According to the classification of neutropenia, 26 patients suffered from severe, 16 moderate, and 14 from mild neutropenia. Single episodes of transient neutropenia have been seen in 19 patients. Twenty-six had experienced chronic neutropenia and 11 cases had recurrent neutropenia (Table 2). Also, 32 out of these patients showed leukopenia (57.1%), 24 had anemia (42.9%), 11 thrombocytopenia, and only 3 patients had monocytosis. The most common infections during the course of the illness were respiratory infections, which were seen in 48 patients (85.7%). Other manifestations were: pneumonia (30 cases), otitis media (28 cases), acute diarrhea (28 cases), abscess (24 cases), oral candidiasis (23 cases), oral ulcers (17 cases), cutaneous infections (16 cases), and sinusitis (12 cases). Other less frequent infections were: periodontitis or conjunctivitis (5 cases), cystitis (2 cases), meningitis (2 cases), and osteomyelitis (1 case). Abscesses were detected in different sites, including: perianal (8 cases), cutaneous (7 cases), submandibular (4 cases), mastoidal (3 cases), dental (3 cases), liver (2 cases), peritonsillar (2 cases), lung (1 case), and soft tissue (1 case). The non-specific signs of hepatomegaly and splenomegaly had already been found in 41.1% and 32.1% of the patients, respectively. All of the patients with single episodes of neutropenia had had infectious complications during the neutropenic episode, including: pneumonia (5 cases), otitis media (4 cases), diarrhea (2 cases), oral ulcers (2 cases), cutaneous infections (2 cases), abscess (2 cases), sinusitis (1 case), and oral candidiasis (1 case).

Neutropenia may occur in any PiD as a consequence of either an intercurrent infection or an autoimmune disease.[3,4] All of our patients with Shwachman-Diamond syndrome, cyclic neutropenia and Kostmann disease had associated neutropenia. In addition, a number of our patients with predominant antibody deficiency disorders had associated neutropenia. It seems that autoimmune neutropenia is a common cause of neutropenia in some primary specific immunodeficiencies.[3,5,6] An increased susceptibility to infections was detected in our patients. For patients presenting with unexpected neutropenia, the clinical history and examination of the peripheral blood smear were the most important parts of the diagnostic evaluation. Examination of the oral cavity, perianal region, and skin is necessary in order to assess the clinical impact of chronic neutropenia. The presence of gingivitis, ulcer, and abscess implies clinically significant neutropenia.[7,8] Persistent or severe infections should always raise a suspicion, which deserves further evaluation, of an underlying immune deficiency syndrome and neutropenia, because a delay in diagnosis may result in a serious organ damage or even death of the patient.[6,9]

Nima Rezaei, Abolhassan Farhoudi, Zahra Pourpak, Asghar Aghamohammadi, Mostafa Moin, Masoud Movahedi, Mohammad Gharagozlou

Department of Allergy and Clinical Immunology of Children's Medical Center, Immunology, Asthma and Allergy Research Institute, Tehran University of Medical Sciences, Tehran, Iran

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Key words: neutropenia, immunologic deficiency syndromes, infection, Iran.

Correspondence: Zahra Pourpak, MD, PhD, Immunology, Asthma and Allergy Research Institute, Children's Medical Center, No. 62, Dr. Gharib St., Keshavarz Blvd., Tehran 14194, Iran, P.O.Box: 14185-863. Phone: international +98.21.6935855. Fax: international +98.21.6428995. E-mail: rezaei_nima@hbi.ir/ zpourpak@hbi.ir

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Chronic Myeloid Leukemia

Clonal cytogenetic abnormalities in patients with chronic myeloid leukemia in complete cytogenetic response to imatinib mesylate

The emergence of clonal chromosomal abnormalities in Philadelphia-negative cells during treatment with imatinib in patients with Philadelphia-positive chronic myeloid leukemia has been reported. We add information to this issue presenting a series of 29 patients in complete cytogenetic response after imatinib treatment, three of whom developed clonal aberrations.

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Chronic myeloid leukemia (CML) is a chronic myeloproliferative disorder (CMPD) characterized by the t(9;22)(q34.1;q11.2) that juxtaposes the *ABL* and *BCR* genes with generation of the Philadelphia chromosome (Ph').[1] The molecular consequence is the *BCR-ABL* oncogene, that encodes a BCR-ABL oncoprotein ($p210^{BCR/ABL}$) with increased tyrosine kinase activity which is necessary and sufficient for leukemogenesis.[2] Imatinib mesylate (STI571, Glivec®, Novartis Pharma, Switzerland), a tyrosine kinase inhibitor selective for ABL, BCR-ABL, c-KIT, PDGFRα and ARG proteins has demonstrated good results in CML. As first line therapy, imatinib is superior to interferon (IFN)-α, inducing complete hematologic responses in 95% of patients, major cytogenetic responses in 85% and complete cytogenetic responses (CCR) in 73%.[3] Imatinib has been proven to be better tolerated, although long-term side effects and influence on long-term survival are not yet known. Recently, some cases of clonal cytogenetic abnormalities in Ph' negative cells of patients with CML treated with imatinib have been

Table 1. Clinical data of three patients with a diagnosis of CML treated with imatinib showing clonal cytogenetic abnormalities in Ph' negative cells.

Case	*1*	*2*	*3*
Age (years)	60	61	47
Gender	F	M	M
Duration of disease (months)	49	41	28
Phase of disease at diagnosis	CP	CP	CP
Duration of treatment with IFN (months)	10	1	NT
Phase of disease at starting STI	AP	CP	CP
Time from STI to CCR (months)	24	3	16
Duration of CCR (months)	6	+23	+22
Time from STI to detection of abnormalities (months)	24	11	12
Imatinib dose (mg)	400	400	400

F: female; M: male; CP: chronic phase; AP: accelerated phase; NT: no treatment.

Table 2. Cytogenetic and morphologic features of three cases diagnosed with CML treated with imatinib showing clonal cytogenetic abnormalities in Ph' negative cells.

Case	*Conventional cytogenetics*	*FISH (% abnormal cells)**	*Morphology*
1	46,XX[20]	-7(18%)	No dysplastic features
2	45,XY,-7[10] /46,XY [10]	-7(44%)	Multilineage dysplasia not present at diagnosis
3	48,XY,+Y,+8[3] /46,XY[17]	-7(16%) +8(36%), XYY(24%)	Megakaryocytic dysplasia not present at diagnosis

**Two hundred nuclei were analyzed for each different probe and patient.*

reported.[4–7,10,11] The aberrations more frequently described are trisomy 8 (+8) in up to 53% of cases, monosomy 7 (–7) in 23%, and deletion of 20q (20q-) in 8%,[7] which are findings classically seen in myelodysplastic syndromes, although +8 has also been detected in CMPD. Clonal chromosomal anomalies in Ph' negative cells have also been seen in CML patients undergoing treatment with IFN-α.[8] We describe the presence of clonal abnormalities detected by conventional cytogenetics and fluorescence *in situ* hibridization (FISH) in patients diagnosed with CML who achieved CCR with imatinib.

Thirty-seven patients with a diagnosis of CML Ph$^+$ in CCR were studied (19M/18F). Twenty-nine patients received imatinib; they had a mean age of 52 years at the time of starting imatinib, and a mean time of treatment of 25 months. These patients were stratified into four groups: one group of 9 patients received imatinib as first-line therapy, a second group of 12 patients had been previously treated with IFN-α, a third group of 6 patients received IFN-α plus autologous stem cell transplantation (autoSCT) and a fourth group of 2 patients received imatinib after an allogeneic SCT (alloSCT). The remaining 8 patients were treated only with IFN-α, and three underwent a transplantation. Conventional cytogenetic studies were performed on bone marrow cells from 24-hour cultures. Karyotypes were described according to the ISCN.[8] All patients included in the study showed a CCR defined as no Ph$^+$ cells in the bone marrow. FISH was performed in all patients on bone marrow cells proceeding from conventional cytogenetic cultures. The following probes were used: CEP8; LSI5q31(EGR1)/D5S23,D5S21; LSI7q31 (D7S486)/CEP7; LSI20q12(D20S108) and CEPX/CEPY (Vysis, Downers Grove, USA). Three of the 29 patients treated with imatinib (10.3%) exhibited clonal cytogenetic abnormalities in Ph' negative cells (Tables 1 and 2). Patient #1 showed a normal karyotype but FISH revealed 18% of nuclei with -7; patient #2 presented -7 by conventional cytogenetics confirmed also by FISH and patient #3 revealed a gain of chromosome Y and 8 in the same clone confirmed by FISH, which also detected a second abnormal clone showing a -7. These findings were not present either at the time of diagnosis or before the start of imatinib. Of the remaining 8 patients not treated with imatinib, none had clonal aberrations. At present, patients #2 and 3 remain in CCR with imatinib and show the cytogenetic anomaly 16 and 18 months later, respectively. Patient 1 relapsed cytogenetically after 6 months of CCR although she remains in chronic phase and maintains hematologic response.

The emergence of additional chromosomal abnormalities in Ph' negative cells of CML patients treated with imatinib has been described in 2 to 15.2% of cases.[4–6,10,11] Nevertheless, those were heterogeneous series and the incidences calculated in non-selected cohorts. The incidence in our series is somewhat high because we only analyzed patients in CCR. Regarding the applied techniques, conventional cytogenetics and FISH results showed some discrepancies. FISH allowed us to detect monosomy 7 in two cases, not previously detected by conventional cytogenetics. There are two possible explanations for this phenomenon: cells carrying -7 have less spontaneous proliferation ability in cell cultures, so metaphases with this abnormality were not seen but interphase FISH could detect non-dividing cells. In addition, FISH is more sensitive than conventional cytogenetics at detecting residual abnormal clones.

Imatinib treatment itself probably induces such abnormalities or favors their adquisition; however, a limited number of cases have been published and the follow-up periods are still very short. In order to understand the real significance of these findings, larger series of cases with long-term follow-up need to be analyzed.

Blanca Espinet,# Ana Carla Oliveira,° Concepción Boqué,° Alicia Domingo,° Esther Alonso,° Francesc Solé*#*

**Laboratori de Citogenetica i Biologia Molecular, Servei de Patologia, Hospital del Mar, IMAS, URNHE-PRBB, Barcelona; °Servei d'Hematologia, ICO, Hospital Universitari de Bellvitge, L'Hospitalet de Llobregat, Barcelona; #Escola de Citologia Hematològica Soledad Woessner-IMAS, Barcelona, Spain*

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Key words: chronic myeloid leukemia, clonal cytogenetic abnormalities, imatinib.

Correspondence: Blanca Espinet, PhD, Laboratori de Citogenètica i Biologia Molecular, Servei de Patologia, Hospital del Mar, Passeig Marítim 25-29, E-08003 Barcelona, Spain. Phone: international +34.93.2483035. Fax: international +34-93-2483131. E-mail: bespinet@imas.imim.es

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Acute Myeloid Leukemia

The incidence of submicroscopic deletions in reciprocal translocations is similar in acute myeloid leukemia, *BCR-ABL* positive acute lymphoblastic leukemia, and chronic myeloid leukemia

We compared the incidence of submicroscopic deletions accompanying balanced translocations using interphase fluorescence *in situ* hybridization (FISH) in 245 patients with chronic myeloid leukemia (CML), 79 patients with acute lymphoblastic leukemia (ALL) and *BCR-ABL* (n=70) or *MLL* rearrangements (n=29), and 412 patients with acute myeloid leukemia (AML) with *CBFB-MYH11* (n=122), *PML-RARα* (n=108), *AML1-ETO* (n=112), or *MLL* rearrangements (n=98). The incidence of submicroscopic deletions was 2-9% depending on the entity.

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Submicroscopic deletions adjacent to the breakpoints of balanced translocations were identified in 9%-16% of all cases of chronic myeloid leukemia (CML) by interphase fluorescence *in situ* hybridization (FISH).[1,2] The rate of hematologic and cytogenetic responses to imatinib was statistically significantly lower in patients with deletions.[1]

So far only a few studies, with limited numbers of cases, have examined the incidence and prognostic impact of submicroscopic deletions in balanced translocations in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) (Table 1). We, therefore, determined the incidence of submicroscopic deletions in the most common balanced rearrangements in leukemia using interphase FISH. This study was based on 245 cases with CML, 79 patients with ALL, and 412 patients with AML, who were referred to our laboratory from January 2000 to April 2004. The cohort of patients with AML comprised 112 patients with *AML1-ETO*, 108 patients with *PML-RARα*, 122 patients with *CBFB-MYH11*, and 96 patients with different *MLL* rearrangements. The cohort with ALL comprised 70 patients with *BCR-ABL* positive ALL and 29 patients with different *MLL* rearrangements. Cytogenetic analysis was performed in all cases. FISH was performed on interphase nuclei and/or metaphases on bone marrow smears or blood smears. We used a BCR-ABL two color/two fusion probe (Cancer Genetics, CGPT 07), a LSI AML1-ETO dual color, dual fusion translocation probe, a LSI *PML-RARα* dual color, dual fusion translocation probe, a LSI CBFB dual color, break apart rearrangement probe (Core Binding Factor β-subunit), and a LSI MLL dual color, break apart rearrangement probe (Abbott, 5J 63-01). At least 100 interphase nuclei were viewed for each case. The analyzing system, ISIS^{R} (MetaSystems, Altussheim, Germany), was used for documentation.

In all cases the leukemia-specific fusion transcripts were also amplified by reverse transcription polymerase chain reaction (RT-PCR). For analysis of *MLL* fusions with partner genes in AML and ALL the respective RT-PCR was performed. In CML we found submicroscopic deletions in 9% of cases (22/245) with interphase FISH. In *BCR-ABL* positive ALL the incidence was 6% (4/70) and in ALL with *MLL* rearrangements it was 3% (1/29). In the different subgroups of AML the incidence of deletions was between 2% and 8% (*AML1-ETO*: 4% (4/112); *CBFB-MYH11*: 2% (3/122); *PML-RARα*: 6% (7/108); *MLL* rearrangements: 8% (8/96) (χ^2, n.s.) (Table 1).

Submicroscopic deletions occur in 9-16% of patients with CML and are clearly associated with an inferior prognosis.[1,2] We determined the incidence of submicroscopic deletions in the most frequent reciprocal translocations in acute leukemias, as well as in CML. Our results in CML (deletions in 9%) and in *BCR-ABL* positive ALL (deletions in 6%) were in the ranges reported in the literature.[1-3] The incidence that was found in ALL with different *MLL* rearrangements was 3%, which is lower than published so far.[4] We observed deletions in 3% of AML with *PML-RARα* and in 4% of AML with *AML1-ETO*. Kolomietz *et al.* did not find deletions in subtypes of AML; to our knowledge their study is the only other examination of submicroscopic deletions in these subtypes.[2] The incidence of submicroscopic deletions in AML with *CBFB-MYH11* was 2% in our study.

These data provide a strong indication that the frequency of these deletions is much lower than previously published (10-33%).[2,5,6] We found submicroscopic del-