a new α-thalassaemia-1 defect due to a partial deletion of the α-globin gene complex. Nucleic Acids Res 1980; 8:4889-98.
3. Winichagoon P, Higgs DR, Goodbourn SEY, Clegg JB, Weatherall DJ, Wasi P. The molecular basis of α-thalassaemia in Thailand. EMBO J 1994; 8:1813-8.
4. Villegas A, Sánchez J, Carreño DL, et al. Molecular characterization of a new family with α-thalassemia-1 (—MA mutation). Am J Hematol 1995; 49:294-8.
5. Villegas A, Porres A, Sánchez J, et al. Red blood cell phenotypes in α-thalassemia in the Spanish population. Haematologica 1998; 83:99-103.
6. González-Redondo JM, Díaz-Chico JC, Malcorra-Azpiazu JJ, Basda-Aguirre, Huisman THJ. Characterization of a newly discovered α-thalassaemia-1 in two Spanish patients with HbH disease. Br J Haematol 1988; 70:459-63.
7. Fortina P, Dianzani I, Serra A, et al. A newly-characterized α-thalassaemia-1 deletion removes the entire α-like globin gene cluster in an Italian family. Br J Haematol 1991; 78:529-34.
8. Clegg JB. Hemoglobin synthesis. In: Weatherall DJ, ed. The thalassaemias. Methods in haematology. Oxford: Churchill Livingstone; 1983. p. 54-73.
9. Fishel-Ghodsian N, Vickers MA, Seip M, Winichagoon P, Higgs DR. Characterization of two deletions that remove the entire human ζ-α globin complex (--THAI and --FIL). Br J Haematol 1988; 70:233-8.

Most frequent mutations of β-thalassemia in Rosario, Argentina

The β-thalassemia syndromes are genetic disorders characterized by absence or decrease in β chain synthesis, producing an alteration in the α and β chain relationship.[1] From the molecular point of view β-thalassemia is very heterogeneous. More than 100 mutations have been described.[2] Each ethnic group has a variety of mutations; in the Mediterranean region there are more than 30 mutations, but only 8 of them are common. Our population is composed of different ethnic groups, with immigration principally from Italy and Spain. This study was an attempt to gather information regarding the presence of the most frequent mutations in our population.

Sir,

Seventy-three non-consanguineous patients, above 1 year of age, diagnosed as having heterozygous β-thalassemia (72 patients), and β^s/β^{Th} (1 patient), were studied at the Hospital Provincial del Centenario, Rosario, Argentina, from 1996 through 1998. Two of the 73 heterozygous β-thalassemia patients were the parents of 4 children: 2 with Cooley's anemia, 1 with heterozygous thalassemia, and 1 hematologically normal.

Patients with Hb A_2 between 4-6% and Hb F lower than 5% were included. Peripheral blood DNA extraction was performed using the salting out method,[3] and these mutations were assessed: –codon 39C→T (β^o39), –IVS1-110 G→A (β^+), –IVS1-1 G→A (β^o), –IVS1-6 T→C (β^+), –IVS2-1 G→A (β^o) and IVS2-745 C→G (β^+) by means of a modified polymerase chain reaction technique: the Amplification Refractory Mutation System.[4]

The ethnic origin of the patients studied was: 89% Italians, 9.6% Spaniards and 1.4% Greek.

Forty patients (54.8 %) were β^o39; 16 (21.9 %) β+1-110; 6 (8.2 %) β^o1-1; 3 (4.1%) β+2-745 and 2 (2.7 %) β^o2-1. There were no β^+1-6 mutations. Genotype could be assessed in 91.7%.

The mutation in the double heterozygote patient was β^o39, which is the most frequent mutation in our population. The parents of the children affected by Cooley's anemia were: β^+1-110 (mother) and β^o2-1 (father), so, these patients were double heterozygous, with only one allele (β^+1-110) having a high frequency in our population. None of the six mutations under study was identified in the patient of Greek origin.

In the patients under study the prevalence of the β^o allele and the β^+ allele was 73% and 27%, respectively.

At present there is little information and very few data published about the distribution of hemoglobinopathies in Argentina.[5-7] The frequencies of the mutations studied are similar to those reported for other regions of our country which, in their turn, are similar to those reported for the Mediterranean region.[8-10] Strikingly, considering the ethnic origin, there were no patients with the β^+1-6 mutation, which reaches a frequency of 5.9% in Buenos Aires and 10.3% in Italy.

In conclusion, this study provides data for our region, for which there were no records up to now. Although there are few homozygous cases in our population, the information could be useful in cases of prenatal diagnosis.

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Key words

β-thalassemia mutations.

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References

1. Weatherall DJ. The thalassemias. In: Stamatoyannopoulos G, Nienhuis A, eds. The Molecular Basis of Blood Diseases. Philadelphia: WB Saunders Co;

1994. p. 157.
2. Kulozik AE. β-thalassaemia: molecular pathogenesis and clinical variability. Eur J Pediatr 1992; 151:78-84.
3. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215.
4. Old JM, Varawalla NY, Weatherall DJ. Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet 1990; 336:834-7.
5. Roldán A, Gutiérrez M, Cygler A, et al. Molecular characterization of β-thalassemia genes in an Argentine population. Am J Hematol 1997; 54:179-82.
6. Soria NW, Tulián CL, Plassa F, Roth GA. β-thalassemia and hemoglobin types in Argentina: determination of most frequent mutations. Am J Hematol 1997; 54:160-3.
7. Noguera N, Bragós I, Morisoli L, Milani A. Screening for hemoglobinopathies in neonates in Argentina. Haematologica 1999; 84:387-9.
8. Schiliró G, Di Gregorio F, Samperi P, et al. Genetic heterogeneity of β thalassemia in Southeast Sicily. Am J Hematol 1995; 48:5-11.
9. Cao A, Gossens M, Pirastu M. β-thalassaemia mutations in Mediterranean populations. Br J Haematol 1989; 71:309-12.
10. Vania A, Gentiloni Silverj F, Fruscella R, Plantamura M, Cianciulli P, Ballati G. Thalassemic syndromes in Latium: epidemiological evaluation. Haematologica 1998; 83:525-32.

Mobilization of peripheral blood progenitor cells with G-CSF alone in lymphoma patients: steady-state circulating progenitor cell count predicts the autograft yield

Nowadays the first choice procedure to collect hematopoietic progenitor cells for autografts is the harvest of peripheral blood progenitor cells (PBPC) mobilized with cytokines, associated or not with chemotherapy. A large amount of data is available on factors affecting mobilization, such as sex, age, number of previous chemotherapy regimens, radiotherapy, and exposure to fludarabine.[1-3] The individual response to mobilization is, however, highly variable from patient to patient and only a few studies have investigated biological parameters that could predict successful mobilization of PBPC for a single patient when a given regimen is employed. Findings of this study show that when G-CSF is used as a single mobilizing agent, the baseline PBPC has a predictive value.

Sir,

We investigated some steady state parameters in 41 patients (21 F, 20 M) affected by non-Hodgkin's (n=31) and Hodgkin's (n=10) lymphoma. The patients had a median age of 40 years (range 15-58) and all patients had been homogeneously treated and mobilized at a median time of 4 months (range 2-19) from the end of a single line of chemotherapy. At the time of the harvest all patients were in partial or complete remission and none had bone marrow involvement. Total nucleated cells (TNC), CD34$^+$ cells and CFU-GM were evaluated in PB and bone marrow (BM) the day before starting a mobilization protocol consisting of a daily G-CSF dose of 16 µg/kg, given as a single subcutaneous injection from day –3. PB CD34$^+$ cells were monitored from day 0 and leukaphereses were performed with PB CD34$^+$ cells ≥ 10/µL and continued until at least 2.5×10^6/kg b.w. CD34$^+$ cells were collected. Steady-state PB and BM characteristics are summarized in Table 1. There were no significant differences between patients with non-Hodgkin's and Hodgkin's lymphoma (data not shown). Baseline BM TNC, CD34$^+$ cell count and CFU-GM/mL did not correlate with the yield of the harvest evaluated as CD34$^+$ cells collected/kg b.w. Otherwise steady-state PB CD34$^+$ cell count and

Table 1. Baseline peripheral blood and bone marrow characteristics.

		Steady-state		
	Peripheral blood		*Bone marrow*	
TNC x10^6/mL	median	5	median	22.9
	range	3-11.8	range	4.7-72.8
CD34+ x10^3/mL	median	1.25	median	83.16
	range	0-16	range	1.1-763
CFU-GM/mL	median	10.6	median	576
	range	0-224	range	69-7000

TNC: total nucleated cells; CFU-GM: colony-forming units granulocyte macrophage.

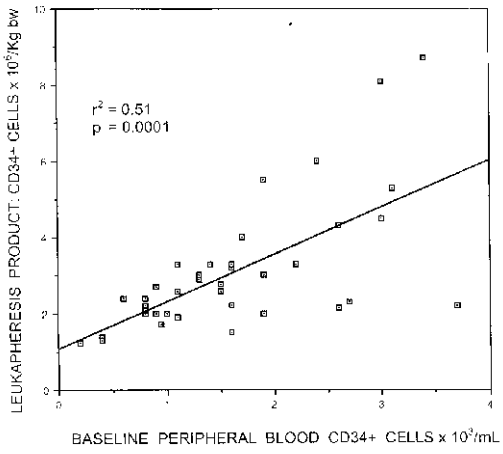

Figure 1. Relationship between steady-state CD34$^+$ peripheral cells and CD34$^+$ cells collected/kg b.w. Enumeration of CD34$^+$ cells was performed incubating 1×10^6 cells with the phycoerythrin conjugated (PE) moAb HPCA-2 (CD34) and the FITC moAb HLE-1 (CD45); after lysis of erythrocytes samples were washed twice and analyzed using a FACScan flow cytometer (Becton Dickinson). Forty-five thousand CD45$^+$ events or at least 100 CD34 positive events were acquired according to the ISHAGE protocol, using a cumulative gating strategy to identify true CD34$^+$ cells and minimize the number of non-specifically stained events (*Sutherland DR et al. J Hematother 1996; 5:213*). Negative controls for each sample were also analyzed (using FITC and PE irrelevant isotypic immunoglobulins).