

DETEKCIA PHILADELPHIA CHROMOZÓMU U PACIENTOV S CHRONICKOU MYELOIDNOU LEUKÉMIOU V PREŠOVSKOM KRAJI (1995-2004)

DETECTION OF PHILADELPHIA CHROMOSOME IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA FROM THE PREŠOV REGION IN SLOVAKIA (1995-2004)

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Súhrn

Autori analyzovali výsledky cytogenetických vyšetrení zameraných na detekciu Philadelphia chromozómu u pacientov chronickou myeloidnou leukémiou v Prešovskom kraji za obdobie rokov 1995-2004. **Súbor a metodika:** V práci boli analyzované výsledky 72 cytogenetických analýz vzoriek kostnej drene pacientov so suspektou diagnózou CML. **Výsledky:** Philadelphia chromozóm bol detegovaný u 94,4% pacientov so suspektou diagnózou CML. V jednom analyzovanom prípade bola zistená komplexná translokácia zahŕňujúca chromozómy 8, 9 a 22. U jedného pacienta boli detegované početné numerické a štrukturálne chromozómové aberácie. Mozaikový karyotyp bol detegovaný u 5,9% pacientov. **Záver:** Výsledky analýz potvrdzujú diagnostický a prognostický význam detekcie Philadelphia chromozómu u pacientov s klinickou diagnózou CML. Konvenčná cytogenetická analýza zostáva naďalej štandardnou metódou pre potvrdenie diagnózy, monitorovanie terapeutickéj odpovede a minimálnej reziduálnej choroby u pacientov s chronickou myeloidnou leukémiou.

Kľúčové slová: chronická myeloidná leukémia, Philadelphia chromozóm, karyotypizácia, cytogenetická analýza.

Summary

Authors analyzed results of cytogenetic examinations with attention on detection of Philadelphia chromosome performed in patients with CML in the Prešov region in 1995-2004. **Material and methods:** Authors analyzed results of cytogenetic examinations performed in 72 samples of bone marrow cells in patients with suspected diagnosis of CML. **Results:** Philadelphia chromosome in bone marrow cells of patients with suspected diagnosis CML in the Prešov region (1995-2004) was detected in 94,4% of cases. In one patient a complex translocation involving chromosomes 8, 9 and 22 was identified. One patient has showed extra numerical and structural chromosomal aberrations. Mosaic karyotype of the Ph chromosome was found in 5,9% of cases. **Conclusion:** The results of cytogenetic analyses confirm diagnostic and prognostic significance of Philadelphia chromosome in patients with clinical diagnosis CML. Conventional cytogenetic analysis remain the standard method for purposes of diagnosis, monitoring of the therapeutic response and minimal residual disease in patients with chronic myeloid leukemia.

Key words: chronic myeloid leukemia, Philadelphia chromosome, karyotyping, cytogenetic analysis.

Úvod

Chronic myeloid leukemia (CML) is one of the commonest hematological malignancies seen in clinical practice. It was the first recognized form of cancer to have a strong association with a recurrent chromosomal abnormality, the t(9,22) translocation, which generates the so - called Philadelphia (Ph) chromosome. (Saglio and Cilloni, 2004) Philadelphia chromosome is a characteristic chromosomal marker that is associated with chronic myelogenous leukemia. 95% of patients with CML show this abnormality, a remaining 2-3% have a very similar abnormality. The Ph chromosome is also found in acute lymphoblastic leukemia (ALL, 25-30% in adult and 2-10% in pediatric cases) and occasionally in acute myelogenous leukemia (AML). (Kurzrock et al., 2003) Cytogenetically, Ph chromosome is the result of the reciprocal translocation between chromosome 9 and 22 with 9q34 and 22q11 breakpoints. On molecular level, two hybrid genes are formed by this translocation

BCR/ABL, which is in the vast majority of the cases localized on the Ph chromosome. It is generally accepted that inception of BCR/ABL hybrid gene and its product plays one of the main role in pathogenesis of CML. (Michalová et al., 2002)

The disease has a chronic phase (CP-CML) that lasted for an average of 4 years before transforming into an advanced phase (AP-CML) that degenerates into acute leukemia (mostly myeloid and approximately 20% lymphoid subtype). (Tefferi et al., 2005) It is the result of abnormal and excess cell proliferation due to deregulated bcr-abl tyrosinase kinase activity as a result of Philadelphia chromosome. (Singhal et al., 2004)

Standard or conventional cytogenetics involves light microscopic examination of chromosomes to identify either numerical or structural abnormalities. Cytogenetic characteristics of CML is the presence of the Philadelphia chromosome. Philadelphia chromosome is designated Ph (or Ph1) chromoso-

me and the translocation is termed $t(9,22)(q34,q11)$. The Ph chromosome is derived from a normal 22 chromosome that has lost part of its long arm as a result of a balanced reciprocal translocation of DNA involving one of the 22 and one of the 9 chromosomes. Thus the Ph chromosome (22q-) appears somewhat shorter than its normal counterpart and the 9q+ somewhat longer than the normal 9. Figure 1 Standard cytogenetic studies of the bone marrow disclose the Philadelphia chromosome, $t(9,22)(q34,q11)$ in approximately 95% of patients at diagnosis of CML.(Tefferi et al., 2005) In remaining 5% the Philadelphia chromosome might be either masked (submicroscopic bcr/abl fusion) or part of a complex variant chromosomal translocation involvement of other chromosome breakpoints in addition to 9q34 and 22q11. These latter „Philadelphia chromosome - negative“ and Philadelphia chromosome - positive cases are readily identified by either FISH or RT-PCR, thus giving the molecular methods superior sensitivity.(Morel et al., 2003)

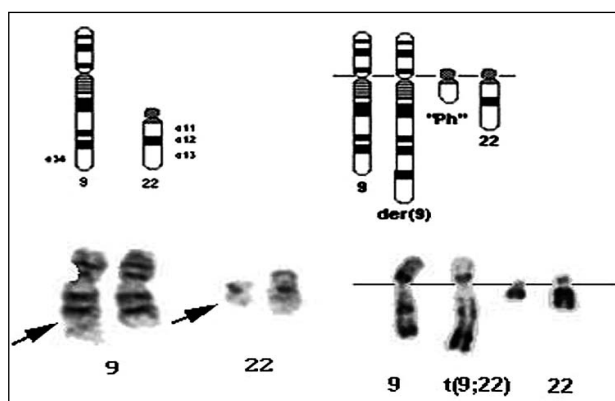


Figure 1: $t(9,22)(q34,q11)$ diagram and breakpoints, G-banding(left), R-banding(right).

In most instances, the $t(9,22)$, or a variant thereof, is the sole chromosomal anomaly during the chronic phase (CP) of the disease.(Johansson et al., 2003) The absence of Philadelphia chromosome does not exclude the possibility of CML and if the clinical scenario dictates, a more sensitive genetic test (e.g. FISH or PCR) should be performed. Additional chromosomal abnormalities of clonal evolution precede the development of the blastic or acute phase in 70% to 80% of CML cases.(Oudat et al., 2001) Most patients with CML will develop additional cytogenetic abnormalities (extra Philadelphia chromosome, trisomy 8, isochromosome 17q, trisomy 19) during blast transformation. Such clonal evolution is more frequent in myeloid compared to lymphoid blast crisis and may be associated with inferior prognosis. Furthermore the specific cytogenetic profile at the time of blast transformation may help distinguish lymphoid (chromosome 7 abnormalities) from myeloid (trisomy 8, isochromosome 17q, trisomy 19) subtype.(Tefferi et al., 2005) These secondary changes usually precede the hematologic and clinical manifestations of more malignant disease by several months and thus may serve as valuable prognostic indicator.

Material and methods

The results were obtained after a period of 10 years (1995-2004) in the Prešov region (Slovakia) in patients with suspected diagnosis of CML by conventional cyto-

genetic analysis of bone marrow cells. Bone marrow cells were cultivated 24 h in RPMI medium with 10% fetal calf serum without stimulation. In some cases, lymphocytes of peripheral blood were cultivated for 72 h and chromosomal preparations were examined, also. Mitoses were harvested after hypotonic treatment with 0,075 M KCl, and slides were prepared using conventional techniques. At least 15 G-banded cells were examined on each sample, if available. Cytogenetic examination in some patients was performed repeatedly. Cytogenetic analyses were performed on Wright's G-banded chromosomes according to ISCN nomenclature. (ISCN, 1995).

Results

The aim of our study was to confirm the presence of Philadelphia chromosome and determine their frequency in patients with CML . The number of cytogenetic examinations of bone marrow cells performed in Department of clinical genetics in the Prešov region (Slovakia) in 1995-2004 are presented in Table 1. From the overall number of 347 cytogenetic examinations, 72 samples was with suspected diagnosis CML. Philadelphia (Ph) chromosome in bone marrow cells of patients with suspected diagnosis CML in the Prešov region in 1995-2004 was detected in 68/72 (94,4%) of cases. In one patient a complex translocation involving chromosomes 8, 9 and 22(karyotype 46, XY, $t(8,9,22)(q13,q34,q11)$) was identified. One patient has showed extra numerical and structural chromosomal aberrations. Mosaic karyotype of the Ph chromosome was found in 5,9% of cases. In analyzed complete the age of patients varied from 19-74 years old (median age 46,4 years).

Cytogenetic studies were helpful in diagnosis of the disease. Karyotyping provided additional information for the differential diagnosis. Our results suggest that standard cytogenetic studies have significance at the time of CML diagnosis. On basis of these observations, it is reasonable to perform bone marrow cytogenetics at the time of diagnosis in patients with clinical diagnosis CML.

Table 1 : Chromosomal analysis of bone marrow cells of patients with CML in the Prešov region (Slovakia)

Year	The overall number of cytogenetic examinations	The number of cytogenetic examinations with susp. dg.CML	Normal karyotype	Pathological karyotype
1995	9	2	0	2
1996	30	11	1	10
1997	43	14	1	13
1998	48	8	1	7
1999	53	3	0	3
2000	47	10	1	9
2001	28	6	0	6
2002	30	9	0	9
2003	31	3	0	3
2004	28	6	0	6
All	347	72	4	68

Discussion

Chronic myeloid leukemia is a clonal stem cell disease caused by an acquired somatic mutation that fuses, through chromosomal translocation, the abl and bcr genes on chro-

mosome 9 and 22, respectively.(Tefferi et al., 2005) The Philadelphia (Ph) chromosome, resulting from the balanced translocation, t(9,22)(q34,q11.2) is the diagnostic hallmark of chronic myeloid leukaemia.(Wan et al., 2003) CML is the first disease that was associated with a consistent cytogenetic abnormality, the Philadelphia chromosome which is a shortened chromosome 22 and represents a reciprocal translocation between chromosomes 9 and 22, t(9,22)(q34,q11).(Tefferi et al., 2005) The t(9,22) fuse the c-ABL gene on chromosome 9 with the BCR gene on chromosome 22, resulting in the production of chimeric oncoproteins.(Giugliano et al., 2003)

Presentation may be at any age, but the peak incidence is at age 40-60 years, with a slight male predominance. In analyzed complete of patients with clinical diagnosis CML in Prešov region (1995-2004) the age of patients varied from 19-74 years old (median age 46,4 years) that confirm this affirmation. Philadelphia chromosome was detected in 27 men and 41 women. In our survey a male predominance was not confirmed. Other authors found, that the median age of patients with Ph+ CML is 67 years age, it can occasionally occur in children (2-3% of all childhood leukaemia).(Faderl et al., 1999) Goldman et al. also postulated that this leukemia is very rare in children.(Goldman, 1997)

Most cases of chronic myeloid leukaemia occur sporadically. The only known predisposing factor is irradiation.(Goldman, 1997)

The Ph1 chromosome in the bone marrow cells of patients with suspected diagnosis of CML in the Prešov region in 1995-2004 was detected in 68/72 (94,4%) of cases. In one patient a complex translocation involving chromosomes 8, 9 and 22 (karyotype 46, XY,t(8,9,22) (q13,q34,q11) was identified. One patient has showed extra numerical and structural chromosomal aberrations. Mosaic karyotype of the Ph chromosome was found in 5,9% of cases. Our findings are similar to the data from literature concerning standard cytogenetic analysis in CML. Werner et al. detected the Ph1 chromosome in 120/128 (93%) cases with histopathologic diagnosis of chronic myeloid leukemia (CML).(Werner et al., 1995)

A prognostic value of cytogenetic analysis was demonstrated in a number of studies.The diagnosis of chronic myeloid leukemia in chronic phase can be made from study of the peripheral blood film, but the marrow is usually examined for confirmation. Detection of Ph chromosome, BCR/ABL rearrangements and expression of its aberrant transcript in now utilized as the definitive diagnosis of CML.

The complete absence of Philadelphia chromosome actually indicates a poor prognosis.(Kurzrock et al., 2003) Significant associations have been reported between cytogenetic responses of Ph-positive CML and remission duration or survival.(Oudat et al., 2000) Basal karyotype information is important in interpreting subsequent clonal evolution, emergence of new cytogenetically abnormal Philadelphia chromosome-negative clones, and cytogenetic monitoring of treatment effect.(Tefferi et al., 2005) During the transformation the additional chromosomal abnormalities to the Ph chromosome could be seen in some patients and are consider as unfavorable prognostic sign.(Michalová et al., 2002)

Rapid developments have occurred both in laboratory medicine and in therapeutic interventions for the management of patients with chronic myelogenous leukemia. With a wide array of laboratory tests available, selecting the appropriate test for a specific diagnostic or therapeutic setting has become increasingly difficult. A several commonly used laboratory assays, including cytogenetics, molecular cytogenetics (FISH), and polymerase chain reaction (PCR) have its advantages and disadvantages. Minimal residual disease (MRD) in leukaemia might be detected and variably quantified by various laboratory techniques. In general, the sensitivity of MRD testing is influenced by the type of assay used, sample source and size, and prevalence of cancer cells in the test sample.(Butturini et al., 2003) Testing for MRD in CML has been shown to predict relapse.(Hughes et al., 2003)

Tefferi et al. do not recommend replacing bone marrow cytogenetics with FISH-based methods in assessing treatment effect in CML. FISH plays a complementary role in providing information on patients in whom cytogenetic studies are inadequate because of poor metaphase yield.(Tefferi et al., 2005)

New molecular cytogenetic techniques are increasingly applied as a routine investigative tool in haematological malignancies, both at diagnosis and subsequent monitoring.(Wan et al., 2003) Su et al. found that combined use of CGH, chromosome painting, and classic cytogenetic analysis allows better evaluation of the genomic aberration involved in CML blastic transformation, and offers new directions for its further molecular investigations.(Su et al., 1999) Vin Sheth et al. observed that advanced molecular techniques like FISH and PCR cannot replace the conventional cytogenetic study but are useful as supportive and confirmative diagnostic tools.(Vin Sheth et al., 2002)

In conclusion, new molecular techniques improved specificity in minimal residual disease detection and allow the identification of variant or atypical patterns, in clinical practice results should not be taken in isolation. They should be interpreted in the light of information gathered through conventional cytogenetics, FISH and molecular genetic studies.(Wan et al., 2003)

Conclusion

Chronic myeloid leukemia represents a unique model to understand the molecular mechanisms underlying the onset and progression of a leukemic process. Philadelphia chromosome is a specific cytogenetic marker, which detection is necessary for differential diagnosis and clinical management of patients with clinical diagnosis CML. It is important, that Ph chromosome occurs in preleukaemic phase and have important diagnostic and prognostic significance. Bone marrow karyotyping is useful for specific identification of cytogenetic profile. Standard cytogenetics for CML is indicated at the time of diagnosis and hematologic relaps, and is reasonable to consider during follow-up bone marrow examination for any indication. Conventional cytogenetic analysis remain the standard method for purposes of diagnosis, monitoring of the therapeutic response and minimal residual disease in patients with chronic myeloid leukemia.

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