whether the advantages of fast engraftment and avoidance of general anesthesia outweigh the theoretical risk of donor leukemogenesis related to a short course of G-CSF and whether PBSC transplant offers advantages in terms of clinical outcome over marrow transplantation in children with leukemia.

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Key words

Peripheral blood stem cells, allogeneic transplantation in children, G-CSF priming

Appendix

Patients' data for this report were contributed by the following institutes and physicians: Department of Pediatrics, University of Turin (R. Miniero, A. Busca); Department of Pediatrics, IRCCS Policlinico S. Matteo, University of Pavia (F. Locatelli, F. Bonetti); Department of Pediatrics, University of Bologna (A. Pession, R. Rondelli); Department of Pediatrics, University of Milan, Monza (C. Uderzo, A. Balduzzi); Department of Pediatrics, Ospedale Pausilipon, Naples (M. Ripaldi).

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References

1. Gratwohl A, Baldomero H, Hermans J. Haemopoietic precursor cell transplant activity in Europe 1994. Bone Marrow Transplant 1996; 17:137-48.
2. Bearman SI, Appelbaum FR, Buckner CD, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 1988; 6:1562-8.
3. Bensinger WI, Weaver CH, Appelbaum F, et al. Transplantation of allogeneic peripheral blood stem cells mobilized by recombinant human granulocyte colony-stimulating factor. Blood 1995; 85:1655-8.
4. Schmitz N, Bacigalupo A, Labopin M, et al. Transplantation of peripheral blood progenitor cells from HLA-identical sibling donors. Br J Haematol 1996; 95:715-23.
5. Korbling M, Chan KW, Anderlini P, et al. Allogeneic peripheral blood stem cell transplantation using normal patient-related pediatric donors. Bone Marrow Transplant 1996; 18:885-90.
6. Villa M, Madero L, Diaz MA, et al. Allogeneic peripheral blood progenitor cell (PBPC) transplantation in children. Bone Marrow Transplant 1996; 18:231-3.
7. Diaz MA, Alegre A, Villa M, et al. Allogeneic peripheral blood progenitor cell (PBPC) transplantation in children with haematological malignancies. Br J Haematol 1997; 96:161-4.
8. Kong Li C, Man Pan Yuen P, Wai Chik K, et al. Allogeneic peripheral blood stem cell transplant in children. Med Pediatr Oncol 1998; 30:147-51.
9. Szydlo R, Goldman JM, Klein JP, et al. Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings. J Clin Oncol 1997;15:1767-77.

4.2 Nippon mutation in a non-Japanese patient with hereditary spherocytosis

Sir,

The erythrocyte protein 4.2 (P4.2) is a major component in the erythrocyte skeletal network that participates directly in band 3-cytoskeleton linkage.[1,2] The main isoform has an apparent molecular weight of 72 kD on sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). P4.2 absence has seldom been observed in hereditary spherocytosis (HS).[3] Since the first description in 1974, most HS patients with absent red blood cell P4.2 have been found in Japan. A band 4.2 gene (*EPB4.2*) point mutation (GCT→ACT: Ala→Thr at codon 142) defining the Nippon allele has frequently been shown.[4] This occurs in the heterozygous state in around 3% of healthy subjects and is specific to the Japanese population.[5] We report the first case of HS associated with 4.2 Nippon mutation in a non-Japanese patient.

The proband (a 30-year-old female) was born in a small mountain village in central Italy. Her family has no Japanese ancestry and have lived there for several generations. Although consanguinity between parents cannot be formally demonstrated, it is strongly suspected. The proband showed splenomegaly and had suffered from moderate hemolytic anemia since birth. The pink test, an osmotic fragility test, was increased. Based on these findings, moderate HS was diagnosed (Figure 1). Her parents and siblings were clinically and hematologically normal (Figure 1). Red cell P4.2 was completely missing in the proband on SDS-PAGE (Figure 2A).[6] Linkage analysis eliminated ankyrin and band 3 genes as candidate genes.[7,8] The thirteen exons of the EPB4.2 gene were amplified by PCR using primers reported by Takaoka *et al.*[9] and submitted to single-strand conformational polymorphism (SSCP) analysis. In the proband, nucleotide sequence analysis of the *EPB4.2* exon 3 revealed a single base substitution at codon 142 (GCT→ACT: Ala→Thr) in the homozygous state. This mutation defines the Nippon allele. Digestion with *Hga* I confirmed the mutation in the proband and demonstrated that the parents and a sister were heterozygotes. Western blot analysis of red cell P4.2 revealed, as reported in the Japanese patients, a minimal trace of an immunoreactive P4.2 doublet of 74 and 72 kD when a large number of proteins were loaded onto SDS-PAGE (Figure 2B). Protein analysis in the other family members was normal except for the presence of a 74 kD peptide on Western blotting in the parents

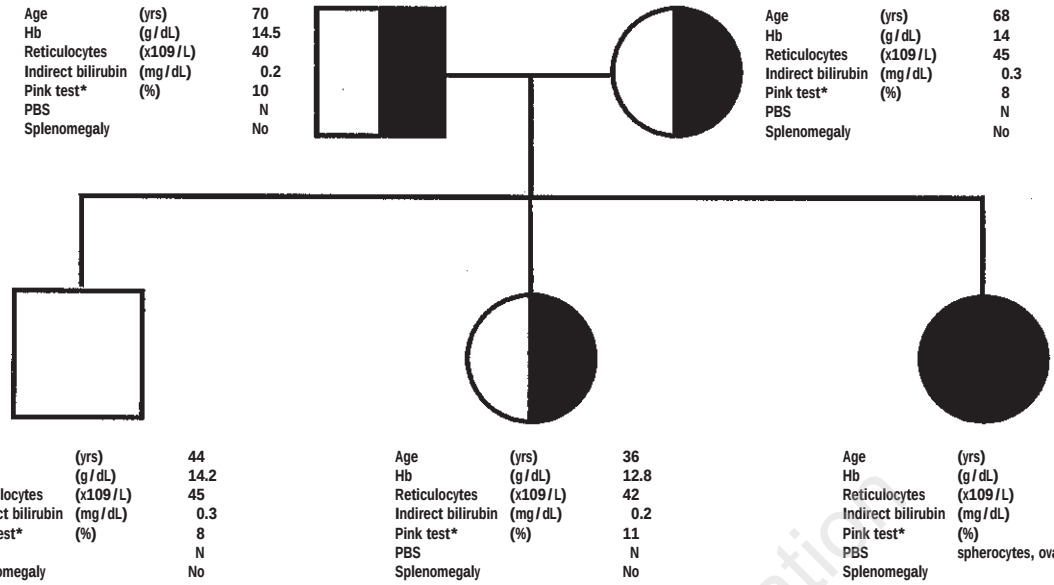

Figure 1. The family pedigree with clinical and hematologic data. *normal value: < 28.5%. PBS: peripheral blood smear.

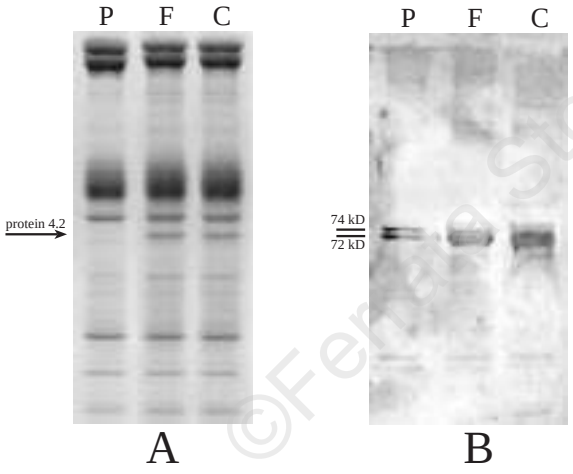

Figure 2. Red blood cell membrane protein analysis. P: proband. F: father. C: control. (A) SDS-PAGE according to Laemmli with Coomassie blue staining. (B) Western blot with polyclonal anti-protein 4.2 antibodies. Apparent molecular weights are expressed as kD.

and in a sister (Figure 2B).

This is the first description of a case of HS due to Nippon allele in a non-Japanese patient. The presence of a mutation in two very different ethnic groups strongly supports the view that the mutation arose from an independent mutational event. It is likely that our mutation occurred in a hot spot CpG dinucleotide[10] more recently than the mutational event producing the original Nippon mutation.

Discussion has arisen as to whether or not the Nippon mutation could be a simple polymorphism in linkage disequilibrium with the determinant of the P4.2 deficiency.[4,5] In fact, although some mechanisms have been hypothesized to explain the P4.2 absence, *in vitro* expression, as well as analysis of the membrane binding properties of the mutant, which would definitively establish the effect of the mutation, have never been performed. Nevertheless, the simple polymorphism, implying a common ancestor of Nippon allele with identical mutant alleles, can be excluded if a new origin of the mutation is established. Our mutation, in a different genetic background and causing identical phenotypic effects, suggests that the Nippon mutation is directly responsible for the erythrocyte P4.2 absence.

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Key words

Hereditary spherocytosis, protein 4.2, red cell membrane, gene mutation

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References

1. Cohen CM, Dotimas E, Korsgren C. Human erythrocyte membrane protein band 4.2 (Pallidin). Semin Hematol 1993; 30: 119-37.
2. Rybicki AC, Schwartz RS, Hustedt EJ, Cobb CE. Increased rotational mobility and extractability of band 3 from protein 4.2-deficient erythrocyte membranes: evidence of a role for protein 4.2 in strengthening the band 3-cytoskeleton linkage. Blood 1996; 88:2745-53.
3. Iolascon A, Miraglia del Giudice E, Perrotta S, Alloisio N, Morlé L, Delaunay J. Hereditary spherocytosis: from clinic to molecular defects. Haematologica 1998; 83:240-57.
4. Bouhassira EE, Schwartz RS, Yawata Y, et al. An alanine-to-threonine substitution in protein 4.2 cDNA is associated with a Japanese form of hereditary hemolytic anemia (protein 4.2^{NIPPON}). Blood 1992; 79:1846-54.
5. Yawata Y. Red cell membrane protein 4.2: phenotypic, genetic and electron microscopic aspects. Biochim Biophys Acta 1994; 1204: 131-48.
6. Miraglia del Giudice E, Iolascon A, Pinto L, Nobili B, Perrotta S. Erythrocyte membrane protein alterations underlying clinical heterogeneity in hereditary spherocytosis. Br J Haematol 1994; 88:52-5.
7. Jenkins PB, Gallagher PG, Forget BG. Analysis of a PstI polymorphism of the human erythrocyte band 3 gene (EPB3). Br J Haematol 1993; 85: 816-8.
8. Miraglia del Giudice E, Francese M, Nobili B, et al. High frequency of de novo mutations in ankyrin gene (ANK1) in children with hereditary spherocytosis. J Pediatr 1998; 132:117-20.
9. Takaoka Y, Ideguchi H, Matsuda M, Sakamoto N, Takeucki T, Fukumaki Y. A novel mutation in the erythrocyte protein 4.2 gene of Japanese patients with hereditary spherocytosis (protein $4.2^{FUKUOKA}$). Br J Haematol 1994; 88:527-33.
10. Youssoufian H, Kazazian HH, Phillips DG, et al. Recurrent mutations in haemophilia A give evidence for CpG mutation hotspots. Nature 1986; 324: 380-3.

Nosocomial infections due to Enterococci in patients with acute leukemia

Sir,

Enterococci have recently emerged as serious pathogens in a growing number of nosocomial infections and progressive high level aminoglycoside resistance (HLAR) and vancomycin resistance (VRE) have been detected.[1,2] Two phenotypes, Van A (vancomycin and teicoplanin resistant) and Van B (susceptible to teicoplanin) predominate. VRE infections can spread either by direct patient-to-patient contact or indirectly via hands of personnel or contaminated environmental surfaces.[3]

In our study we examined febrile episodes in acute leukemic patients, admitted to our Department from January 1995 to June 1997, in order to evaluate the incidence and antimicrobial susceptibility of enterococcal blood isolates.

All patients presenting with fever (axillary temperature > 38°C) during neutropenia (absolute granulocyte count < 1.0×10^9/L) were treated empirically with a beta-lactam antibiotic plus an aminoglycoside; glycopeptide was added later if Gram positive bacteria were isolated from culture or if no response was obtained to the initial antibiotics after 72-96 h. Blood cultures were obtained from a peripheral vein (pv) or from both a pv and central venous catheter (CVC) by at least two different venipunctures. Bacteremia and CVC related bacteremia were defined according to previously reported criteria.[4,5] Febrile episodes were classified according to the EORTC statement.[6]

All bacterial isolates were identified and tested for their antimicrobial susceptibility with the automated Vitek system. HLAR and VRE were verified by the E-test. During 29 months, 146 patients were enrolled and a total of 345 febrile episodes occurred (Table 1).

Of 148 organisms isolated in 132 bacteremias (16 were polymicrobial), Gram positive microbes were responsible for 53% of the cases of sepsis: *S. aureus* in 14%, coagulase negative staphylococci in 48%, streptococci in 20%, enterococci in 14 %, other in 4%. Enterococci were detected in at least two different blood cultures in all patients (26 positive blood cultures out of 41 performed).

Table 1. Characteristics of the 345 evaluable febrile episodes in acute leukemic patients.

Characteristics	*Value*
No. of enrolled patients	146
No. of episodes	345
Sex (male/female)	81/65
Mean age (range)	50 (14-77)
AML	111
ALL	35
No. (%) of episodes in:	
remission-induction	159 (46)
consolidation	52 (15)
relapse or refractory disease	134 (39)
Median days of granulocytopenia (< 1,000 cells/mm^3)	18.6
No. (%) of episodes with	
< 100 cells/mm^3	253 (73)
100-500 cells/mm^3	52 (15)
500-1,000 cells/mm^3	40 (12)
No. (%) of episodes with antibacterial prophylaxis	169 (49)
No.(%) of episodes with:	
central venous catheter	127 (37)
shock	5 (1.5)
Classification of episodes (%):	
MDI with bacteremia	132 (38)
MDI without bacteremia	15 (5)
CDI	70 (20)
FUO	128 (37)

MDI: microbiologically documented infection; CDI: clinically documented infection; FUO: fever of unknown origin.