a stem cell disease caused by clonal proliferation of myeloid precursor cells. 15% of leukemias seen in adult are CML. It is more common in males (M) than females (F) (M/F: 2/1.2) (1-5).

CML was first described by Virchow and Bennetin in 1845. The discovery of the Philadelphia (Ph) chromosome by Nowel and Hungerford in 1960 provided a better understanding of the pathogenesis of the disease (6). In 1973, the broken regions t(9; 22) (q34; q11) of the chromosome were identified by Rowley (7). In 1980, this translocation has been found to cause the formation of the *BCR/ABL* fusion gene. This fusion results in the *BCR/ABL1* chimeric gene form encoding the P190 *BCR/ABL1* and P210 *BCR/ABL1* proteins, depending on the breakpoints in the *BCR*. In most of the CML cases, weights 210-kDa have increased tyrosin kinase activity p210, an oncogenic protein, is synthesized (8-11). Ph chromosome can also be detected in acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). Ph chromosome is a diagnostic factor for CML and a prognostic indicator for ALL and AML (12,13).

With the introduction of imatinib mesylate, a tyrosine kinase inhibitor (TKI), in 1998, the lifespan of these patients increased (13,14). In the follow-up of their treatment, hematological, cytogenetic and molecular responses are evaluated (13,15). Patients who develop treatment unresponsiveness or loss of response during follow-up should be detected early and effective treatment changes should be made (15). Hematological follow-up includes leukocyte, platelet count, basophil myeloblast, promyelocyte, myelocyte examination and splenic evaluation in environmental blood. Cytogenetic monitoring is done by evaluating Ph positive metaphase phases as percentage. Molecular monitoring is done by real-time polymerase chain reaction (RT-PCR). Molecular follow-up should be performed every 3 months until the major molecular response (MMR) is obtained and confirmed, then repeated in every 3-6 months (15-17).

In this study, pathology experience was shared in order to evaluate molecular follow-up in CML patients and to observe their contribution to the treatment.

## Materials and Methods

Seventy-six patients with a diagnosis of myeloproliferative neoplasia (MPN)/CML were included in this study. The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University (protocol number: 2019/195, date: 23.01.2020). Bone marrow biopsy specimen evaluated and patients with who had molecular response at the consecutive 3$^{rd}$, 6$^{th}$ and 9$^{th}$ month follow up were included in the study. In addition, patients with follow-up between 9-24 months and with whom we could reach clinical information were included in the study. Patients who were excluded from follow-up and could not obtain sufficient clinical information were excluded from the study. Follow-up was performed with clinical information in the form of uneventful survival, death and relapse according to the European Leukemia Network (ELN) criteria. Bone marrow biopsies were evaluated with 2 mm thick HE sections after decalcification processing and routine tissue processing. The sections were applied CD34, MPO, Glycophorin, CD117 antibodies immunohistochemically and Reticulin stain histochemically. Additional immunohistochemical stains were requested when the differential diagnosis was suspected. Sections were evaluated under a light microscope.

Total RNA isolation was performed from the peripheral blood sample of the cases. Samples were studied within 24 hours to ensure that the RNA copies were not degraded. cDNA synthesis was performed with reverse transcriptase from the total RNA obtained. BCR-ABL amplification was performed with quantitative RT-PCR method using specific primers

and probes (Ipsogen BCR-ABL1 MBcr IS-MMR kit). MMR was performed according to international scale (IS) 0.05. According to the instructions for use of the kit, IS≤0.5 MMR is present, 0.05 0,0 IS≤0.15 gray zone response is uncertain, IS≥0.15 MMR is evaluated as no response.

The demographic features, treatment and lifetimes of the cases were achieved by scanning files and sometimes by contacting clinical physicians.

**Statistical Analyses**

Descriptive statistics were performed in the SPSS statistics program. Data was expressed as number, percentage and mean. Kaplan-Meier method and log-rank test were used in survival analyzes. P<0.05 was considered as statistically significant.

## Results

Forty four of our patients were female (57.9%) and 32 of them were male (42.1%). The patients were between the ages of 28-81 (mean 53.29). Bone marrow biopsy sample of all these cases were also evaluated. Sixty-nine patients were diagnosed with CML (90.8%) and 7 (9.2%) were diagnosed with MPN (subtype undetermined) (Figure 1). Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase.

BCR-ABL was studied in the 3rd, 6th and 9th months of the cases. In the third month, MMR was observed in 31 patients (40.8%), MMR was not observed in 32 patients (42.1%), and treatment response in 13 patients (17.1%) was evaluated as gray zone uncertain. In the sixth month, 42 patients (55.3%) had MMR, 26 patients (34.2%) had no MMR, and at 8 patients (10.5%) the gray zone was reported as uncertain. In the 9th month, MMR was observed in 51 patients (67.1%), while MMR was not observed in 15 patients (19.7%), and the response was uncertain (13.2%) in 10 patients (Figure 2, 3). In 58 (76.3%) of 76 patients, 1st generation TKI were used, while in 19 (23.7%) 2nd generation TKI have been used. Due to the development of side effects in 3 of these patients, 2nd generation TKI, 15 of them were treated depending on the resistance to treatment or the desired response 2nd generation TKI was used.

The cases were followed up between 9-24 months. The mean follow-up period of the patients was 20.5 months. During this period, uneventful survival was observed in 65 patients according to ELN criteria, death in 5 patients (6.6%) and relapse in 7 patients (7.9%). Total survival rate is 93.42%.

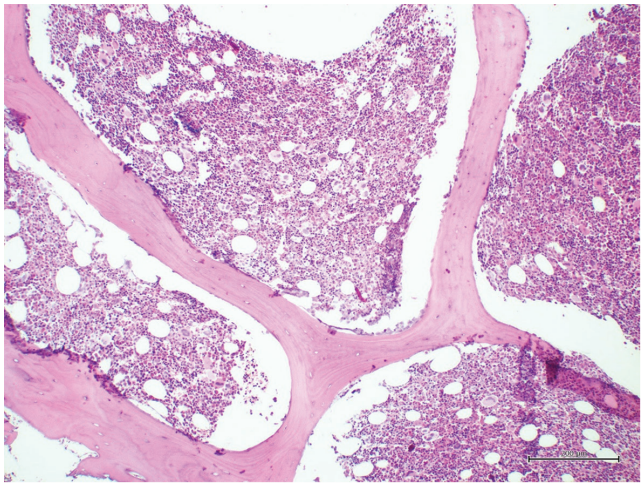


**Figure 1.** CML in bone marrow biopsy H&EX200
CML: Choronic myeloid leukemia

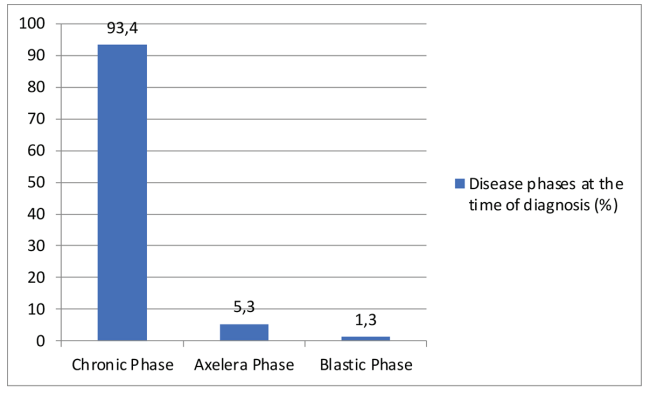



**Figure 2.** Disease phases at the time of diagnosis of cases

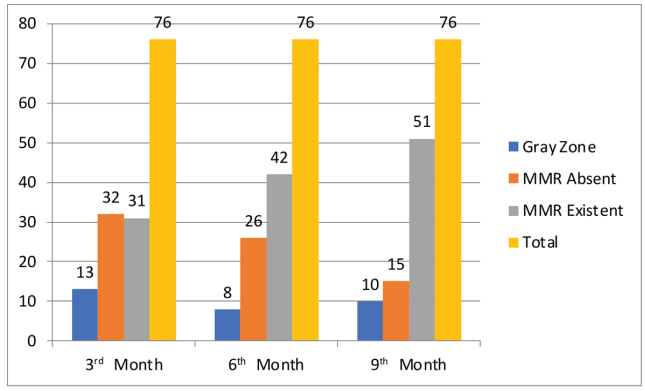



**Figure 3.** MMR distribution of cases
MMR: Major molecular response

None of the 5 cases who died in the third and 6th month follow-up were found to have MMR in the ninth month follow-up, 3 of 5 death cases were not followed by MMR, 1 was followed by MMR, and 1 was evaluated as an uncertain gray zone. The survival times of those with and without MMR during the 3rd, 6th and 9th month follow-up are shown in the Kaplan-Meier graphs (Figure 4, 5). In the 3rd month follow up of 7 cases who relapsed, 3 cases had no MMR, 2 cases had MMR, and 2 cases were reported as uncertain gray zone. In the 6th and 9-month follow-ups, 3 cases had MMR, 3 cases had no MMR and 1 was reported as gray zone.

## Discussion

Analysis by molecular methods is an important part of pathology laboratories (18). In our country, molecular units are spreading rapidly in pathology laboratories in recent years. In this article, we wanted to present MMR follow-up as a pathology experience and emphasize the importance of the clinicopathological approach in the diagnosis as well as in the follow-up of the patient.

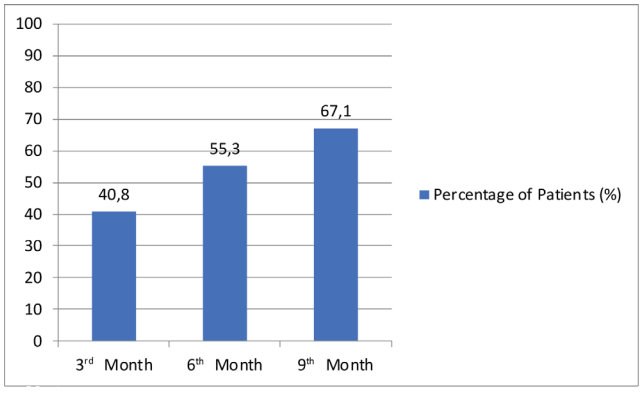
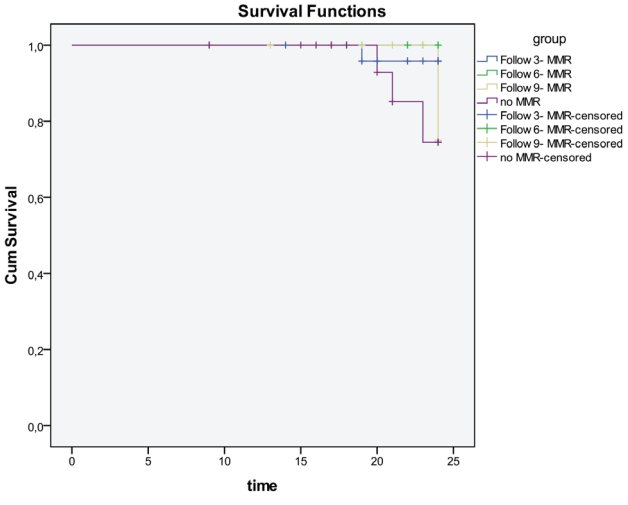



**Figure 4.** MMR rates of cases by months
MMR: Major molecular response




**Figures 5.** Evaluation of uneventful survival according to MMR (p≤0.05)
MMR: Major molecular response

In addition to the diagnosis and treatment of CML, the Ph chromosome also provided a better understanding of the pathophysiology and molecular biology of the disease (3,4,6,19). Ph chromosome arises as a result of reciprocal translocation. This translocation occurs as a result of the fusion of the ABL1 (Abelson) protooncogene in the 9th chromosome and the *BCR* gene located in the 22nd chromosome. Compared to the normal *ABL* gene, the *BCR-ABL1* hybrid gene synthesizes a chimeric fusion protein with high tyrosine kinase activity. As a result of the fusion of these two genes, the c-ABL protooncogene is activated (4,14,15). Oncogenic *BCR-ABL1* proteins affect cell proliferation, adhesion, migration and DNA repair mechanisms by altering various signal pathways (4,14,19,20).

It is more common in males than females (M/F: 2/1.2) (1,3,4,14,20). However, in this series, this ratio was reversed, it was observed more in women (M/F: 1/1.3). This rate may have been due to regional characteristics. Since patients without clinical follow-up are excluded from the study, it may be because the female patients are more compatible with the follow-up.

CML progresses in chronic, accelerated and blastic stages. Most of the patients (about 85%) are in chronic stage at the time of diagnosis. Seventy-one (93.4%) of our cases were in chronic phase, 4 were in accelerated phase (5.3%) and 1 (1.3%) was in blastic phase. The findings are consistent with the literature (1,3,4,14,15,19,20).

During the treatment, it is necessary to improve the quality of life of patients, to better treat patients who do not get a response or have a loss of response over time, and to prevent further stages.

CML has been the first and the most successful example of targeted therapies in hemato-oncological diseases with the use of TKI (3,4,15,21). The treatment response criteria recommended by the ELN, the National Comprehensive Cancer Network and the Turkish Society of Hematology (THD) should be applied for the follow-up of treatment. THD treatment

recommendations are also applied in the hematology clinic (15,22).

Molecular response tracking is done by RT-PCR. Molecular follow-up is important in the treatment follow-up of the CML patient since a patient with a complete cytogenetic response may still have leukemia cells (15,16,17,23). It should be repeated every 3 months until MMR is obtained, then it should be repeated every 6 months unless there is a loss of response during treatment (15,16). It is practical and easy to follow the molecular margins from bone marrow sampling. In the follow-up of the disease, it is more sensitive than cytogenetic follow-up in order to evaluate the minimal level of disease. Furthermore, molecular follow-up according to IS has ensured standardization by eliminating differences between laboratory evaluations (15,16,23). MMR (Ipsogen *BCR-ABL1* Mbcr IS-MMR kit) kit is used in department of pathology. There are publications about the positive effects of early detection of MMR on prognosis in cases (17,23,24). In the third month, MMR was observed in 31 patients (40.8%), in the sixth month; MMR was observed in 42 patients (55.3%), and MMR was observed in 51 patients (67.1%) in the 9$^{th}$ month. None of the 5 cases who died in the third month follow-up were found to have MMR. MMR could not be obtained in any of the patients who died during the sixth month follow-up. In the third and sixth months, the statistical are significant in terms of survival between the group with MMR and the group without MMR ($p \leq 0.05$, $p \leq 0.05$). In the 9$^{th}$ month follow-up, the difference found between the MMR and non-MMR groups in terms of survival in the log-rank test is not significant ($p \geq 0.05$). Our findings are consistent with publications emphasizing the importance of early detection of MMR in terms of prognosis (17,23).

Molecular follow-up does not provide information about bone marrow morphology or chromosomal changes. Developing mutations can lead to resistance to treatment. Many mutations that can cause resistance have been identified today. Cytogenetic monitoring should be performed to detect mutations (4,15,25). In other words, in cases of resistance or non-response to treatment, only molecular follow-up may not be sufficient, cytogenetic follow-up should be performed.

Despite the importance of molecular monitoring in predicting long-term results and evaluating treatment success, minor fluctuations in patients' *BCR-ABL1* transcript levels should not be over-interpreted (25). In our cases, the values reported as gray zone continued to be monitored if there were no side effects or mutations without treatment changes.

In histopathological examination, an increase in megakaryocytes is observed in hypercellular bone marrow, myeloid hyperplasia and small megakaryocyte morphology (1,2). There were similar bone marrow findings in our cases. There may be reticulin fibrosis detected in the bone marrow with a reticulin stain and gradually increases during the disease (1,2,15). In our cases, increased reticulin fiber is evident.

## Conclusion

Molecular follow-up, which is a sensitive method for evaluating treatment effectiveness and monitoring its response, is increasing. Getting MMR in the early period suggests that it will be good in its prognosis. Patients who cannot obtain MMR in the early period should be monitored more carefully and treatment changes should be made if necessary.

### Ethics

**Ethics Committee Approval:** The study protocol was approved by the Ethics Committee from Aydın Adnan Menderes University (protocol number: 2019/195, date: 23.01.2020).

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: İ.H.E., M.Ç.B., A.Z.B., İ.Y., Concept: İ.H.E., A.Z.B., İ.Y., Design: F.K.D., Data Collection or Processing: İ.H.E., Analysis or Interpretation: F.K.D., Literature Search: İ.H.E., M.Ç.B., Writing: F.K.D., M.Ç.B.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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