short report
Haematologica 1997; 82:596-599

SIMULTANEOUS OCCURRENCE OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA AND CHRONIC MYELOID LEUKEMIA WITH FURTHER EVOLUTION TO LYMPHOID BLAST CRISIS

JORDI ESTEVE, FRANCISCO CERVANTES, SUSANA RIVES, MARÍA ROZMAN, MARÍA ÁNGELES ZARCO, EMILIO MONTSERRAT

Department of Hematology and Postgraduate School of Hematology "Farreras Valentí", Department of Medicine, Hospital Clínic, University of Barcelona, Barcelona, Spain

ABSTRACT

The coexistence of chronic myeloid leukemia (CML) and B-cell chronic lymphocytic leukemia (CLL) in the same patient is rare. A 71-year-old woman developed a B-lineage lymphoid blast crisis at 18 months after diagnosis of Ph-positive CML. At this time, a lymphoid cell population with morphologic and immunophenotypic features of CLL was demonstrated. The retrospective review of the tests performed at diagnosis and thereafter disclosed the presence of lymphoid nodules in the initial bone marrow biopsy in the absence of lymphocytosis. Subsequently, there was an appearance of moderate lymphocytosis in the following months.

Therefore, diagnosis of CML and coexistent CLL was established. Although a transient remission of blast crisis was achieved, blast cells reappeared two months later and the patient died shortly afterwards. Molecular studies of the immunoglobulin heavy chain gene (J_H) rearrangement pattern point to the origin of the diseases in two different cell clones. In addition, previously published cases of simultaneous CLL and CML are reviewed.

Key words: chronic myeloid leukemia, chronic lymphocytic leukemia, lymphoid blast crisis

The coexistence of chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL) in the same patient has only been reported occasionally,[1-12] with most cases corresponding to patients who developed CML during the evolutive course of CLL.[1,2,4-7,10,12] In the latter cases, the leukemogenic effect of the treatment employed and the increased risk of a second neoplasias associated with CLL have been claimed as possible mechanisms in explaining the sequence of events described above. In other cases, however, both disorders were diagnosed simultaneously, thus presenting a strong case against the role of previous CLL therapy in the appearance of CML.[3,8,9,11] The origin of the two diseases in the same or in two different cell clones can only be determined by means of molecular studies, but very few of these studies have been performed to date.[9-11]

The aim of the present report is to describe a patient who had CML and CLL simultaneously, and in whom CML eventually evolved into a B-cell lineage blast crisis. In addition, we report the results of the molecular studies which were performed in order to determine the origin of the two proliferating cell lines.

Materials and Methods

Immunophenotypic analysis

Double direct immunofluorescence phenotype was performed using a FACscan flow cytofluorometer (Becton Dickinson Immunocytometry Systems, San José , CA, USA) after incubation of the mononuclear cell fraction of peripheral blood (PB) and bone marrow (BM) samples with a pannel of monoclonal antibodies labelled with fluorochromes against B, T-cell, and myeloid antigens (including Tdt, CD19, CD10, CD22, CD23, CD3, CD5, CD7, CD11b, CD13, CD33, CD14, CD15, HLA-DR, CD34 and surface M immunoglobulin heavy chain and κ and λ light chains, purchased from Becton-Dickinson, San José, CA, USA), followed by red cell lysis and analysis.

The expression of cytoplasmatic antigens was studied using the alkaline phosphatase anti-alkaline phosphatase technique. Additionally, immunohistochemical analysis using monoclonal antibodies CD20 and CD45RO was performed in the initial bone marrow biopsy in order to ascertain B-cell lineage of lymphoid nodules.

Correspondence: Jordi Esteve, MD, Department of Hematology, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain. Tel./Fax international +34.3.2275428.
Acknowledgments: this work was supported in part by grants 1995-SGR 00150 and 00152 from the Generalitat de Catalunya and FISss 96/0236 from the Ministerio de Sanidad y Consumo, Spain. Jordi Esteve holds a Research Fellowship from the Hospital Clínic of Barcelona.
Received May 20, 1997; accepted July 22, 1997.

Molecular analysis

The clonality study of the lymphoid population was carried out by assessing the rearrangement of the heavy chain immunoglobulin gene (JH) pattern by Southern blot analysis and the polymerase chain reaction (PCR) technique. Southern blot analysis was performed after digestion of genomic DNA from peripheral blood and/or bone marrow samples with the *EcoRI* and *HindIII* restriction enzymes and hybridization with a P^{32}-labelled JH probe with the random primer method. PCR analysis of the third complementary region (*CDRIII*) of the JH region was performed after 30 cycles of amplification using a primer based on a consensus sequence from the third framework portion (FR3) of the variable region (VH), and an antisense primer homologous to a conserved 3' sequence of the six joining (J) segments.[13] The finding of a discrete band of an expected length of 80 to 120 base pairs after electrophoresis of the PCR product on a 4% agarose and/or 12% polyacrylamide gel was indicative of the existence of a monoclonal population carrying the same rearrangement pattern in this region.

Case Report

A 71-year-old woman was referred to our center after the discovery of leukocytosis with immature myeloid cells in peripheral blood in a routine analysis. There was no previous history of exposure to radiation or myelotoxic drugs.

The physical examination was normal. The Hb was 129 g/L, WBC count was 12×10^9/L (45% neutrophils, 1% band cells, 4% eosinophils, 5% basophils, 22% lymphocytes, 8% monocytes, 1% metamyelocyte and 14% myelocytes), and the platelet count 172×10^9/L. The serum LDH level was 614 U/L (N:250-450), vitamin B12 was 1600 (N:200-1000 pg/mL), and the LAP score was 4 (N:30-60). A bone marrow aspirate was hypercellular with no fat cells and an increased proportion of the myeloid series in all maturative stages; the percentage of lymphocytes was 10%. A bone marrow-biopsy revealed a marked increase in the overall cellularity with hyperplasia of the myeloid series. Two small lymphoid nodules were observed in a central medullary location along with a few sheets of mature-looking lymphoid cells scattered throughout the bone marrow.

The cytogenetic study of the bone marrow showed the Ph chromosome in all metaphases analyzed, whereas Southern blot analysis of a leukocyte-enriched fraction of peripheral blood, after hybridization with a *bcr* probe, demonstrated *bcr/abl* rearrangement.

The patient was diagnosed as having CML and subsequently treated with hydroxyurea. In the following months the WBC counts were maintained between 10 and 20×10^9/L, with the percentage of lymphocytes ranging from 25% to 50%.

The patient remained asymptomatic until May 1996, 18 months from diagnosis, when her general condition suddenly deteriorated and 38% blast cells were observed in peripheral blood. The WBC was 78×10^9/L and the percentage of lymphocytes was 17%. A bone marrow aspirate disclosed 70% blast cells, whose immunophenotype corresponded to B-lymphoid lineage ($CD34^+$, TdT^+, $CD10^+$, $CD19^+$, and $CD22^+$), and 30% lymphocytes with a mature appearance.

The immunophenotypic study of the latter population demonstrated expression of mature B cell markers (CD19, CD22) and coexpression of CD5 and CD23, weak IgM expression on the cell surface, a restricted pattern of the κ light chain, and absence of immature markers such as TdT and CD10.

Southern blot for JH analysis of DNA from peripheral blood and bone marrow samples obtained at blast crisis showed the existence of a band which was differentiated from the germline band, indicating the existence of a monoclonal B-cell population. PCR amplification of CDRIII in the above samples yielded a double discrete band typical of a monoclonal rearrangement pattern of the JH region. Therefore, the diagnosis of B-cell lymphoid blast crisis of CML in association with B-CLL was made.

Retrospective analysis of initial bone marrow-biopsy showed the B-cell nature of lymphoid nodules seen in the biopsy, as demonstrated by the CD20 expression of most lymphocytes in the nodule and the negativity of a pan-T cell marker, CD45RO. Additionally, DNA was extracted from the biopsy and a PCR of the CDRIII region was performed showing a unique band of around 100 bp, similar to the smaller band seen in the PCR product of blast crisis DNA. Doxorubicin (30 mg/m^2 i.v./day × 2), vincristine (2 mg weekly × 4) and prednisone (60 mg/m^2 during the first week and then gradually reducing the dose) were administered, which lead to the normalization of the peripheral blood hematological values showing no blast cells in either blood or bone marrow.

At that time, no blast cells in bone marrow were present, but the lymphoid population, with typical CLL morphology and phenotype, persisted. After having achieved a complete response, consolidation therapy with the same regimen as used to induce remission was given. However, a few weeks later, an increased proportion of blast cells in the bone marrow was observed, and a palliative treatment with 6-mercaptopurine was instituted. The patient died shortly afterwards, at five months after blast crisis diagnosis.

A review of previously reported cases in literature concerning coexistent myeloproliferative and lymphoproliferative disorders was performed through a MedLine® search (Table 1).

Table 1. Patients previously reported with coexistence of CLL and CML.

Author (year)	*Age/sex*	*Interval CLL-CML*	*Treatment for CLL*
Carcassonne et al. (1968)	59/F	36 mos.	CLB + PDN
Whang-Peng et al. (1974)	62/M	36 mos.	TBI
Whang-Peng et al. (1974)	74/M	24 mos.	None
Vilpo et al. (1980)	58/M	Simultaneous	
Khojasteh et al. (1981)	55/M	61 mos.	CLB
Schreiber et al. (1984)	55/M	87 mos.	CLB
Teichmann et al. (1986)	47/F	72 mos.	CLB + PDN, Vincristine + Bleo COP
Hashimi et al. (1986)	82/F	62 mos.	None
Leoni et al. (1987)	55/M	Simultaneous	
Browett et al. (1988)	69/M	Simultaneous	
Zollino et al. (1991)	66/M	72 mos.	CLB
Maher et al. (1993)	69/M	Simultaneous	
Nanjangud et al. (1996)	43/M	73 mos.	TBI

CLB: chlorambucil; PDN: prednisone; Bleo: bleomycin; TBI: total body irradiation. COP: cyclophosphamide + vincristine + prednisone

Discussion

The coexistence of myeloproliferative and lymphoproliferative disorders in the same patient is uncommon. However, different lymphoproliferative disorders, including Waldenström's macroglobulinemia,[14] Hodgkin's disease,[15] non-Hodgkin's lymphoma,[16] multiple myeloma[17] and especially CLL have been observed in association with myeloproliferative disorders like CML,[1-12] but can also be associated with idiopathic myelofibrosis[18] and polycythemia vera.[19,20] In most cases, the diagnosis of the myeloproliferative disorder followed that of the lymphoid disorder,[1,2,4-7,10,12] whereas in other cases, both diagnoses were made simultaneously.[3,8,9,11] In the cases previously reported, the interval between the diagnoses of CLL and CML ranged from 24 to 87 months (see Table 1).

In the attempt of explaining the association between CLL and CML, several mechanisms have been suggested. Thus, the leukemogenic effect of the treatment employed for CLL might be related to the appearance of CML in those cases supervening after treatment of the lymphoproliferative disorder. In this sense, the mutagenic effect of chlorambucil (the cytolytic agent most commonly employed in such patients) and radiotherapy is well-known.[12,21-24] However, the latter mechanism would not apply in those patients in whom the diagnoses of the myeloid and lymphoid disorders are simultaneously established. The impaired immune surveillance associated with CLL might also contribute to the increased risk of second neoplasias registered in these patients.[25] In addition, a coincidental occurrence of CML and CLL in the same patient can not be ruled out. Finally, an alternative explanation for the association above could be that both diseases originate from a unique stem cell capable of differentiating into two different cell lines; however, molecular studies aimed at ascertaining such a possibility are scarce.[9-11] Zollino *et al*.[9] analyzed the expression of molecular markers specific of both proliferations (*bcr/abl* rearrangement for CML and the monoclonal pattern of JH rearrangement for CLL) in differently cell-enriched blood fractions from a patient with CML following CLL, detecting *bcr/abl* rearrangement in the granulocytic but not in the mononuclear cell fraction, probably composed mainly of CLL cells; on the contrary, they observed a characteristic band corresponding to a monoclonal lymphoid population after JH rearrangement analysis in the lymphoid-enriched but not in the myeloid blood fraction. Therefore, these authors could determine the different origin of CLL and CML in their patient. Likewise, Maher *et al*. and Browett *et al*. reported similar findings in two additional cases of simultaneous CML and CLL.[9,11]

The patient reported herein had CML and CLL in an early stage at the time of presentation; she further developed B-lineage lymphoid blast crisis, which prompted us to study the JH rearrangement pattern as a specific molecular marker of the clone. PCR of the *CDRIII* region in a DNA sample extracted from the initial bone marrow biopsy showed a unique discrete band, probably corresponding to the CLL population. When this analysis was repeated in mononuclear cell fractions obtained at blast cell crisis, a double pattern band was clearly observed. There were also the appearance of a larger-sized band, which was not seen after amplification of chronic phase DNA, and suggested the existence of a different monoclonal population, probably corresponding to the blast crisis clone. Thus, the analysis of JH rearrangement pattern of sequential samples supports the origin of both disorders in two different cell clones.

References

1. Carcassonne Y, Gascard E. Leucèmie myèloïde succédant à una leucèmie lymphoïde. Marseille Médical 1968; 105:339-41.
2. Whang-Peng J, Gralnick HR, Johnson RE, Lee EC, Lear A. Chronic granulocytic leukemia (CGL) during the course of chronic lymphocytic leukemia (CLL): correlation of blood, marrow and spleen morphology and cytogenetics. Blood 1974; 43:333-9.
3. Vilpo JA, Klemi P, Lasilla O, de la Chapelle A. Concomitant presentation of two chronic leukemias: evidence for independent clonal evolution. Am J Hematol 1980; 8:205-11.
4. Khojasteh A, Perry MC, Taylor HM. Chronic myelocytic leukemia developing as a second cancer in a patient with chronic lymphocytic leukemia. CA-A Cancer J Clinicians 1981; 31:172-6.
5. Schreiber ZA, Axelrod MR, Abebe LS. Coexistence of chronic myelogenous leukemia and chronic lymphocytic leukemia. Cancer 1984; 54:697-701.
6. Hashimi L, Al-Katib A, Mertelsmann R, Mohamed AN, Koziner B. Cytofluorometric detection of chronic myelocytic leukemia supervening in a patient with chronic lymphocytic leukemia. Am J Med

1986; 80:269-75.
7. Teichmann JV, Sieber G, Ludwig WD, Karow J, Ruehl H. Chronic myelocytic leukemia as a second neoplasia in the course of chronic lymphocytic leukemia. Leuk Res 1986; 10:361-8.
8. Leoni F, Ferrini PR, Castoldi GL, Pata M, del Prete GF, Tomasi P. Simultaneous occurrence of chronic granulocytic leukemia and chronic lymphoid leukemia. Haematologica 1987; 72:253-6.
9. Browett PJ, Pattinson J, Pinkney J, Hoffbrand AV, Norton JD. Gene probing analysis demonstrates independent clonal evolution of coexistent chronic myeloid and chronic lymphocytic leukemia. Eur J Haematol 1988; 40:181-4.
10. Zollino M, Genuardi M, Tanci P, et al. Chronic myelogenous leukemia in the course of chronic lymphocytic leukemia: evidence for an independent clonal origin. Leuk Res 1991; 15:269-73.
11. Maher VE, Gill L, Townes PL, et al. Simultaneous chronic lymphocytic leukemia and chronic myelogenous leukemia. Cancer 1993; 71:1993-7.
12. Nanjangud GJ, Saikia TK, Chopra H, Kadam PR, Advani SH. Development of Ph positive chronic myeloid leukemia in a patient with chronic lymphocytic leukemia treated with total body irradiation: a rare association. Leuk Lymphoma 1996; 22:355-9.
13. Trainor KJ, Brisco MJ, Story CJ, Morley AA. Monoclonality in B lymphoproliferative disorders detected at the DNA level. Blood 1990; 75:2220-2.
14. Majado MJ, González C, Marín-Blàzquez MD, Morales A, Moreno M. Aparicièn de una leucemia mieloide crònica en el curso de una macroglobulinemia de Waldenström. Sangre (Barc) 1992; 37:465-6.
15. Punt CJA, Rozenberg-Arska M, Verdonck LF. Successful treatment with chemotherapy and subsequent allogeneic bone marrow transplantation for myeloid blastic crisis of chronic myelogenous leukemia following advanced Hodgkin's disease. Cancer 1987; 60:934-5.
16. Cazzola M, Bergamaschi G, Melazzini M, Ponchio L, Rosti V, Molinari E. Chronic myelogenous leukemia following radiotherapy and chemotherapy for non Hodgkin's lymphoma. Haematologica 1990; 75:477-9.
17. Dumouchel P, Casassus Ph, Giacomini T, Lortholary P. Coexistence d'un myèlome et d'une leucémie myéloïde chronique. Sem Hôp (Paris) 1983; 59:2191-3.
18. Varadi S, Vijaykumar P. Myeloproliferative disorder developing in chronic lymphocytic leukemia. Clin Lab Haematol 1982; 4:67-73.
19. Bosch F, Cervantes F, Lòpez-Guillermo A, Carreras E, Montserrat E, Rozman C. Polycythemia vera following non-Hodgkin's lymphoma. Leuk Lymphoma 1992; 8:501-2.
20. Manoharan A, Catovsky D, Clein P, et al. Simultaneous or spontaneous occurrence of lympho- and myeloproliferative disorders: a report of four cases. Br J Haematol 1981; 48:111-6.
21. Kaldor JM, Day NE, Pattersson F, et al. Leukemia following chemotherapy for ovarian cancer. N Engl J Med 1990; 322:1-6.
22. Kaldor JM, Day NE, Clarke A, et al. Leukemia following Hodgkin's disease. N Engl J Med 1990; 322:7-13.
23. Moloney WC. Radiogenic leukemia revisited. Blood 1987; 70:905-8.
24. Santucci MA, Saglio G, Tura S. Pathogenesis and progression of chronic myeloid leukemia. Haematologica 1996; 81:63-76.
25. Hyman GA. Increased incidence of neoplasia in association with chronic lymphocytic leukemia. Scand J Haematol 1969; 6:99-104.