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Case report

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A rare complex variant translocation t(9;22;6;17;1) in chronic myeloid leukemia: case report

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The presence of the Philadelphia chromosome (Ph) in chronic myelogenous leukaemia (CML) is a specific cytogenetic change resulting from a reciprocal translocation between chromosomes 9 and 22. In 5–10% of newly diagnosed cases there are variant translocations (vPh) involving more chromosomes. This paper presents the case of a CML patient with a complex variant translocation involving chromosomes 1, 6, 9, 17 and 22. A molecular analysis did not reveal any mutation in the kinase domain of *BCR-ABL1* gene or the mutation of *TP53* gene. After the first-line treatment with imatinib no cytogenetic or molecular response was obtained. The change of treatment to dasatinib resulted in a minimal cytogenetic response (minCyR) followed by a minor cytogenetic response (mCyR). The application of nilotinib in the third-line treatment resulted in a complete molecular response (CMolR) and therapy success. The likely reason for the failure of the first- and second-line treatment was the loss of a fragment of the 17p13 region as a result of a variant translocation. The change can be a functional equivalent of the loss of one copy of *TP53*. The analysis of presented case confirms the significance of the detailed evaluation of the composition of vPh complex variant translocations as well as importance of combination cytogenetic and molecular diagnostics in CML treatment monitoring. It makes possible to adequate diagnose higher-risk patients and apply effective treatment strategies if an aberration is identified.

Key words: chronic myelogenous leukaemia, complex variant translocation of the Philadelphia chromosome, treatment of chronic myelogenous leukaemia

Introduction

Chronic myelogenous leukaemia (CML) belongs to the group of myeloproliferative disorders. The CML development is associated with the presence of the Philadelphia chromosome (Ph) and *BCR-ABL1*, a fusion gene with oncogenic properties. As a result of reciprocal translocation t(9;22), the *ABL1* protooncogene from the long arm of chromosome 9 (q34) is transferred to the *BCR* gene on the long arm of chromosome 22 (q11). The shorter chromosome 22 (Ph) carries a new oncogene, *BCR-ABL1*, which encodes tyrosine kinase with constitutive activity. The autophosphorylation of tyrosine in BCR-ABL1 kinase and the activation of the Ras/MAPK signalling pathway in a pluripotent bone marrow stem cell leads to the increased proliferation of a leukemic clone and contributes to the neoplasm growth. The product of chromosomes 9 and 22 translocation is the BCR-ABL1: p210 protein (in 99% of cases) or, less frequently, p190 or p230, which differ in terms of their mass as well as biological properties [1].

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The presence of the Ph chromosome is a specific cytogenetic abnormality which occurs in over 90% of CML cases, regardless of the disease progression stage. But in 5–10% of newly diagnosed patients there may occur complex translocations involving, except chromosomes 9 and 22, one or more other chromosomes. These are variant translocations (vPh). The mechanism of vPh formation is not entirely clear, but two alternative possibilities are taken into consideration. In a one-step mechanism, it is assumed that breaks occur simultaneously in several different chromosomes involved in the translocation, while in a two- step mechanism there are two subsequent translocations, a classical one followed by the one involving additional chromosomes.

The second mechanism of vPh formation could result in a worse prognosis because it is analogous to the mechanism of clonal evolution. Currently, it is believed that patients with Ph variants have a good response to treatment and their prognosis is similar to the one for patients with a typical translocation involving t(9;22) [2]. A cytogenetic analysis enables the detection of secondary chromosomal aberrations that accompany the primary translocation involving t(9;22) and indicate clonal evolution. Most often, there occur additional copies of chromosome 8 and 19, chromosome Ph and isochromosome 17q. The trisomy of chromosomes 21 and 17, the monosomy of chromosomes 7, 17 and Y as well as the translocation involving t(3;21)(q26;q22) are less frequent. The frequency of these changes increases during disease progression , respectively up to 5–10% in the chronic phase, up to 30% in the acceleration phase and up to 80% in the blast crisis phase [3, 4]. Among the described aberrations only some abnormalities, such as i(17)(q10), –7/del(7q) and 3q26.2, are related to prognosis deterioration [5]. According to the current European LeukemiaNet (ELN) recommendations, the detection of an additional aberration in a primary test should be a warning, but if a secondary aberration in Ph+ clone arises during the therapy, it indicates its failure [6].

The molecular diagnostics of CML using PCR (polymerase chain reaction) techniques identifies the type of *BCR-ABL1* transcript which level may be adequately monitored during the response to the tyrosine kinase inhibitors (TKI) treatment. Most translocations involving t(9;22) are characterised by the fusion between exons e13 or e14 of the *BCR* gene with exon a2 of the *ABL1* gene. This combination of gene fusions is described, respectively, as transcripts b2a2 (e13a2) and b3a2 (e14a2). About 2–5% of patients carries rare transcript variants [2].

The current CML treatment is based on tyrosine kinase inhibitors that block the binding site of ATP kinase, inhibits the phosphorylation of tyrosine residues, and prevent the activation of a cell signal. Targeted therapy include: imatinib (first-generation drug), dasatinib, nilotinib, bosutinib (second--generation drugs) and third-generation inhibitors (e.g. ponatinib). Multi-centre studies confirmed that imatinib treated patients achived a high response rate of a complete cytogenetic response and survival free from progression (over 80%) [7]. If a patient has not responded to the first-line treatment, next-generation drugs are used. Indications for the use of other drugs involve resistance to or intolerance of the first-line treatment. Mutations of the *BCR-ABL1* kinase domain are the major cause of resistance to the TKI treatment. [8]. The most unfavourable is a point mutation T315I, which is the main cause of resistance to imatinib, dasatinib, nilotinib and bosutinib.

Chromosome analysis is necessary to determine the effectiveness of the TKI treatment, which evaluates the cytogenetic response level. A total cytogenetic response (CCyR) is defined by no metaphases with Ph+ clone in tested bone marrow, a partial response (PCyR) means the presence of Ph+ in 1–35% of the metaphases , a minor response (mCyR) –in 36-65%, a minimal response (minCyR) – 66–95% metaphases with Ph+, and there is no cytogenetic response (noCyR) when Ph+ is present in over 95% of analysed metaphases. The level of the molecular response to the TKI treatment is evaluated by *BCR--ABL1* transcript type and its quantity. Therefore, without the identification of transcript variant , the monitoring of patients with rare variant transcript would give a false negative result suggesting that patient achived complete molecular response (CMolR). CMolR means that there is no mRNA *BCR-ABL1* in RQ--PCR (real-time quantitative – PCR) test or in RT-PCR (reverse transcriptase – PCR) in two consecutive blood samples. In major molecular response (MMR), the quantitative ratio of *BCR-ABL1* to *ABL1* or another reference gene is ≤0.1% on the international scale (IS) [9]. If the resistance to the first-line treatment occurs, the mutation of the *BCR-ABL1* oncogene kinase domain should be evaluated.

Case study

A 52-year old man was diagnosed with chronic myelogenous leukaemia in October 2015, which was confirmed by cytogenetic and molecular tests. The CBC results were as follows: WBC (white blood cells) – 176 thousand, Hb (haemoglobin) – 7.4g%, Ht (haematocrit) – 23.4, PLT (thrombocytes) – 327 thousand. Additionally, there was a shift towards myelocytes and MBL (monoclonal B lymphocytosis) of 6% in the peripheral blood smear.

There were no deviations from the norm in biochemical tests, except for the high activity of LDH (lactate dehydrogenase). A baseline molecular test confirmed the presence of *BCR-ABL1* transcript p210 of the b2a2 type, while the kinase domain analysis performed after the failure of the first-line treatment excluded the presence of any mutation in the kinase domain of this gene.

The cytogenetic examination performed at the diagnosis revealed the presence of a complex translocation involving t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21) (fig. 1).

The fluorescence in situ hybridisation (FISH) confirmed the presence of fusion *BCR-ABL1* on changed chromosome 22 and

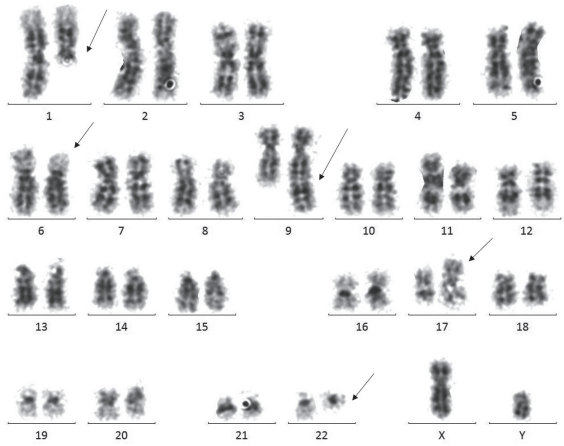

Figure 1. Karyotype: 46, XY, t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21)

the absence of the second fusion signal (visible on changed chromosome 9 in typical cases). It was confirmed that the *BCR* gene signal (from chromosome 22) had been reduced (dim) and transferred to the short arm (p) of chromosome 6 (fig. 2). The presence of a complex variant translocation between chromosomes 1, 6, 9, 17 and 22 was confirmed by additional tests with painting probes. They demonstrated the translocation of the short arm of chromosome 6 to the short arm (p) of chromosome 17 and the transfer of a part of chromosome 1 to chromosome 9q. This change was accompanied by the deletion of the fragment including *ABL1* gene from translocation chromosome 9.

At the same time, it was confirmed that the breakpoint on the short arm of chromosome 17 involved in the complex translocation resulted in the alternation of the structure of one copy of the *TP53*. In the FISH image, this change was visible

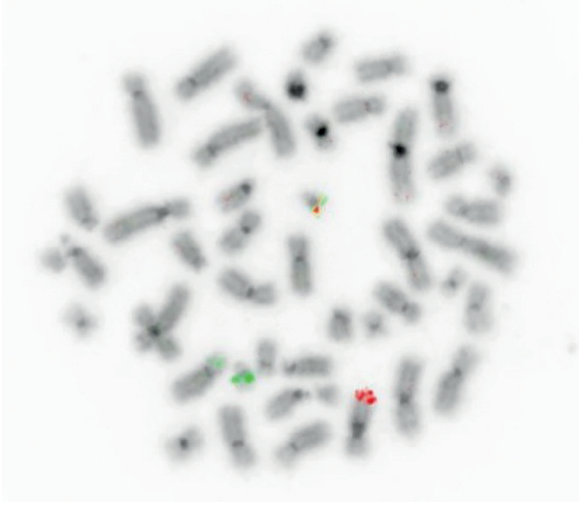

Figure 2. 46,XY,t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21).ish der(6) t(9;22;6;17;1)(BCR dim+) der(9)t(9;22;6;17;1)del(9)(q34q34)(ABL1-, BCR-),der(22)t(9;22;6;17;1)(BCR+,ABL1+)

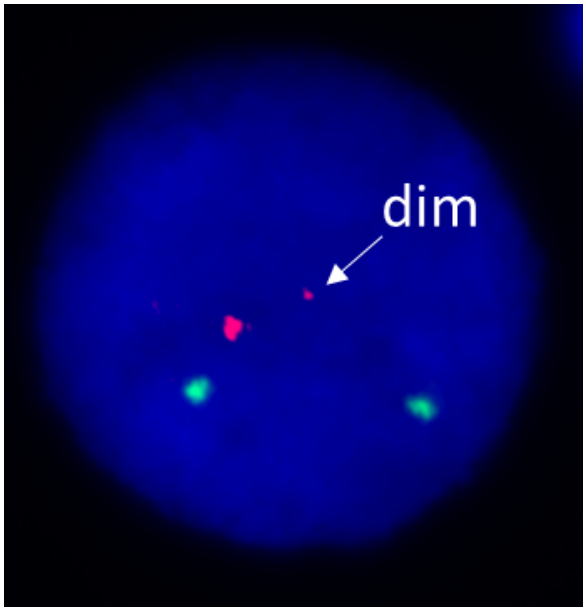

Figure 3. Difference in TP53 signal size on interphase. FISH with TP53/CEP 17 Probe Kit (Abbott): TP53 – red signal, chromosome 17 centromere – green signal. Apparent reduction of one TP53 signal (dim)

as a reduced signal of the probe specific for *TP53* gene (dim) (fig. 3).

NGS was employed to search for the mutations of oncogenes and suppressor genes typical for neoplasms. The sequencing of 50 genes, including *TP53*, using Ion AmpliSeq Cancer Hotspot Panel v2 on the Ion SS sequencer (Thermo Fisher Sci.) did not reveal any mutations of the 207 amplicons tested. The sequencing results (depth x1881) did not reveal any mutations of the 207 amplicons tested. The results of sequencing (depth x1881) for *TP53* gene were analysed for the presence of pathogenic mutations according to the COSMIC database and the additional sequences obtained were analysed using the Integrative Genomics Viewer.

Course of treatment

The first-line treatment involved cytoreduction with hydroxycarbamide. The treatment with imatinib at a dose of 400 mg/d was launched in November 2015. According to the cytogenetic analysis performed after the imatinib therapy, the aberration involving t(9;22;6;17;1)(q34;q11;p11.2;p11.2;q21) was still present in all cells (noCyR). The molecular evaluation of quantity revealed a high amount of *BCR-ABL1* transcript at the level of 1.4467%. During monitoring, a new aberrations, such as marker chromosome in Ph+ clone and additional chromosome 8 in Ph- clone, appeared. These additional aberrations indicated the progression of cytogenetic changes.

Due to no cytogenetic or molecular response as of July 2016, the second line treatment with dasatinib was introduced. After change of treatment minCyR at the level of 85% and 5 months later – mCyR at the level of 61% were achieved.

In May 2017, nilotinib at a dose of 800 mg/d was used as the third-line treatment. After 6 months there was a reduction of the number of cells with t(9;22;6;17;1) to 4% and a PCyR was observed (fig. 4). As of January 2018, the level of *BCR-ABL1* transcript also dropped to 0.0871%, which confirmed a MMolR. Subsequent molecular tests (April 2018, April and July 2019) showed a reduction in the level of *BCR-ABL1* transcript (respectively, 0.0527%, 0.0285% and 0.0019%) until a CMolR was achieved in August 2019, which was also maintained in the tests of January 2020.

Conclusions

The prognostic value of cytogenetic aberrations in CML has been changing along with the development of modern therapy methods. The prognostic value of deletion in the region of *ABL1-BCR* fusion on chromosome 9 ceased to be important along with the marginalisation of the treatment using interferon alpha, in which it was an independent negative prognostic factor [10]. In the era of advanced TKI therapies, the significance of other prognostic factors has also been evolving. The detection of additional aberrations in a baseline test should be treated as a warning, while the appearance of a secondary aberration in the Ph+ clone during the therapy indicates its failure. It should be remembered that both variant and secondary translocations may be a result of sub-microscopic changes, which are invisible in classical karyotype analysis. In this situation, it is important to use cytogenetic molecular techniques, such as fluorescence in situ hybridisation (FISH) [15, 16]. At present, ELN guidelines promote more detailed tests for patients with an increased cytogenetic risk, but they do not clearly indicate that physicians should diversify initial therapies in everyday practice [6].

In the presented case, complex translocation involving chromosomes 1, 6, 9, 17 and 22 was described. The formation of a *BCR-ABL1* fusion gene on Ph chromosome was accompanied by the loss of *ABL1* on changed chromosome 9. The complex translocation also resulted in the disruption of the structure of the short arm of chromosome 17. The *TP53* gene, located in this region, is one of the most important tumor suppressors. The loss of *TP53* function may be the main factor causing resistance to the treatment with tyrosine kinase inhibitors and may influence the disease progression [12–14]. The involvement of chromosome 17 in the variant translocation as confirmed by the authors caused the loss of a fragment of 17p13 with an atypical *TP53* aberration, which may be functionally equivalent to the loss of one copy. At the same time, the absence of the *TP53* mutation was confirmed. The loss of *TP53*, which usually occurs as a result of the formation of i(17q), is a warning indicating the possibility of the TKI treatment failure [5]. The loss of *TP53* as a result of vPh has also been described by other authors [13]. In presented case, no atypical transcript or mutation in the *BCR-ABL1* fusion gene after the failure of the first-line treatment in molecular analysis was confirmed.

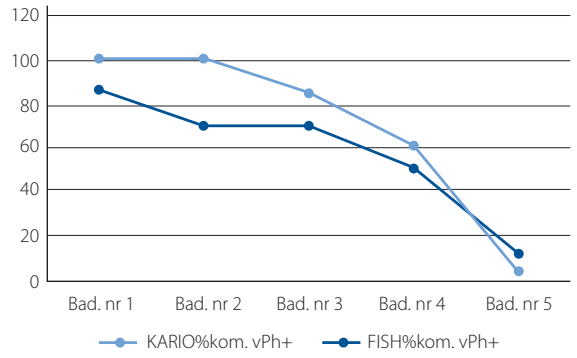

Figure 4. Changes in the proportion of Ph+ cells in karyotype and FISH tests over the course of treatment

It is generally believed that the imatinib treatment failure in patients with chronic CML is most often caused by the presence of the *BCR-ABL1* oncogene mutation [18]. Attempts to demonstrate the impact of *BCR-ABL1* transcript on the treatment results did not show any significant differences in this respect, although it was demonstrated that patients with b2a2 transcript had a higher event-free survival (EFS) [18, 19].

During the first-line treatment, the patient did not respond to imatinib and then revealed a weak response to the second-line treatment (dasatinib). Patients for whom two consecutive lines of treatment were proven unsuccessful are known to cause the most therapeutic problems, but in this case the third-line treatment with nilotinib turned out to be effective [20].

The nilotinib therapy has already been confirmed successful in patients with *TP53* deletion who were resistant to imatinib [21]. Recent tests have demonstrated that nilotinib is more effective than imatinib in increasing the level of p53 in serum in patients with chronic myelogenous leukaemia [22]. Thus, the failure of the first- and second-line treatment in this case is most likely caused by the loss of one functional copy of the *TP53* gene, which was confirmed in the FISH test. In the absence of a mutation in the *BCR-ABL1* kinase domain and no mutation in the *TP53* gene, therapy failure may have been related to loss of TP53 function as a result of complex translocation. The analysis of this case confirms the legitimacy of an expanded cytogenetic examination in the presence of vPh or atypical secondary changes during CML diagnostics as they make it possible to detect patients with an increased risk of the disease.

In conclusion, presented case confirms the importance of common analysis diagnostic and monitoring results using cytogenetic and molecular analysis methods, including the expanded possibilities offered by NGS. It should be emphasized that the limitation of a genetic aberration analysis to one innovative technique, regardless of its advancement level, causes a risk that some important data which influence the therapy and its results might be ignored. To date, there has been no standard NGS diagnostic method. Hence, close collaboration between diagnostic and clinical centres is essential to develop the most effective treatment strategies for higher-risk patients.

Conflict of interest: none declared

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