CLINICAL STUDY

Detection of Philadelphia chromosome in patients with chronic myeloid leukemia from the Presov region in Slovakia (1995–2004)

Boronova I, Bernasovsky I, Bernasovska J, Sotak M, Petrejcikova E, Bozikova A, Seliga P[1]

Department of Biology, Faculty of Humanities and Natural Science, University of Prešov, Slovakia. **boronova@unipo.sk**

Abstract

Philadelphia chromosome (Ph) is a characteristic chromosomal marker that is associated with chronic myelogenous leukemia (CML). Philadelphia chromosome in bone marrow cells in patients with suspected diagnosis of CML in the Prešov region (1995–2004) was detected in 94.4 % of cases. In one patient a complex translocation involving the chromosomes 8, 9 and 22 was identified. One patient has showed extra numerical and structural chromosomal aberrations. The mosaic karyotype of Ph chromosome was found in 5.9 % of cases. The conventional cytogenetic analysis remains the standard method for the purpose of diagnosis and monitoring of the therapeutic response and minimal residual disease in patients with chronic myeloid leukemia *(Tab. 1, Fig. 1, Ref. 18)*. Full Text (Free, PDF) *www.bmj.sk*.
Key words: chronic myeloid leukemia, Philadelphia chromosome, karyotyping, cytogenetic analysis.

Chronic myeloid leukemia (CML) is one of the commonest hematological malignancies seen in clinical practice. It is the first form of cancer that was first recognized to have a strong association with a recurrent chromosomal abnormality, namely the t(9;22) translocation generating 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, the 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 chromosomes 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 cases localized on Ph chromosome. It is generally accepted that the inception of BCR/ABL hybrid gene and its product plays one of the main roles in the pathogenesis of CML (Michalová et al, 2002).

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

Both standard and conventional cytogenetics involve light microscopic examination of chromosomes to identify either numerical or structural abnormalities. CML is cytogenetically characterised by the presence of the Philadelphia chromosome. Philadelphia chromosome is referred to as Ph (or Ph1) chromosome and the translocation is marked as t(9;22)(q34;q11). The Ph chromosome is derived from a normal chromosome 22 that has lost a part of its long arm as a result of balanced reciprocal translocation of DNA involving one of the chromosomes 22 and one of the chromosomes 9. Thus the Ph chromosome (22q-) appears somewhat shorter than its normal counterpart and the 9q+ somewhat longer than the normal 9 (Fig. 1).

Department of Biology, Faculty of Humanities and Natural Science, University of Presov, and [1]Department of Surgery, Hospital FNsP R.A. Rayman Presov, Slovakia

Address for correspondence: I. Boronova, Dept of Biology, Faculty of Humanities and Natural Science, University of Presov, ul. 17. novembra, SK-081 16 Presov, Slovakia.
Phone: +421.51.7734188

Standard cytogenetic studies of the bone marrow diclose the Ph1 chromosome, t(9;22)(q34;q11) in approximately 95 % of patients with the diagnosis of CML (Tefferi et al, 2005). In the remaining 5 % the Philadelphia chromosome might be either masked (submicroscopic bcr/abl fusion) or a part of complex variant chromosomal translocation where some other chromosome breakpoints are involved 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 a superior sensitivity (Morel et al, 2003).

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 where the clinical picture 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 to distinguish the 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 a more malignant disease by several months and thus may serve as valuable prognostic indicator.

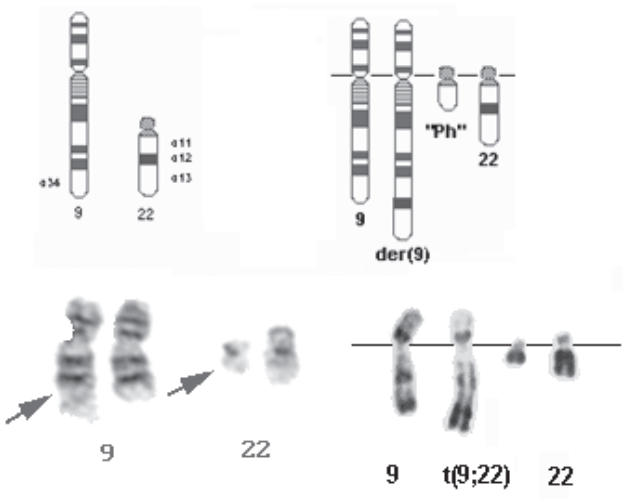

Fig. 1. t(9;22)(q34;q11) diagram and breakpoints, G – banding (left), R – banding (right).

Patients 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 cytogenetic analysis of bone marrow cells. Bone marrow cells were cultivated for 24 hours in RPMI medium with 10 % fetal calf serum without stimulation. In some cases, lymphocytes of peripheral blood were cultivated for 72 hours, and chromosomal preparations were examined too. 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 in each sample, when available. Cytogenetic examinations in some patients were 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 at the Department of Clinical Genetics (Hospital in Prešov) in the Prešov region (Slovakia) over the period of years 1995–2004 are presented in Table 1. Out of the overall number of 347 cytogenetic examinations, 72 samples were with the suspected diagnosis of CML. Philadelphia chromosome in 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 the chromosomes 8, 9 and 22 (karyotype 46,XY, t(8;9;22)(q13;q34;q11) was identified. One patient has showed some extra numerical and structural chromosomal aberrations. Mosaic karyotype of the Ph chromosome was found in 5.9 % of cases. In the analyzed group the age of patients varied from 19–74 years (median age was 46.4 years).

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

Discussion

Chronic myeloid leukemia is a clonal stem cell disease caused by an acquired somatic mutation that fuses through chromosomal translocation of the abl and bcr genes on chromosome 9 and 22, respectively (Tefferi et al, 2005). The Philadelphia chromosome resulting from the balanced translocation of 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) fuses the

Tab. 1. Chromosomal analysis of bone marrow cells of patients with CML in region Prešov (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	23
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
Total	347	72	4	68

c-ABL gene on chromosome 9 with the BCR gene on chromosome 22 resulting in the production of chimeric oncoproteins (Giugliano et al, 2003).

The presentation may be at any age but the peak incidence is at the age of 40–60 years with a slight male predominance. In the analyzed group of patients with the clinical diagnosis of CML in Prešov region (1995–2004) the age of patients varied from 19–74 years (median age was 46.4 years), the fact of which confirms the latter affirmation. Philadelphia chromosome was detected in 27 men and 41 women. In our survey the male predominance was not confirmed. Other authors indicate that the median age of patients with Ph+CML is 67 years, it can occasionally occur in children (2–3 % of all childhood leukaemia) (Faderl et al, 1999). Goldman et al (1997) also postulated that this type of leukemia is very rare in children. Most cases of chronic myeloid leukaemia occur sporadically. The only known predisposing factor is irradiation.

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 %) 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 some extra numerical and structural chromosomal aberrations. The mosaic karyotype of the Ph1 chromosome was found in 5.9 % of cases. Our findings are simillar to the data from literature concerning the standard cytogenetic analysis in CML. Werner et al (1995) detected the Ph1 chromosome in 120/128 (93 %) cases with histopathologic diagnosis of chronic myeloid leukemia (CML). The 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 the study of peripheral blood film but the marrow is usually examined for confirmation. The detection of Ph chromosome, BCR/ABL rearrangements and the expression of its aberrant transcript are now utilized as the definitive diagnosis of CML.

A complete absence of Ph1 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 the subsequent clonal evolution, emergence of new cytogenetically abnormal Ph1 chromosome-negative clones and cytogenetic monitoring of treatment effect (Tefferi et al, 2005). During the transformation, some chromosomal abnormalities additional to Ph chromosome could be seen in some patients, the fact of which is considered to be an unforable prognostic sign (Michalová et al, 2002).

Rapid developments have occured both in laboratory medicine and in therapeutic interventions within the managment of patients with chronic myelogenous leukemia. With a wide array of laboratory tests available, the selection of the appropriate test for 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 prevalence of cancer cells in the test sample (Butturini et al, 2003). Testing for MRD in CML has beeen shown to predict the relapse (Hughes et al, 2003). Tefferi et al (2005) do not recommend replacing bone marrow cytogenetics with FISH-based methods in assessing the treatment effect in CML. FISH plays a complementary role in providing information in patients with poor metaphases. New molecular cytogenetic techniques are increasingly applied in haematological malignancies as a routine investigative tool in both diagnosis and subsequent monitoring. Su et al (1999) indicate that combined use of CGH, chromosome painting and classic cytogenetic analysis allows better evaluation of genomic aberrrations involved in CML blastic transformation and offer new directions for its further molecular investigations. Vin Sheth et al (2002) observed that advanced molecular techniques like FISH and PCR cannot replace the conventional cytogenetic study but are useful as supportive and confirmative diagnostic tools.

In conclusion, new molecular techniques have improved the specifity in minimal residual disease detection and allow us to identify the variant or atypical patterns. In clinical practise 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 leukemic process. Philadelphia chromosome is a specific cytogenetic marker, the detection of which is necessary for differencial diagnosis and clinical management of patients with the clinical diagnosis of CML. It is of importance that Ph

chromosome occurs in the preleukaemic phase and has a great diagnostic and prognostic significance. Bone marrow karyotyping is useful for specific identification of the cytogenetic profile. Standard cytogenetics for CML is indicated at the time of diagnosis and hematologic relaps, and it is reasonable to consider it during the follow-up bone marrow examination for any indication. The conventional cytogenetic analysis remains the standard method for the purposes of diagnosis and monitoring of the therapeutic response and minimal residual disease in patients with chronic myeloid leukemia.

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