Laboratory characteristics of patients with variant Phiadelphia chromosome positive leukemia

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Abstract

Background Variant Philadelphia chromosomes are characterized by the involvement of another chromosome in addition to chromosome 9 or 22. To detect the difference between variant Philadelphia chromosomes positive leukemia and classic Philadelphia chromosomes positive leukemia. And to help diagnose and treat variant Philadelphia chromosomes positive leukemia.

Methods In this study, Peripheral blood and bone morrow cell morphology test was used to analysis bone morphology of variant Ph positive patients. Karyotype analysis was used to find out variant Ph chromosomes. Flow cytometry analysis was used for immunology analysis. BCR/ABL was detected by PCR to monitor change of molecular genetics in variant Ph positive patients.

Results From 48 patients with Ph positive leukemia, we found out 3 variant Ph positive leukemia. Compared with the classic Ph positive leukemia patients, the hemogram in the variant Ph positive leukemia patients was more variant for presenting hypomyelodysplasia or hyperactive. Compared with classic chronic myeloid leukemia which neutrophilic myelocyte, metamyelocyte and stab granulocyte is increasing in, both the morphological testing of bone marrow cells smear and flow cytometry analysis were indicated that proportion of myeloblast and promyelocyte increased in variant Philadelphia chromosomes leukemia. What's more, the variant Ph with breakpoint 4q31 was the first report in leukemia patient. Same as the classic Ph positive leukemia patient, formal and effective treatment could prolong the survival of patients with variant Ph positive leukemia.

Conclusions Compared with classic Ph positive leukemia, the hemogram was more variant in variant Ph. The myeloid morphology in the patients with variant Ph was more immature than that of in the patients with classic Ph. Reporting new cases of complex variant translocations, which can refer to new breakpoints that can eventually be recurrent and important for the understanding of this leukemia. Formal and effective treatment are necessary for variant Ph positve leukemia.

Background

Philadelphia chromosome is the result of a reciprocal translocation involving the long arms of chromosomes 9 and 22, referred to as t (9; 22) (q34.1; q11.2). It's not only a hallmark of chronic myeloid leukemia(CML), but it is also happened in patients with acute lymphoblastic leukemia(ALL) and mixed phenotype acute leukemia[1, 2]. The Phildelphia chromosome is involved in the break-point cluster region-Abelson tyrosine kinase fusion gene, which encodes a constitutively active tyrosine kinase protein [3]. The Philadelphia chromosome is detected by karyotyping in around 90% of chronic myeloid leukemia (CML) patients, however, 5–10% may have variant types [4]. Variant Philadelphia chromosomes (Variant Ph) are characterized by the involvement of another chromosome in addition to chromosome 9 or 22. It can be a simple type of variant when one other chromosome is involved, or complex, in which two or more chromosomes take part in the translocation. Variant translocation could be formatted via either the one step or two step mechanisms[5].

Most cases of CML are diagnosed in the chronic phase. The classic CML is Ph positive CML. In Ph positive CML, the leukocyte in peripheral blood was increasing obvisously and continuously. In patients with chronic myelogenous leukemia, the bone marrow is active or hyperactive, with an increased proportion of G/E(granulocyte/erythrocyte) [3]. The myelocytes proliferated as neutrophilic myelocyte, metamyelocyte and stab granulocyte. Erythrocytes proliferate at early stage and are inhibited at late stage. The thickness of granulocytes in bone marrow biopsy sections is usually 5–10 layers, compared with 2–3 layers in normal cases. When the acute myeloid line was changed, the protocells expressed the differentiation antigens associated with granular monocyte line, mononuclear line, megakaryocyte line and red line. The location of the BCR gene break point can affect the phenotype of the disease.

Imatinib should be considered in the same way as it is used for all patients with Ph-positive CML. Nalatinib and dasatinib can prevent imatinib resistance due to tyrosine kinase mutations in most but not all Phiadelphia chromosome positive leukemia[5]. Few studies have reported the variant Ph in acute myeloid leukemia (AML). The BCR/ABL1 fusion protein (p210) was observed in 95% of Ph positive CML, whereas the p190 was appear in Ph positive ALL[6–8].

The mechanism of variant Ph generation and the molecular bases of biological differences between classic Ph and variant Ph chromosomes are not fully understood[9]. Few comprehensive studies have reported the clinical characteristics of variant Philadelphia chromosomes or the molecular cytogenetic characterization involved among leukemia patients. Although they both have variant Phiadelphia translocation, the laboratory diagnostic characterization and prognosis are some different[10]. To find out the difference between classic Ph and variant Ph chromosomes leukemia and report new cases of complex variant translocations which may be involved new breakpoints, can eventually be help for diagnosing and treating these leukemia.

Methods

Patients

Retrospective analysis were taken among the patients diagnosed and treated in affiliated hospital of North Sichuan Medical College accepted normative laboratory examinations concluding complete blood count (CBC), bone morrow cell

morphology test, flow cytometry analysis, karyotype analysis of bone marrow cells, molecular genetic analysis from Jan.1.2018 to Dec.30.2019. The diagnosis of Ph+ leukemia was made on the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia according to morphology, immunology, cytogenetic, and molecular criteria, and multiple organ failure was also diagnosed[3].There are 47 Ph+ leukemia(in table1) and among them 3 patients with variant Ph(in table2, two patients with CML and one patients with AML).Both of them are male and their mean age is 50.7 years old. Ethics committee of affiliated hospital of North Sichuan Medical college approved this study.

Peripheral blood and bone morrow cell morphology test

Smears of peripheral blood or bone marrow cells were stained with Wright-Giemsa solution and observed by light microscopy. Bone marrow smear examination included the degree of hyperplasia and the proportion of leukemia cells. The degree of hyperplasia activity was determined according to the proportion of nucleated cells and mature erythrocytes: 1:20 was active, l: 10 was significantly active, and l: 1 was hyperactive. At the same time, 200 nucleated cells were counted and their morphology and proportions were analyzed[3,11].

Karyotype analysis

Conventional cytogenetic analysis was performed on G-banded metaphase cells after having cultured bone marrow 24 hours using standard techniques. At least 20 metaphases with good quality banding were evaluated for each case when satisfactory cell cultures were available. A 320-band ideogram was considered as the standard level of resolution for the purpose of the present study. The karyotype was documented according to the International System for Human Cytogenetic Nomenclatures (ISCN 2013) [7]

Flow cytometry analysis

Using heparin anticoagulation bone marrow 2 ml, immune phenotype were analyzed by a four-color immune classification method for patients with leukemia cell, and the fluorescent tags were with monoclonal antibody (McAb) and various type are from Beckman Coulter company product of the United States, flow cytometry detection was using American Beckman Coulter company. Collect and analysis of 10 000 nucleated cells to identified leukemia cell group, with setting by CD45 / SSC gate. The expression of each antigen in this cell group was analyzed, and the fluorescence intensity of a certain antigen expression in the abnormal cell group was > 20% as positive, and the positive dividing line for CD34 was 10%.

PCR for BCR/ABL

BCR-ABL t(9;22) quantitative assay was performed in Sichuan kingmed center laboratory, Sichuan, China. Briefly, patient medulla RNA was isolated and reverse transcribed to complementary DNA (cDNA). The BCR/ABL1 and ABL1 reference gene sequences were amplified in duplicate using multiplexed quantitative real-time PCR. This assay can detect the major, minor, and micro BCR/ABL breakpoints and has an analytical sensitivity of better than 0.002%.[5]

Results

Characteristics of variant Ph positive leukemia patients are some different

Three cases (6.38%) with variant t (9;22) were found out from 47 Ph positive CML & AML (Table 1). Table 2 shows the list of the variant Philadelphia translocation cases. WBC in blood of both patient No.1 and

patient No.3 increased significantly, but was not change in patient No.2. What's more, patients No.2 and No.3 were calinical diagnosed as CML, and patient 1 as AML. Because of his own beliefs, patient No.2 refused further examination and treatment. It was lacks of evidence including flow cytometry analysis and molecular genetic analysis for clinical diagnose.

Morphological tests of bone marrow cells smear

Compared with normal bone marrow image, bone marrow images with Ph+ leukemia patients had some characteristics: one CML case were hypomyelodysplasia like aplastic anemia (AA), and another CML case and one AML case is hyperactive, which is consistent with CBC of the three case (Fig1). Compared with classic Ph positive leukemia, the hemogram in the variant Ph positive patients was more variant. The proportion of myeloblast and promyelocyte also increased significantly.

Chromosomal abnormalities about variant Phiadelphia chromosome

Compared with classic Ph positive leukemia chromosome karyotype, complex karyotype were among three variant Ph positve leukemia. The complex translocation occured among chromosome 4, chromosome 9 and chromosome 22, or among chromosome 5, chromosome 9 and chromosome22. (Fig2)

Flow cytometry analysis

According to the fluorescence intensity analysis of SSC and CD45, gate analysis was set on the CD45/SSC point diagram. The results showed that abnormal cell population could be seen in the distribution area of the protocells, accounting for about 84.8% of the nuclear cells expressing CD13, CD33, CD64, CD117, CD9, CD123, CD2, MPO. The proliferation of medullary system was obviously inhibited (Fig3). These surface antigens suggested an increase in myeloblast and promyelocyte. The myeloid morphology of the variant Ph positive leukemia was more immature than that of the classic CML. It was consistent with morphological findings of bone marrow cells smear.

PCR for BCR/ABL1

In our study, Reverse transcription-polymerase chain reaction (RT-PCR) showed the presence of p210-type BCR-ABL fusion transcript in bone morrow with patients No.2 and patients No.3. What's more, Y253F/H mutation was existed in patients No.2 (the figure not show) (Fig4). P210-type BCR-ABL fusion transcript often appeared in CML including variant Philadelphia chromosome positive leukemia and the classic CML.

Treatment outcome and prognosis

It was indicated that patient No.1 have no treatment so he were dead 3 month after diagnose (table 2). Another two man were treated by dasatinib or imatinib & hydroxyurea. So they are alive up to now.

Therefore, formal and effective treatment could prolong the survival of patients with variable Philadelphia chromosome positive leukemia.

Discussion

In about 5–10% of CML cases, a complex translocation leaded to the formation of a variant Ph chromosome [12, 13]. Three cases (6.38%) with variant t (9;22) were found out from 47 Ph positive CML & AML (Table 1).Three cases of variant Ph positive leukemia patients were observed about their laboratory tests and clinical characteristics. Few comprehensive studies have reported the clinical characteristics of variant Philadelphia chromosomes or the molecular cytogenetic characterization involved among leukemia patients. We reported it in our study foucus on the difference between Ph positive leukemia and variant Ph positive leukemia in laboratory characteristics.

From the morphological test of bone marrow cells smear, two patients were similar with classic Ph chromosome positive CML for high WBC. Their bone marrow were hyperactive. Interestingly, another patient's bone marrow were infertile like AA. This type is rare in previous reports. Based on the evidence, his treatment was different with high WBC CML. The variable blood test results suggest we need to collect more cases to summarize the laboratory characteristics of the variant Philadelphia chromosome.

Variant Philadelphia translocations involving chromosome 4 were a very rare events and the translocation in the presented case involving 4q31 have not been reported previously. The distribution of the break-points was non-random with the chromosomal bands most susceptible to break being: 1p36, 3p21, 5q31, 6p21, 9q22, 10q22, 11q13, 12p13, 17p13, 17q21, 17q25, 19q13, 21q22, 22q12 and 22q13 [14]. So 4q31 is first report up to now. Reporting new cases of complex variant translocations, which can refer to new breakpoints can eventually be recurrent and important for the understanding of this leukemia. What's more, studing new variant translocations and cryptic translocation can help for illuminating the differences and similarities between classic Ph positive leukemia and variant Ph positive leukemia, especially analyzing the diversity. Variant translocation could be via either the one-step or two-step mechanisms. some researchers [15–18] have introduced a one-step mechanism, wherein chromosome breakpoints occurs on three different chromosomes simultaneously in a three-, four-, or five-way translocation, then reciprocally rejoin at the same time. Others [19, 20] have confirmed a two-step mechanism, in which a standard two-way t(9;22) was followed by subsequent translocation involving additional chromosomes, and some recent studies [21] have reported both mechanisms in the same patient. We should use fluorescence in situ hybridization to judge the three patients' breakages in further study.

The karyotype and the combination of different FISH probes are essential to characterize complex variant Ph translocations [21–22]. In our article, conventional

and molecular cytogenetic studies have allowed the characterize three complex variant Ph translocations. And the chromosomes most mainly involved were chromosomes 4 and 5. 85% of variant 9, 22

translocations located in the G-light bands (CG-richest areas)[22]. The CG richness areas reflected increasing in the density of the CpG islands, genes, repetitive elements, and recombination[23].

In our study, one case was variant Ph positive AML. But this patient was dead because of his own beliefs and give up treatment. And from his laboratory tests, both variant Ph and classic Ph in his bone marrow. Based on his CBC, the WBC were increased significantly. These data was indicated that his prognosis is poor. Two case were variant Ph positive CML. Molecular biological tests can help us choose which drugs to treat. Because of the Y253F/H mutation patient No.2 was treated by dasatinib. However, patient No.3 was treated by imatinib & hydroxyurea result of no mutations of BCR/ABL1 gene. Different chromosomal translocations may cause different gene mutations, which may affect the treatment of patients. Thus, it suggests that chromosomal aberration and genetic mutations in the bone marrow of patients with variant Philadelphia chromosome positive leukemia are critical to their diagnosis. This also accords with the tumor precision medicine project. CG richness varies with many other features of the genome[19, 23–24].The available results provide evidence that the variant translocation in CML patients may affect response to imatinib therapy and patient prognosis [25, 26].So patients with a complex karyotype should be closely monitored for both side effects and disease progression under tyrosine kinase inhibitor treatment[28].

Conclusion

In conclusion, the laboratory diagnostic characterization studies has allowed us:

i) Compared with classic Ph positive leukemia ,the hemogram was more variant. The myeloid morphology of the variant Philadelphia chromosome positive leukemia was more immature than that of the classic CML. ii) Complex karyotype were among variant Ph positve leukemia. Reporting new cases of complex variant translocations, which can refer to new breakpoints that can eventually be recurrent and important for the understanding of this leukemia. iii)Formal and effective treatment are necessary for variant Ph positve leukemia[29-30].

Abbreviations

Abbreviation	Unabbreviated form
WBC	White blood cell
Hb	Hemoglobin
Plt	Platelet
CML	Chronic myeloid leukemia
AML	Acute lymphoblastic leukemia
Che	Chemotherapy
AA	Aplastic anemia

Declarations

Ethics approval and consent to participate

The authors have no ethical conflicts to disclose, and Ethics committee of affiliated hospital of North Sichuan Medical University approved this study.

Consent for publication

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

Availability of data and material

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors of this paper have no conflict of interests, including specific financial interests, relationships, and affiliations relevant to the subject matter or materials included.

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Authors' contributions

QS and YG as co-first author contributed equally to this work QS designed the study, and wrote the manuscript. YG is the funder of our study. And he collected and analyzed data. All authors read and approved the final manuscript.

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Tables

Table 1 Characteristics of Ph positive leukemia patients

The red marked 3 variant Ph positive leukemia patients

ear)	Diagnosis	The type of BCR/ABL	Chromosome Karyotype
3	CML	Not tested	46,XX,t(9;22)(q34;q11)
2	CML	Not tested	48,XY,+8,t(9;22)(q34;q11),t(15;17)(q22;q11),+mar
.	CML	Not tested	46,XY,t(9;22)(q34;q11)
7	CML	p210	46,XY,t(9;22)(q34;q11)
7	ALL	p190	46,XY,t(9;22)(q34;q11)
0	CML	Positive	46,XY,t(9;22)(q34;q11)
.	CML	Positive	46,XX,t(9;22)(q34;q11)
7	CML	p210	46,XY,t(9;22)(q34;q11)
2	AML	Not tested	46,XY,t(7;11)(p15;p15)
.	CML	p210	46,XY,t(9;22)(q34;q11)
3	CML	p210	46,XY,t(9;22)(q34;q11)
5	ALL-B	p210	46,XY,t(9;22)(q34;q11)[4]
5	CML	p210	48,XX,t(9;22)(q34;q11),+8,+15,+mar,-17
3	CML	p210	46,XY,t(9;22)(q34;q11)
2	CML	p210	46,XY,t(9;22)(q34;q11)
7	ALL	p190	46,XY,t(9;22)(q34;q11)[3]/46,XY[7]
2	CML	p210	46,XY,t(9;22)(q34;q11),9qh+
3	ALL	p190	46,XY,t(9;22)(q34;q11)[2]/46,XX[3]
5	CML	Not tested	46,XY,t(9;22)(q34;q11)
0	CML	Not tested	46,XY,t(9;22)(q34;q11)
5	CML	Not tested	46,XY,t(9;22)(q34;q11)
5	CML	Not tested	46,XY,t(9;22)(q34;q11)
7	CML	Not tested	46,XY,t(9;22)(q34;q11)
2	CML	p210	46,XY,t(9;22)(q34;q11)
3	ALL	Positive	46,XY,t(9;22)(q34;q11)[5]/46,XY[1]
3	CML	p210	46,XY,t(9;22)(q34;q11)
5	CML	Not tested	46,XY,t(9;22)(q34;q11)
0	MDS	Not tested	46,XY,t(9;22)(q34;q11)[6]/46,XY[4]
3	CML	Not tested	46,XY,t(9;22)(q34;q11)
3	CML	Not tested	46,XX,t(9;22)(q34;q11)
3	CML	Not tested	46,XX,t(9;22)(q34;q11)
.	CML	Not tested	46,XX,t(9;22)(q34;q11)
.	CML	Not tested	46,XY,t(9;22)(q34;q11)
0	ALL	Not tested	46,XX,t(9;22)(q34;q11)[20]
3	CML	Not tested	46-49,XY,t(4;9;22)(q31;q34;q11),+5,+der(9;22)(q34;q11)

5	CML	Not tested	46,XY,t(9;22)(q34;q11)[20]
7	CML	Not tested	46,XY,t(9;22)(q34;q11)
2	CML	p210	46,XY,t(5;9;22)(q35;q34;q11)
1	CML	p210	46,XY,t(9;22)(q34;q11)[10]
5	CML	Positive	46,XX,t(9;22)(q34;q11)
4	CML	p210	46,XX,t(9;22)(q34;q11)
2	CML	Positive	46,XY,t(9;22)(q34;q11)[10]
0	ALL	p190	46,XY,t(9;22)(q34;q11)
0	CML	p210	44,XY,-7,-18,t(8;11;14)(p23;p11.2;q11.2),t(9;22)(q34;q11)
5	ALL	Not tested	49,XX,+7,+15,-9□-17,-22□+t(9;22)(q34;q11)×2
4	CML	p210	52-54,XXY,+4,+5,+7,+14,+21,+9,t(9;22)(q34;q11),t(9;14)(p13;q10)/46,XY
7	CML	p210	46,XY,t(5;9;22)(q;13;q34;q11)
5	CML	p210	46,XY,t(9;22)(q34;q11)

Table 2 Characteristics of variant Ph+ leukemia patients

Patients	sex	Age(year)	WBC(10^9/)	Hb(g/L)	Plt(10^9/L)	Diagnosis	Chromosome Karyotype	Therapeutic strategy	outcome
1	M	63	47.77	95	117	AML?	46-49,XY,t(4;9;22)(q31;q34;q11),+5, +der(9;22)(q34;q11)	no	dead
2	M	52	9.11	81	320	CML	46,XY,t(5;9;22)(q35;q34;q11)	dasatinib	alive
3	M	37	176.39	126	269	CML	46,XY,t(5;9;22)(q13;q34;q11)	Imatinib +hydroxyurea	alive

Figure

S

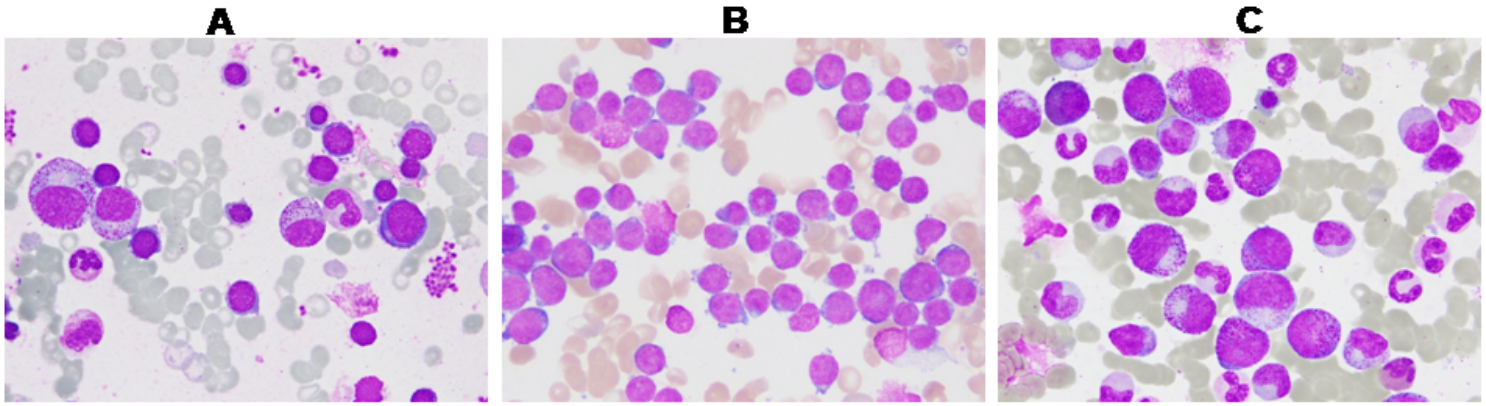

Figure 1

Morphological results of bone marrow cells smear A normal bone marrow image□B and C bone marrow cells smear of patient1 and patient3.

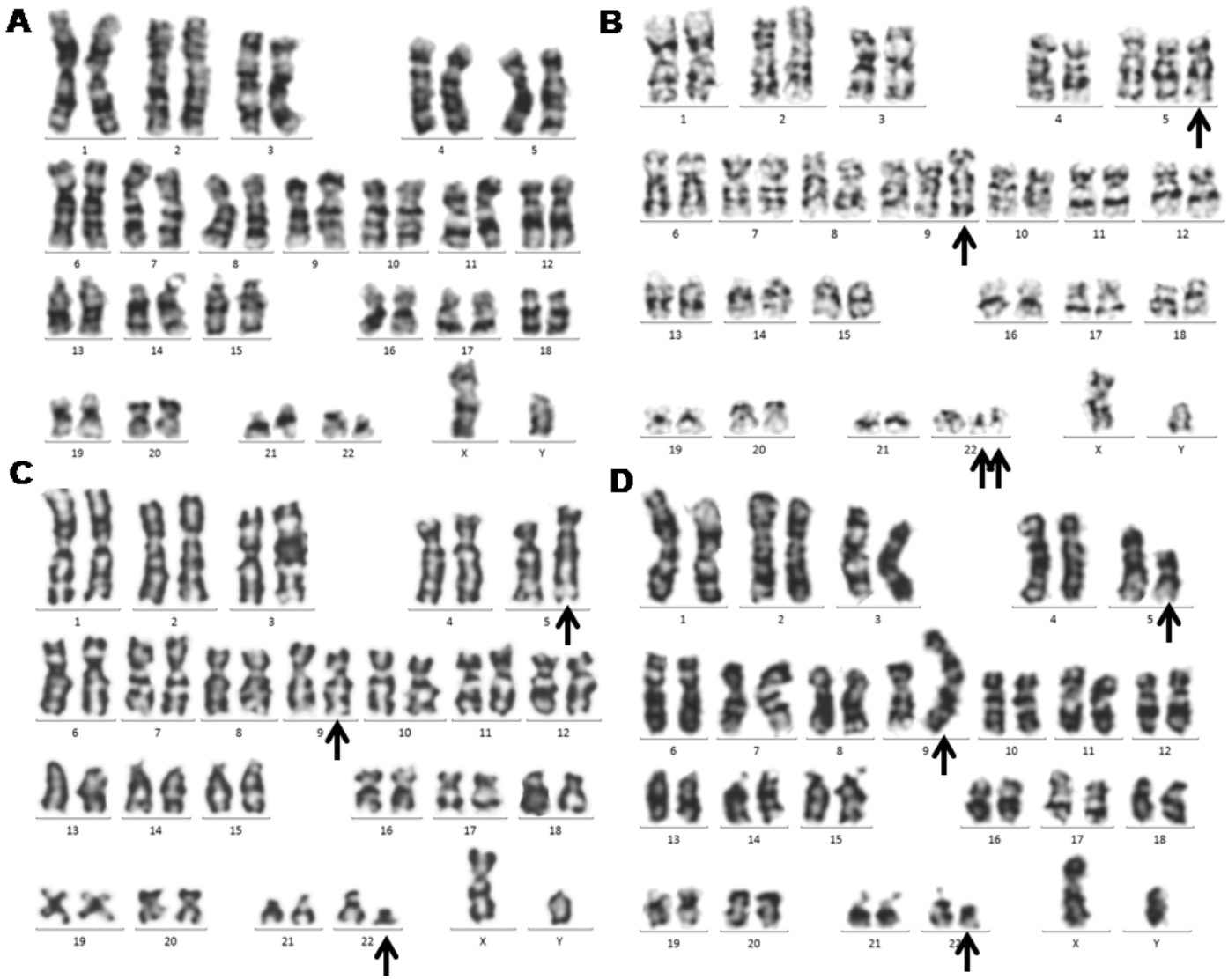

Figure 2

Chromosomal abnormalities of Ph+ leukemia patients A classic ph chromosome 46,XY,t(9;22)(q34;q11);B patient 1 karytype of BM,46-49,XY,t(4;9;22)(q31;q34;q11),+5,+der(9;22)(q34;q11);C patient 2karytype of BM,46,XY,t(5;9;22)(q35;q34;q11);D patient 3karytype of BM,46,XY,t(5;9;22)(q;13;q34;q11) Arrow- heads highlight all derivative chromosomes.

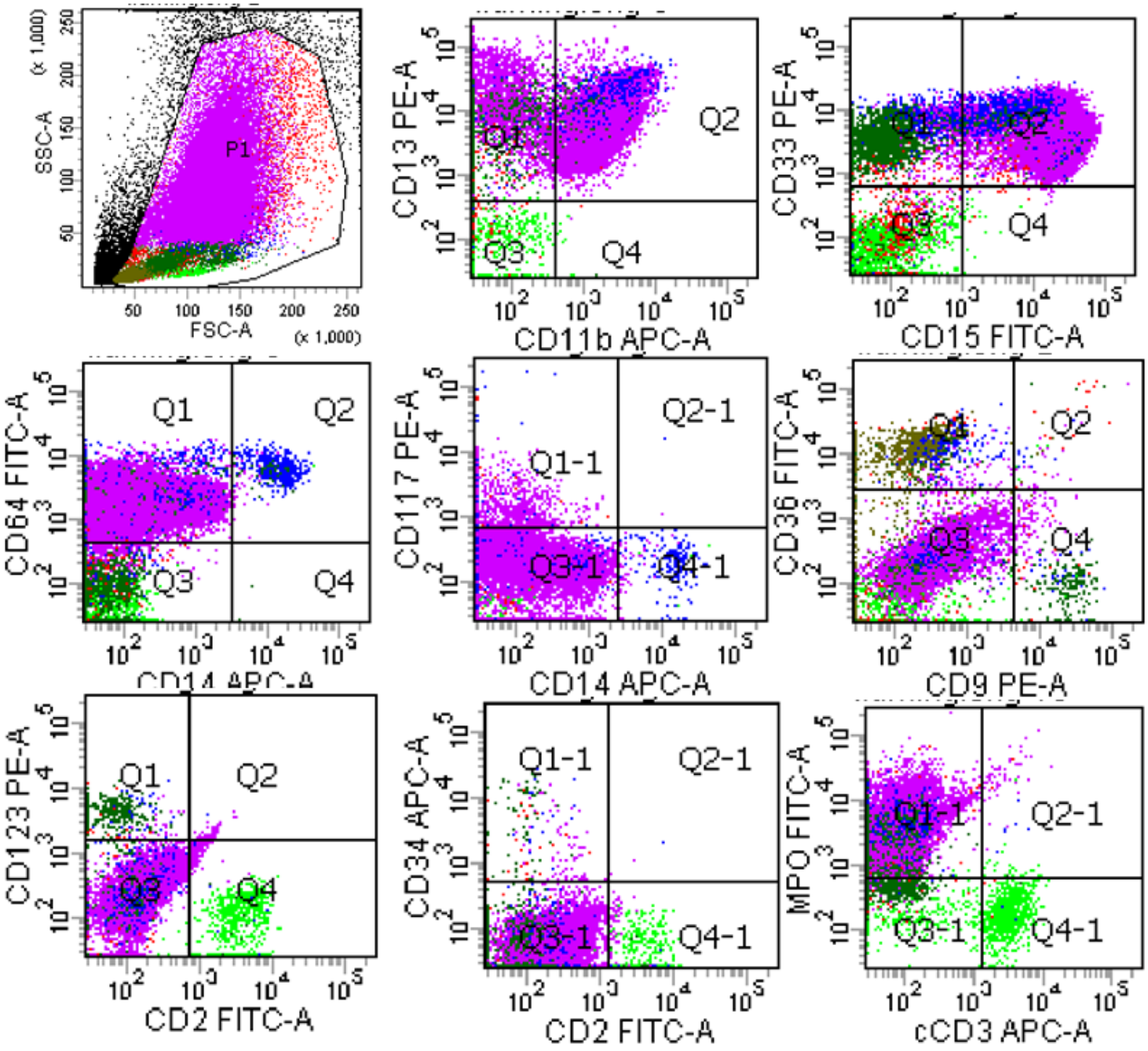

Figure 3

Flow cytometry analysis of variant Ph+ leukemia patients PCR for BCR/ABL 84.8% of the nuclear cells expressing CD13, CD33, CD64, CD117, CD9, CD123, CD2, MPO.

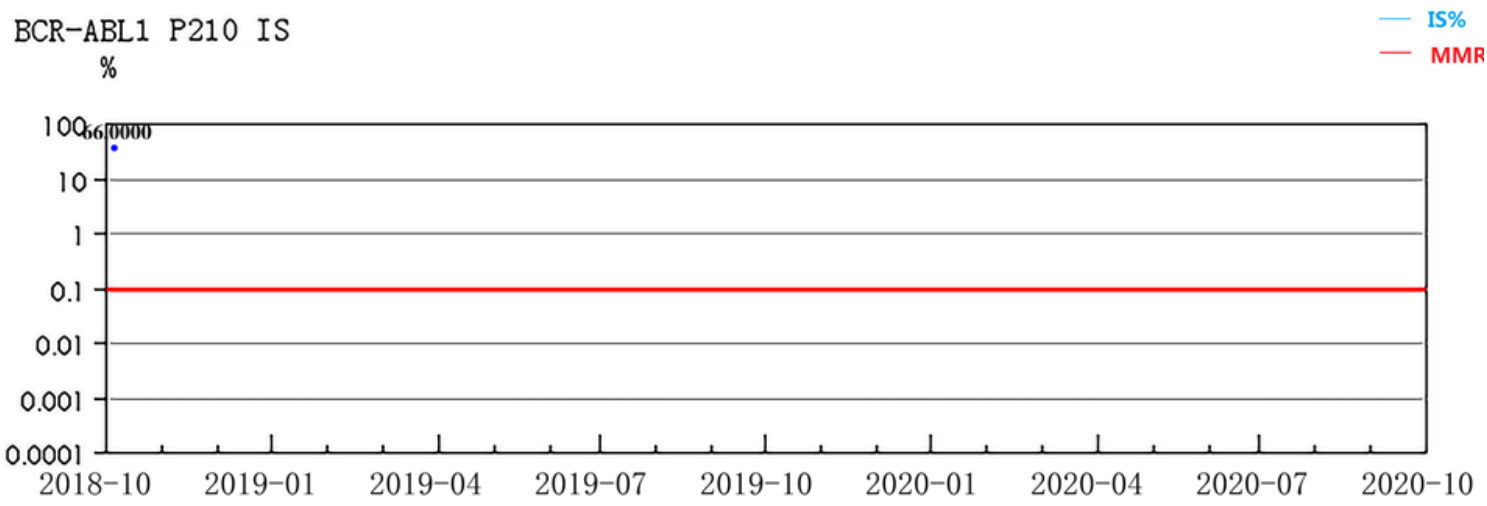

Figure 4

PCR for BCR/ABL1 P210-type BCR–ABL fusion transcript appeared in bone morrow with patients2 and patients3.