Correspondence

Silverio Perrotta, M.D., Dipartimento di Pediatria, Seconda Università di Napoli, via S. Andrea delle Dame 4, 80138 Naples, Italy. Phone: international +39-081-5665421 – Fax: international +39-081-446533 - E-mail: nobili@unina.it

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.

Table 2. Characteristics of patients with enterococcal bloodstream infections.

Case	Age\sex	Diagnosis	Status	Cells/mm³	CVC	Pathogen	Antibiotic therapy	Outcome	Antimicrobial susceptibility VA	TEC	AM	GM
1	50/m	AML	I	<100	Y	*E. faecium*	CAZ+AN+VA	Died	S	S	S	S
2	49/m	AML	I	<100	Y	*E. faecalis*+	CAZ+AN+VA	Improved	S	S	R	S
				<100		*C. albicans*						
3	63/m	AML	R	<100	Y	*E. faecium*	CRO+AN+VA	Improved	S	S	R	S
4	19/m	ALL	R	<100	Y	*E. faecium*	CRO+AN+VA	Improved	S	S	S	S
5	22/m	AML	I	<100	N	*E. faecium*	CRO+AN+TEC	Improved	S	S	R	R
6	53/f	AML	I	<100	N	*E. faecalis*	CAZ+AN+VA	Improved	S	S	R	R
7	56/f	AML	R	<100	Y	*E. faecium*	CAZ+AN+VA	Improved	S	S	R	R
8	64/f	AML	C	<100	N	*E. faecalis* +	CRO+AN+VA	Improved	S	S	R	R
				<100		*P. aeruginosa*						
9	34/f	AML	R	<100	N	*E. faecium*	CAZ+AN+TEC	Died	S	S	S	R
10	66/f	AML	I	<100	Y	*E. faecalis*	CAZ+AN+VA	Died	S	S	R	R
11	50/m	AML	I	<100	N	*E. faecium*	CAZ+AN+VA	Died	R	R	R	R

Status: I=remission induction, C=consolidation, R=relapse or refractory disease; CVC=central venous catheter. Antibiotic therapy and susceptibility: CAZ=ceftazidime, CRO=ceftriaxon, AN=amikacin, GM=gentamicin, AM=ampicillin, VA=vancomycin, TEC=teicoplanin. R=resistant, S=susceptible.

The characteristics of patients with enterococcal infections are listed in Table 2. In two patients we documented a probable CVC-related infection (tips were not cultured), 4 had a previous infection (FUO) and two had been previously treated with glycopeptides. The mean hospital stay before enterocccal detection was 21 days (range 16-28).

Antimicrobial susceptibility tests showed a high prevalence of HLAR and one VRE (Table 2) Van A type (vancomycin MIC 168 µg/mL, teicoplanin MIC 32 µg/mL) occurred in one patient with AML, without previous infection. Multiple positive VRE blood cultures were obtained after 13 days of fever. The hospital stay before isolation of VRE was 26 days and the patient was treated with vancomycin for 12 days before he was isolated and treated with chloramphenicol for 10 days. The patient died and the room was cleaned and disinfected. Mortality is high (36%) although clinical data suggest that only in the patient with VRE was death directly attributable to this infection, because it occurred as a direct consequence of the presenting infection.

Our data show that severe neutropenia, cephalosporins, multiple antibiotics and the duration of stay in hospital are likely contributing factors to the development of these infections, even if no difference was observed among the strains.

Although a rapid increase of VRE has been reported in the USA ,[3] the incidence remains low in Europe and in Italy.[7,8] As reported by others, our study confirms that the highest incidence and resistance rates occur in oncology-hematology patients.[8,9] Since the total number of enteroccal bloodstream infections was substantially low during the study period, the first detection of a Van A type resistance in our cancer center is an alarming sign. There is no clearly effective treatment.[10] Immediate infection control measures and vigilant surveillance for multiresistant strains are essential.

*Rosa Fanci, *Patrizia Pecile, Alberto Fabbri, Cristina Paci, Reyna Lorena Martinez, Giovanni Longo*

*Department of Hematology and University of Florence, *Department of Bacteriology and Virology, Careggi Hospital, Florence, Italy.*

Key words

Acute leukemia, enterococcal infections, vancomycin susceptibility, neutropenic patient.

Correspondence

Rosa Fanci, MD, Cattedra di Ematologia, Az. Ospedaliera Careggi, viale Morgagni, 85, 50134 Florence, Italy. Phone: international +39-055-4277476 – Fax: international + 39-055-412098 – E-mail: afabbri@val.it

References

1. Montecalvo MA, Shay DK, Patel P, et al. Bloodstream infections with vancomycin-resistant enterococci. Arch Intern Med 1996; 156:1458-62.
2. Manso E, De Sio G, Biavasco F, Varaldo PE, Sambo G, Maffei C. Vancomycin-resistant enterococci. Lancet 1993; 342:615-7.
3. Hospital Infection Control Practices Advisory Committee(HICPAC). Recommendations for preventing the spread of vancomycin resistance. Infect Control Hosp Epidemiol 1995; 16:105-13.
4. Freifeld AG, Walch T, Marshall D, et al. Monotherapy for fever and neutropenia in cancer patients: a randomized comparison of ceftazidime vs imipenem. J Clin Oncol 1995; 13:165-7.
5. Menichetti F, Del Favero A, Bucaneve G, et al. Teicoplanin empirical combined antibiotic therapy of bacteraemias in bone marrow transplant patients. J Antimicrob Chemother 1988; 21:(Suppl A)105-11.
6. EORTC International Antimicrobial Therapy Project Group. Combination of amikacin and carbenicillin with or without cefazolin as empirical treatment of febrile neutropenic patients. J Clin Oncol 1983; 1:597-603.
7. Venditti M, Goglio A and the GIMEMA Microbiology

Group. Vancomycin susceptibility in enterococcal blood isolates in Italy: a multicenter retrospective analysis. J Chemother 1996; 8:33-6.
8. Fontana R, Ligozzi M, Mazzariol A, Veneri G, The Italian Surveillance Group for antimicrobial resistance, Cornaglia G. Resistance of enterococci to ampicillin and glycopeptide antibiotics in Italy. Clin Infect Dis 1998; 27:8(Suppl.1):84-6.
9. Montecalvo MA, Horowitz H, Gedris C. Outbreak of vancomycin-ampicillin and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. Antimicrob Agents Ch 1994; 38.1363-7.
10. Roberts RB. Report on the first 100 patients treated with Synercid (RP5900) under the emergency use program for the treatment of vancomycin-resistant *Enterococcus faecium* (VREF) and methicillin-resistant *Staphylococcus aureus* infection. Can J Infect Dis 1995; 6:(Suppl C), July 1995.

Transplacental transmission of EDTA-dependent pseudothrombocytopenia

Sir,

Pseudothrombocytopenia (Pstp) is an *in vitro* laboratory finding usually associated with the use of EDTA in blood collection tubes; it may cause unjustified alarm in the patient and physician, sometimes leading to the use of unwarranted diagnostic procedures or treatment.[1] This rare phenomenon (1:1,000)[2] is related to *in vitro* platelet clumping caused by immunoglobulins (most often IgG) recognizing platelet antigens which are exposed on the membrane only in the presence of EDTA.[3] Over the past 12 years we have collected about 200 cases of Pstp, almost always discovered during routine hematologic tests and sent to us with the diagnosis of asymptomatic thrombocytopenia. The phenomenon is time- and temperature-dependent; once established, it seems to be permanent.[4] So far, a familial occurrence has never been documented. We describe a case of transient congenital Pstp in a baby born to a mother with Pstp. A 38-year old woman was found to have persistent severe thrombocytopenia by routine electronic blood counting; there was no history of antecedent or present bleeding tendency. The automatic platelet count on EDTA-anticoagulated sample was 20×10^9/L; a peripheral blood smear exhibited multiple large platelet clumps. Platelet count performed immediately after withdrawing the blood was 267×10^9/L; counts performed every ten minutes on the same test tube kept at room temperature showed a progressive reduction in platelet number , reaching the nadir at 4 hours. This phenomenon was absent in heparinized blood, and less evident when citrate or oxalate was used as the anticoagulant. We diagnosed EDTA-dependent pseudothrombocytopenia. The phenomenon was still present at a follow-up performed 1 year later. Six years later, the woman became pregnant and delivered a baby without complication. At routine examination, the newborn showed a platelet count of 23×10^9/L, in the absence of any bleeding tendency. Large platelet clumps were evident on the blood smear. A count from heelstick blood, immediately diluted in a buffer solution without EDTA, showed a platelet count of 430×10^9/L. After 1 month, the baby's platelet count was normal, even in the presence of EDTA. Repeat counts performed over the following months showed persistence of Pstp in the mother but absence of the phenomenon in the baby. This case documents transplacental transmission of the plasmatic factor, probably an IgG, responsible for Pstp. It also indicates the need to consider the likelihood of this phenomenon in neonates with asymptomatic thrombocytopenia in order to avoid inappropriate and potentially harmful treatments.

Federico Chiurazzi, Maria Rosaria Villa, Bruno Rotoli

Hematology Division, Federico II University, Napoli, Italy

Key words

EDTA, pseudothrombocytopenia

Correspondence

Bruno Rotoli, M.D., Divisione di Ematologia, Università Federico II, via Pansini 5, 80131 Naples, Italy. Fax: international +39-081-7462165.

References

1. Onder O, Weinstein W, Hoyer LW. Pseudothrombocytopenia caused by platelet agglutinins that are reactive in blood anticoagulated with chelating agents. Blood 1980; 56:177-82.
2. Bartels PC, Schoorl M, Lombarts AJ. Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. Scand J Clin Lab Invest 1997; 57:629-36.
3. Berkman N, Michaeli Y, Or R, Eldor A. EDTA-dependent pseudothrombocytopenia: a clinical study of 18 patients and review of the literature. Am J Hematol 1991; 36:195-201.
4. Bizzarro N. EDTA-dependent pseudothrombocytopenia: a clinical and epidemiological study of 112 cases, with 10-year follow-up. Am J Hematol 1995; 50:103-9.

Acenocoumarol and 6-mercaptopurine: an important drug interaction

Sir,

Many drugs are known to interact with oral anticoagulants (OA),[1] but the greater part of the reported interactions refer to warfarin. Acenocoumarol is the most widely used coumarinic derivative used in Spain as an OA. 6-MP is a metabolite of azathioprine, both used as immunosuppressant drugs in a variety of autoimmune disorders. 6-MP decreases warfarin activity, and severe bleeding was described in a patient on long-term warfarin treatment after discontinuing azathioprine.[2]