

**Figure 1. Nuclear staining for p53 (red) in the bone marrow of a patient with MDS before (left, x200) and after progression (right, x400).**

tional stabilization of the protein which may also abolish its function; c) overexpression of wild-type protein in response to spontaneous genetic errors occurring at a higher frequency in neoplastic tissue; d) the effect of antigen retrieval techniques which can alter detection thresholds; e) mutations occur outside the exons studied.

Several reports have described that p53 alterations are not observed in more benign MDS cases.<sup>2-6</sup> However, this study, in keeping with a study done by Kitagawa *et al.*<sup>9</sup> revealed two RA cases showing p53 overexpression suggesting that p53 abnormality may not be a terminal genetic event during leukemia development. To the best of our knowledge only one other study has described p53 alteration in the RA phase.<sup>10</sup> The presence of DNA from normal cells in less advanced subtypes is likely to affect the sensitivity of the mutation detection and may underestimate the rate of p53 mutation in RA phase.

Taking into account the shorter interval between RA phase and progression, p53 overexpression may have contributed to the pathogenetic process in the progression of MDS in our cases. However, additional and more extended studies are necessary to determine the genetic basis for this immunoreactivity and to clarify the prognostic value of such findings.

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### Key words

*p53 overexpression, refractory anemia*

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### A complex immunodeficiency. Idiopathic CD4<sup>+</sup> T-lymphocytopenia and hypogammaglobulinemia associated with HHV8 infection, Kaposi's sarcoma and gastric cancer

Sir,

We report a case of idiopathic CD4<sup>+</sup> T-lymphocytopenia (ICL) associated with hypogammaglobulinemia in a 70-year-old woman. She developed Kaposi's sarcoma (KS) and her mononuclear cells were found to be positive for *Herpes virus type 8* (HHV8). In 1997 she developed gastric cancer and died from septic shock.

At the beginning of 1990 unusual cases of CD4<sup>+</sup> T-lymphocytopenia in the absence of human immunodeficiency virus (HIV) infection were reported.<sup>1,2</sup> In 1993 the CDC defined the criteria for a new syndrome: ICL. The criteria are low CD4<sup>+</sup> T-lymphocytes (less than 300/ $\mu$ L or below 20% of the total lymphocyte count), no serologic evidence of HIV infection and no defined immunodeficiency diseases or therapy associated with T-cell depletion.<sup>3</sup>

In 1994 a 70-year-old woman with hypertension and *herpes zoster virus* infection was admitted to our section because of fever, skin abscesses due to *Serratia marcescens* and angiomatous abscesses on the left leg. There was no epidemiology suggestive of HIV

**Table 1. Immunohematologic data and lymphocyte subsets of our patient during the follow-up over three years.**

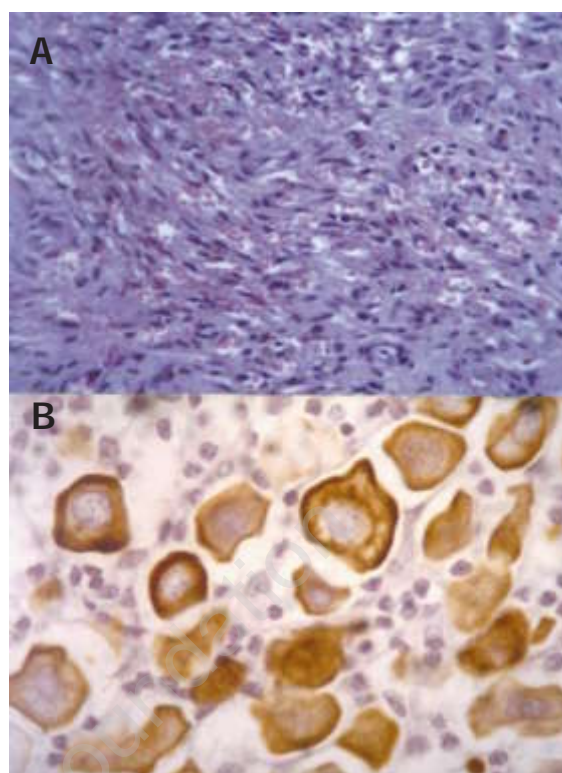
	Dec. 5, 1994	Oct. 13, 1995	Sept. 16, 1996	Feb. 17, 1997	normal range
WBC count ( $\mu\text{L}$ )	4470	4300	6300	5600	4000-10,000
Lymphocytes	5%	13.9%	9.9%	5.2%	20-50%
CD2 ( $\mu\text{L}$ )	187	540	571	267	1600-2400
CD19 ( $\mu\text{L}$ )	37	53	50	22	200-400
CD3 ( $\mu\text{L}$ )	174	493	447	221	1100-1700
CD4 ( $\mu\text{L}$ )	97	265	202	132	700-1100
CD8 ( $\mu\text{L}$ )	73	236	234	93	500-900
CD16 ( $\mu\text{L}$ )	12	61	34	48	200-400
Monocytes	9%	4.9%	6.2%	4.4%	1-7%
Neutrophils	83%	78.6%	82%	87.4%	50-80%
Eosinophils	1%	1.9%	1.7%	3%	0-3%
Basophils	2%	0.2%	0.3%	0%	0-1%
IgG (mg/dL)	239	573	457	379	840-1660
IgA (mg/dL)	44.5	67.2	72.4	101	90-395
IgM (mg/dL)	28.2	36.5	35	29	48-220

infection. Serologic tests for HIV1, HIV2 and p24 were negative. She was negative for *Cytomegalovirus*, for hepatitis B and C, for *Toxoplasma* and also for pharyngeal and rectal viral studies. The immunologic studies demonstrated a marked deficit of immunoglobulin and a low count of CD4<sup>+</sup> T-lymphocytes (Table 1). Skin biopsy of the left leg showed a picture of KS (Figure 1A).

The diagnosis was KS in a patient with ICL associated with hypogammaglobulinemia. The patient was treated with radiotherapy and the KS lesions resolved. The follow-up, every four months, persistently showed hypogammaglobulinemia and CD4<sup>+</sup> lymphocytopenia (Table 1).

In 1996, HHV8 on mononuclear cells and a high level of plasma antibody titer (>1/10,000), were detected. In March 1997 she had acute anemia and the clinical examination showed cervical lymphadenopathy. Histologic examinations of the lymph nodes showed a reactive picture with adenocarcinoma metastases from a gastric adenocarcinoma positive for cytokeratin antibody (Figure 1B). The patient died one month later from septic shock.

In HIV infection, the level of lymphocytes is maintained high by the early increase of CD8<sup>+</sup> in response to the infection; in patients with ICL the number of lymphocytes decreases. Our patient had a low lymphocyte count and low levels of IgG, IgA and IgM. ICL is rare; the increase in the number of patients reported has been related to accurate analysis of lymphocyte subpopulations in subjects at high-risk of HIV infection and in others patients, and also to the increased diligence with which these cases are being investigated.<sup>4</sup>



**Figure 1. A:** skin biopsy, Kaposi's sarcoma: spindle cells with hyperchromatic nuclei aligned in parallel array with "slit-like" spaces containing erythrocytes (hematoxylin-eosin, 63 $\times$ ). **B:** gastric biopsy; the adenocarcinoma cells show strong immunoreactivity for cytokeratin antibody (clone CAM 5.2, Becton Dickinson, San José, CA, USA); the surrounding lymphocytes are negative (streptavidin-biotin peroxidase method, 100 $\times$ ).

So far, epidemiologic studies have excluded retroviral causes of ICL, but the importance of defining the immunodeficiency state in these patients has been underlined.<sup>6</sup>

In 1994, the discovery by Chang *et al.*<sup>7</sup> of HHV8 in KS tissue from AIDS patients implicated this agent as a candidate etiologic cofactor in KS. Recent epidemiological studies give a picture of the patterns of HHV8 infection, but we do not know precisely how the virus is transmitted.<sup>8</sup> The pathogenic effects of HHV8 are better known. Recently Ensoli<sup>9</sup> reported that inflammatory cytokines could be responsible for virus growth<sup>10</sup> and Davis *et al.* showed that the viral gene encodes for cyclin D which shortens the G1 phase of the cells' cycle and results in cellular proliferation. In this case the immunodeficiency status probably favored the activation of HHV8, which resulted in KS and also promoted the neoplastic evolution.

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**Key words**

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**Molecular analysis of granulocytic sarcoma: a single center experience**

Sir,

Granulocytic sarcomas (GS) are extramedullary tumor masses composed of immature myeloid cells, frequently associated with acute forms of leukemia, with particular phenotypic, cytogenetic and molecular features.<sup>1,2</sup> We report our diagnostic and therapeutic experience on a series of 11 patients affected by GS who presented with different disease localizations and symptoms. Six patients' characteristics have been previously reported.<sup>3</sup>

Clinical and laboratory data are reported in Table

1. Six patients were male and five female. Median age was 45 years (range 17-76). The median interval between the GS localization and symptoms and bone marrow involvement was 120 days (range 30-270). GS was diagnosed concomitantly with AML in two patients. All patients were treated with a chemotherapy schedules and radiotherapy as described elsewhere.<sup>4,5</sup> Briefly, patients were enrolled in the ICE protocol. After induction chemotherapy, the patients underwent one course of NOVIA consolidation therapy if a complete remission (CR) was achieved, or underwent 2 courses of chemotherapy with FLAG or FLANG protocols if the patients obtained a partial remission (PR) or relapsed. The elderly patient (#8) was only treated with non-ablative, reduced-dosage chemotherapy.

All the patients were studied by cytogenetic analysis, using a standard technique with Wright's stain banding.<sup>6</sup> At least 20 mitoses were analyzed for each sample, whenever possible.

We detected the presence of a cytogenetic defect in the bone marrow of seven (63%) of our eleven cases: two patients carried the t(15;17), two the inv(16) karyotypic abnormality, two the t(9;22) (of which one with trisomy of chromosome 19), and one trisomy of chromosome 13 and chromosome 19. The six patients with a well characterized translocation were also studied by reverse transcription polymerase-chain reaction (RT-PCR) assay (PML-RAR $\alpha$ , CBF $\beta$ -MYH11 and qualitative and quantitative evaluation of BCR-ABL), as reported.<sup>6,8</sup> We were able to confirm the presence of the molecular abnormality in all six patients.

In two CML patients (#10 and 11), who developed a GS after allogeneic BMT, we tested mixed chimerism by cytogenetic analysis and by molecular approaches: we found that all the bone marrow was from donors, while we evidenced, by quantitative PCR, the presence of an amount of *bcr-abl* transcript ranging from 400,000 to 40,000 per mg of RNA analyzed, on bone needle biopsy.<sup>3</sup>

One patient (#3) relapsed with GS in the bladder several months after allogeneic bone marrow transplantation for AML (FAB M1), suggesting a clonal evolution of the disease. Two patients (#2 and 9) had the karyotypic alteration inv(16)(p13q22), with intestinal involvement. In both cases, CR was achieved and cytogenetically documented, providing evidence that this translocation is associated with a good prognosis.<sup>9</sup>

Regarding clinical outcome, the tumor progressively regressed and disappeared in nine patients (81%) during chemotherapy and/or radiotherapy. The median time of CR was 41 months (range 3-137 months). The median time of overall survival was 32 months (range 4-138 months). The other patients died of disease progression (Table 1).

Our small series with cytogenetic abnormalities gives no evidence of any prognostic difference between patients with or without bone marrow involvement.<sup>3,7</sup>