

Key words

Idiopathic CD4+ T-lymphocytopenia, hypogammaglobulinemia, HHV8 infection, Kaposi's sarcoma, gastric cancer

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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.^{1,2} 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.³

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.^{4,5} 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.⁶ 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 α , CBF β -MYH11 and qualitative and quantitative evaluation of BCR-ABL), as reported.^{6,8} 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.³

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.⁹

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.^{3,7}

Table 1. Granulocytic sarcoma: clinical and laboratory data. The table displays the clinical and laboratory features of the 11 patients analyzed in our study.

Pt. Sex/Age	Symptoms	Localization	Interval (days)	Diagnostic evaluation	Bone marrow at GS	Karyotype	Molecular status	Therapy	Response*	Status
1 F/40	Chest pain dyspnea	Mediastinum	270	CT, NMR, tumor and bone biopsy	No	46,XX (30)	N.D.	ICE-NOVIA	TR	CR (29)
2 M/32	Diarrhea, weight loss, bowel subocclusion	Rectum	90	CT, US, endoscopy tumor biopsy, bone biopsy	AML M4 eos	46,XY(3) 46,XY inv(16)(p13q22) (16)	CBFb+ type A	ICE -NOVIA	TR	CR (28)
3 M/17	ARI hydro- nephrosis	Bladder	90	CT, urography, US, bone biopsy, tumor biopsy	CR +36 mos. after BMT for AML M1	46,XX(30) (BMT)	N.D.	DA	TR	CR (137)
4 M/42	Pain thigh mass	Quadriceps muscles	120	Tumor biopsy, bone biopsy arteriography, US	CML BC	46,XY, t(9;22) (q34;q11)(30)	BCR-ABL type b3-a2	ARA-C radiotherapy busulphan	PoD	DOD (2)
5 M/42	Lumbago, leg paralgesia	L4 Needle biopsy	90	CT, NMR, (q22;q21)(25) Total body CT	AML M3 BCR1	46,XY, t(15;17)	PML-RARa	AIDA radiotherapy	TR	CR (53)
6 M/45	Bowel subocclusion	Appendix, ileum, mesenteric lymph nodes	30	CT, US, Tumor biopsy, laparotomy	After 7 mos. AML M2	46,XY(30)	N.D.	radiotherapy DA-DAT-ABMT	TR	CR (113)
7 F/51	Chest pain, dyspnea	Mediastinum	180	CT, NMR, tumor biopsy, bone marrow	After 6 mos. AML M4	48,XX, +13,+19	N.D.	radiotherapy ICE	PoD	DOD (8)s
8 F/76	Dyspnea	Mediastinum	concomitant	CT, NMR	N.D. AML 35	46,XX, t(15;17) (q22;q21)(30)	PML-RARa BCR1	AIDA like (reduced dosage)	TR	CR (7)
9 M/51	Bowel subocclusion	Ileum	concomitant	CT, NMR, bone marrow, total body CT	N.D. AML M4 eos	46,XY,inv(16) (p13q22),+8(25)	CBFβ+ type A	ICE, FLANGx2	TR	CR (7)
10 F/47	Bone pain	Humerus	120	CT, NMR, bone marrow	CR + 6 mos. after BMT for CML BC	46,XX(30)	BCR-ABL type b3-a2	radiotherapy FLANG	PoD	CR (5).
11 F/45	Bone pain	Left leg	210	CT, NMR, CR +5 mos., needle biopsy, total body CT	48,XY,t(9;22) after BMT for CML BC	BCR-ABL (q34;q4) +19+Ph(26)	type b3-a2	radiotherapy + DLI FLANG	TR	CR (123)

*TR = tumor regression; PoD=persistence of disease.

Pt. = patients' number. Sex = M: male, F: female; age in years. Symptoms = symptoms reported at diagnosis. Localization = localization of granulocytic sarcoma mass. Interval = time between symptoms and diagnosis. Bone marrow = presence of acute or chronic myelogenous leukaemia. AML = acute myeloblastic leukemia. CML = chronic myeloid leukemia. BMT = Bone marrow transplantation. M2, M3, M4 = French American British classification of the disease. Karyotype = cytogenetic analysis with description of chromosomes involved and no. of metaphases analyzed; Therapy = employed; Response = response of the disease to the therapy. Status = period expressed in days or months (mo.) indicating the disease free survival; CR = complete remission. DOD = death due to disease; DLI = donor lymphocyte infusion.

Furthermore, we found no difference in the CR rate between our series of GS patients and a group of AML patients without GS treated with the same therapeutic schedule at our institution over the same period (Visani *et al.*, *in preparation*). Concerning the incidence of karyotypic abnormalities and GS we found that the cytogenetic abnormalities displayed by our patients [inv(16)(p13q22);t(15;17)(q22;q12);t(9;22)(q34;q11)] were those already cited in the literature,^{4,6} but in our small series, the reported high frequency of t(8;21)(q22;q22) in association with GS¹⁰ was not confirmed.

Our results further underscore the importance of a prompt diagnosis of GS in the absence of bone mar-

row involvement. In this respect, we can confirm the validity of cytogenetic evaluation and propose the application of molecular procedures. Prompt diagnosis of GS permits an effective initial chemotherapeutic strategy and a consequently higher rate of CR.

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A rapid prenatal diagnosis of hemophilia A by DNA analysis on crude chorionic villus biopsy

Sir,

We have developed a rapid method for prenatal diagnosis of hemophilia A with a nested PCR on DNA obtained from boiled chorionic villus biopsy (CVS).

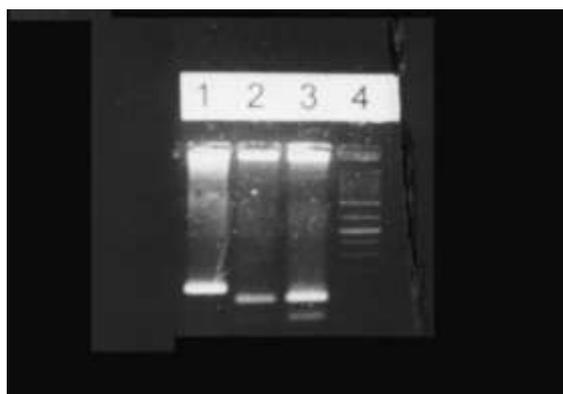


Figure 1. Prenatal diagnosis of hemophilia A by nested PCR analysis. Lane 1: undigested 142 bp fragment. Lane 2: villus, after Bcl-I enzymatic digestion, shows the (99+42) fragments. Lane 3: mother; lane 4: 100 bp ladder DNA marker.

We report on a family with severe hemophilia A due to a large deletion of factor VIII gene spanning from exon 14 to the end of the gene. The hemophiliac did not show a band for all the intragenic polymorphisms located in this deleted region, i.e. for intron (18)/Bcl I RFLP. His sister asked for genetic counselling when she was pregnant; the family study revealed a haplo-type (+/deletion) for intron (18)/Bcl I polymorphism, thus she was diagnosed as a carrier.

CVS biopsy was performed at the 12th week of gestation. Karyotype analysis revealed a male fetus.

In order to obtain a rapid prenatal diagnosis of the fetal genotype, we developed a new method of DNA extraction. A single fragment of the CVS biopsy, without maternal decidua, was boiled in 5 μ L of distilled water, quickly cooled on ice and utilized directly for PCR reaction.

We used two consecutive PCRs to increase the concentration and specificity of the amplified DNA.

Initially, the following external pair of primers was used: Int 18 (A) 5' ATG GCA CTG RAC-AATCTCTA 3' and Int 18 (B) 5' GGTAACATTTCCACTGTCT 3' and 35 step cycles were performed at 94° for 1 min and at 60° for 6 min.

The PCR produced a 1.8 kb fragment containing two Bcl I sites: one constant (1.4 kb) and the other polymorphic (400/300+100 bp).

Fifteen microliters of the amplified fragment were loaded onto 2% agarose gel and visualized under U.V. light with ethidium bromide staining. The villus showed a very faint 1.8 kb amplified band.

This suggested the presence of the whole FVIII gene and therefore of a healthy fetus.

To confirm the diagnosis, 10 μ L of the amplified product from both mother and fetus were used as templates for the second PCR reaction with an internal pair of primers spanning the polymorphic Bcl I site (for details on primers see ref. #1).