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Case Report

ROLE OF BREAK CLUSTER REGION (BCR) - ABELSON MURINE LEUKIMIA (ABL) EXAMINATION IN CHRONIC MYELOGENOUS LEUKEMIA (CML)

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ABSTRACT

Chronic myelogenous leukemia (CML) is a clonal bone marrow stem cell disorder associated with a characteristic chromosomal translocation called the Philadelphia chromosome which caused a proliferation of mature granulocytes (neutrophils, eosinophils and basophils) and their precursors, increasing unregulated growth of predominantly myeloid cells in the bone marrow and its accumulation in the blood. As myeloproliferative disease, Chronic Myelogenous Leukemia or CML is a malignancy of the sixth-highest, reaching 15% of all blood malignancies in adults with an incidence of 1.1 per 100,000 population (Ugroseno, 2012). The CML diagnosis is made based on a presence of Philadelphia chromosome due to the existence of a reciprocal translocation of chromosomes 9 and chromosome 22 t (9.22), and raises the fusion of Break Cluster Region (BCR) gene of chromosome 22 on band q11 by Abelson Murine Leukemia (ABL). The fused BCR-ABL gene has BCR sequences of different length, so it produces a protein that has a different molecular weight. Despite having different length of BCR sequences, however, the length of fuses ABL gene sequence is constant. Associated with this different BCR sequence length are the three variations of the BCR-ABL gene fusion. The first variation is a Major Break Cluster (M-BCR), the BCR gene break is found in exon 2 in e13-E14 region. This type of CML is the fusion of BCR exon b2 or b3 to ABL exon a2, forming two major transcripts of the b2a2 or b3a2, which has a protein product with 210 kD weight or referred to as p210. The second variation is Minor BCR (m-BCR), which has e1a2 fusion. CML with BCR-ABL gene fusion of this type has a protein product with a molecular weight of 190 kDa or called p190. The third variation is micro-BCR (m-BCR), with BCR gene break between exons E19 and e20b that form mRNA transcripts e19a2, with BCR-ABL protein P230. This fused gene can be detected with qualitative multiplex PCR.

Key words: *CML, Philadelphia Chromosome, translocation t(9,22), fuse gene BCR ABL, Qualitative Multiplex PCR*

ABSTRAK

Chronic myelogenous leukemia (CML) adalah kelainan klonal dari stemsel sumsum tulang belakang terkait dengan adanya translokasi kromosom t (9,22) atau yang lebih dikenal dengan kromosom philadelphia. Translokasi kromosom t (9,22) menyebabkan terjadinya proliferasi dari granulosit dewasa (neutrofils, eosinofils dan basofil). Proliferasi sel granulosit ini menyebabkan terjadinya peningkatan dari pertumbuhan yang tidak terkendali pada sel mieloid di bone marrow dan terakumulasi dalam darah. Sebagai suatu kelainan myeloproliferatif, Chronic Myelogenous Leukemia atau CML merupakan malignansi urutan ke enam terbesar, di mana kelainan ini mencapai angka 15% dari seluruh malignansi darah pada orang dewasa dengan insidensi 1.1 per 100,000 populasi penduduk (Ugroseno, 2012). Diagnosis CML dibuat berdasarkan pada adanya kromosom Philadelphia, yang terjadi sebagai akibat adanya translokasi resiprokal antara kromosom 9 dan kromosom 22 atau t (9.22). Translokasi ini menyebabkan munculnya fusi gen BCR(Break Cluster Region) pada chromosome 22 lengan q11 dengan gen Abl atau Abelson Murine Leukemia. Fusi gen BCR-ABL memiliki sekuen gen BCR dengan panjang sekuen yang berbeda, sehingga menghasilkan produk protein dengan berat molekul yang berbeda. Terkait dengan perbedaan panjang sekuen gen BCR, menimbulkan adanya variasi fusi gen BCR-ABL, yakni pertama Major Break Cluster (M-BCR), fusi gen BCR ditemukan pada exon 2 di daerah e13-E14. Tipe ini merupakan fusi gen BCR exon b2 atau b3 dengan gen ABL exon a2, membentuk 2 jenis transkrip utama, yakni b2a2 atau b3a2, di mana produk proteinnya memiliki berat 210 kD atau biasa ditulis dengan p210. Variaso kedua adalah Minor BCR (m-BCR), yang memiliki fusi pada titik e1a2. CML dengan fusi

gen BCR-ABL type ini memiliki produk protein dengan berat molekul 190 kDa atau disebut p190. Variasi ketiga adalah micro-BCR (m-BCR), di mana gene BCR terletak pada exons E19 dan e20b membentuk transkrip mRNA e19a2, dengan produk protein p230. Keseluruhan variasi tersebut dapat dideteksi dengan qualitative multiplex PCR.

Kata kunci: *CML, Kromosom Philadelphia, translokasi t(9,22), fuse gen BCR ABL, PCR Multipleks kualitatif*

INTRODUCTION

Chronic Myelogenous Leukemia or CML is a malignancy of the sixth-highest, reaching 15% of all blood malignancies in adults with an incidence of 1.1 per 100,000 population[1]. Specific characteristics of CML is the presence of Philadelphia chromosome due to the existence of a reciprocal translocation of chromosomes 9 and chromosome 22 t (9.22). The presence of the translocation t (9; 22) raises the fusion of Break Cluster Region (BCR) gene of chromosome 22 on band q11 by Abelson Murine Leukemia (ABL), which causes adult granulocytes proliferation grown without being interrupted by differentiation[1]. Chromosomal translocation, the gene ABL from chromosomes 9, which is replaced by the BCR gene on chromosome 22, is translated as Bcr-Abl fusion protein that has the ability to transform.

The fused BCR-ABL gene has BCR sequences of different length, so it produces a protein that has a different molecular weight. Despite having different length of BCR sequences, however, the length of fuses ABL gene sequence is constant. Associated with this different BCR sequence length are the three variations of the BCR-ABL gene fusion. The first variation is a Major Break Cluster (M-BCR), the BCR gene break is found in exon 2 in e13-E14 region. This type of CML is the fusion of BCR exon b2 or b3 to ABL exon a2, forming two major transcripts of the b2a2 or b3a2, which has a protein product with 210 kD weight or referred to as p210. The second variation is Minor BCR (m-BCR), which has e1a2 fusion. CML with BCR-ABL gene fusion of this type has a protein product with a molecular weight of 190 kDa or called p190. The third variation is micro-BCR (m-BCR), with BCR gene break between exons E19 and e20b that form mRNA transcripts e19a2, with BCR-ABL protein P230. The transcript is even rarer, ie, with a clinical picture of netrophilia and or thrombocytosis.[2]

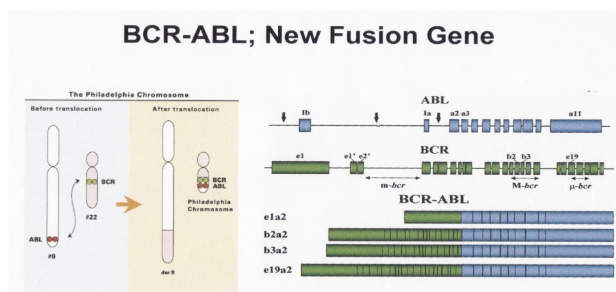

Figure 1. BCR-ABL gene fusion as a result of chromosomal translocation t (9,22) (Goh *et al.*, 2006)

In CML, the form of the BCR-ABL gene fusion will then be transcribed into Bcr-Abl fusion protein that has the ability to phosphorylate a substrate which further activates transduction cascade Ras-signaling pathways[2]. A tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a protein in a cell. The Ras pathway is a signaling center that forwards signals both upstream and downstream, with 3 signaling pathway downstream of Ras, namely Ras-Raf-MAP-kinase pathway, Akt/PKB pathway and RAL pathway. The substrate phosphorylation may activate Ras/Raf/MAPK/ERK JUN kinase, Myc, and JAK-STAT, and form PI3K (phosphatidylinositol-3 kinase)-AKT protein, which plays a role in the inhibition of apoptosis, cell cycle progression, and DNA repair, thus resulted in the deregulation of cell proliferation, through increased leukocytes proliferation.[3]

CML Diagnosis

CML diagnosis is made based on history, physical and laboratory examination, which includes a complete blood count, peripheral blood smear, cytogenetic examination to identify the presence of the Philadelphia chromosome or the inspection of Bcr-Abl gene fusion.[4] Most of these are found adults. CML is rare in children.

Complete blood examination is intended to determine the amount of various kinds of cells in blood. In complete blood count examination, white blood cells often increase, typically > 25 109/L and could even above 100 109/L. Differential cell shows granulocytes in all stages of maturation, ranging from the blast to the mature form. Basophils also increases, but only at 10–15% of the patients who have basophils ? 7% in peripheral blood. Eosinophils also increases lightly. Platelets increases in 30–50% of the patients and only a few per cent were in excess of 1,000 109/L.[1,4]

Cytogenetic examination of philadelphia chromosome or molecular examination of BCR-ABL gene fusion are performed to confirm the diagnosis. In some cases, about 5% of Philadelphia chromosome cannot be detected, so that it requires other methods, such as fluorescent in situ hybridization (FISH) and molecular detection of BCR-ABL gene fusion by examination of Polymerase Chain Reaction (PCR) for establishing CML diagnosis.[5]

CML examination with Polymerase Chain Reaction (PCR)

PCR is an in vitro DNA amplification technique in specific regions bounded by two oligonucleotide primer. Primer is used as a barrier region is propagated single-stranded DNA sequence with its complementary DNA template. The process is similar to the process of DNA replication in vivo, which is semi-conservative. PCR works

to help speed up the diagnosis of malignant diseases such as chronic leukemia with BCR-ABL gene fusion.[1]

Stages of BCR-ABL examination include RNA isolation of peripheral blood samples of CML patients using the High Pure RNA Isolation Kit, cDNA synthesis, using RNA that had been isolated, converted into cDNA using Transcriptor First Strand cDNA Synthesis Kit, and examination of the BCR-ABL gene fusion using multiplex PCR technique.

Multiplex PCR assay for the detection of BCR-ABL gene fusion

Multiplex-PCR is a modification of PCR reaction in order to be faster in detecting gene deletions or duplications. This process amplifies genomic DNA samples using multiple primers and a temperature-mediated DNA polymerase in a thermal cycler. Multiplex-PCR was first described in 1988 as a method to detect deletions in the dystrophin gene.[5] It has also been used with the steroid sulfatase gene.[6] In 2008, the multiplex-PCR was used for analysis of microsatellites and SNPs.[7] Multiplex-PCR consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times the reagents and more time to perform. Cross *et al*, (1994) have used a multiplex-PCR for the identification of BCR-ABL transcripts simultaneously from two or more genes in the same reaction. The goal is to qualitatively detect the BCR-ABL gene fusion. One example of the use of Multiplex PCR Examination is to identify for the presence of BCR-ABL gene fusion by using the following primers:

Sense primers A1: caacagtccttcgacagcag (5'–3') on bcr exon 1
B1: gctacggagaggctgaagaa (5'–3') on bcr exon 11
Anti-sense primer C1: cgtgatgtagttgcttggga (5'–3') on abl exon 3
Primer sequences in nested RT-PCR
Sense primers A2: caacagtccttcgacagcag (5'–3') on bcr exon 1
B2: gtgcagagtggagggagaac (5'–3') on bcr exon 12
Anti-sense primer C2: acaccattccccattgtgat (5'–3') on abl exon 3

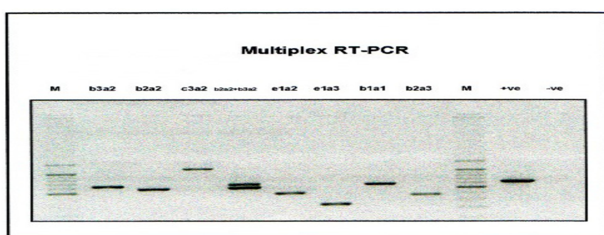

Figure 2. Example of electrophoresis results of BCR-ABL qualitative examination.[8].

The next phase is gel electrophoresis using 2% agarose. Results of electrophoresis was stained with ethidium bromide (by soaking for 15–20 minutes). PCR results is positive when 627 bands are obtained for b3a2 transcript (e13a2), 552 for the b2a2 (e14a2), 378 for b2a3, and 580 for b1a1.[8]

Examination of response to therapy using RQ-PCR

Examination of response to therapy aims to quantitatively detect BCR-ABL fusion gene transcript derived from mRNA isolation of the patients' blood samples, using Real Time PCR or better known as Quantitative-PCR atau Real Time Quantitative–PCR atau RQ PCR. Using RQ-PCR the quantity of PCR products generated from each amplification cycle is done by measuring the strength/increase in fluorescence generated by the fluorescent molecules used in the reaction.

RQ PCR products can be quantified relative to control genes or house keeping gene. In particular, quantification process relies on a standard curve obtained from known concentration of plasmid DNA. RQ-PCR has many advantages over conventional PCR. RQ-PCR allows amplification and detection process carried out simultaneously in one tube so that the process takes place more quickly and efficiently.

In CML treatment management, Real Time Quantitative PCR is used to evaluate molecular response of BCR-ABL therapy, namely by calculating residual BCR-ABL transcripts within 2 weeks after reaching CcyR (Complete Cytogenetic Response) and then performed every 3 months. Molecular response can be divided into complete molecular response (CMR), in which BCR-ABL transcript concentrations cannot be detected by RQ-PCR, and MMR or Major Molecular Response, in which BCR-ABL decreases? 3-log or the ratio of BCR-ABL/ABL is < 0.1.

Resistance and examination of mutation detection with ASO PCR

According to National Comprehensive Cancer Network (NCCN) and LeukemiaNet Guidelines, resistance to tyrosine kinase inhibitors is defined as failure to achieve complete hematologic response (CHR) in 3 months, cytogenetic response (CR) in 6 months, or major cytogenetic response (MCR) in 12 months. Resistance to tyrosine kinase inhibitor of a drug can be divided into three, namely hematologic, cytogenetic and molecular resistance.

Failed therapy is defined as not achieving hematologic response after 3 months of therapy, or loss of complete hematological response at any time, or after 6 months not achieving cytogenetic response, or after 12 months not achieving major cytogenetic response, and or BCR-ABL > 10%, or after 18 months not achieving complete cytogenetic response and or BCR-ABL of > 10%.[1]

According to Branford et al[6] (2003), patients who fail to achieve a 1-log reduction in BCR-ABL transcripts in 3 months or a decrease of > 2-log within 6 months, tend

not to give a significant response and at high risk for progressiveness. Molecular resistance is defined as the lack or loss of complete molecular response (BCR-ABL transcripts cannot be detected) using PCR RQ- or as a lack of major molecular remission (ie, a decrease of BCR-ABL transcripts > 3-log or the ratio of BCR-ABL/ABL < 0.1%, respectively).[9]

According to Corbin *et al*[11] (2003), certain mutations, such as M244V, M351T, and Phe311L also causes resistance to Tyrosine Kinase Inhibitors (imatinib), which, using biochemical and cellular tests, the mutation will show decreased sensitivity to imatinib respectively of 1, 8 and 2.8-fold. Slight shifts in terms of sensitivity causes kinase activity becomes sufficient to cause disease progression. However, in theory, the resistance caused by these mechanisms can be overcome with increased doses of imatinib.

Mutation found in T315I frequently causes of resistance to inhibitors of first-generation (imatinib) or second generation. This mutation is also associated with secondary imatinib resistance that usually occurs in the later stages of the disease and is associated with advanced age, receiving interferon previously, a high Sokal score, and failure to reach the CCR in 12 months.[10]

Detection of ABL kinase domain mutations is performed when in chronic phase the CML patients, after treatment with TKI drugs, provide inadequate therapeutic response (failure to achieve hematologic complete response in the first 3 months, minimal cytogenetic response within 6 months, or a major cytogenetic response within 12 months) or loss of therapeutic response (defined as hematologic relapse, cytogenetic relapse, 1-log increase of BCR-ABL transcripts ratio, and the loss of major molecular response), or the disease becomes progressive (the occurrence of accelerated phase or blastic crisis).[1]

Mutation analysis can be done in various ways. One is by using Allele-Specific Olygonucleotide (ASO)-PCR. This method is a technique that is highly sensitive and specific for the detection of known mutations[1]. This method is even more sensitive than mutation detection with sequencing method because DNA sequencing method can only be useful for point mutation if the proportion of mutated cells is more than 30%. In cases where the number of mutated cells is less than 30% of total cells in the sample of the patients, at least 10 independent clones from patients should be analyzed for mutations detection, a quite expensive and time consuming procedure. In contrast, the ASO-PCR is comparatively more sensitive, specific and "economical", so it is a rapid method for mutation detection.[12]

CONCLUSION

The use of qualitative multiplex PCR to detect BCR-ABL fused gene becomes one of the methods to detect the Chronic Myelogenous Leukemia (CML). It is easier to be performed while the use of karryotyping was complicated to detect this fused genes that called philladelphia chromosome

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