Key words

Chronic refractory ITP, IFN-α2b, immune system

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Long-term disappearance of previous chromosomal abnormalities in myelodysplastic syndromes treated with low dose cytosine arabinoside and granulocyte/macrophage-colony stimulating factor

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Most therapies for elderly patients with myelodysplastic syndromes offer few short responses and little improvement in survival. We describe two patients who, after several cycles of low dose cytosine arabinoside and GM-CSF, achieved and maintained complete remission and became tranfusion independent. Previous chromosomal abnormalities also disappeared and karyotype remains normal.

No uniformly accepted treatment is available for elderly patients with myelodysplastic syndromes (MDS).[1] We present two MDS patients treated with combined low-dose araC and GM-CSF who achieved a complete (CR) clinical, hematological and cytogenetic response.

Case #1. A 71-year-old-woman diagnosed in 1992 as having refractory anemia was referred in 1995 because of severe cytopenias and elevated transfusional requirements. Bone marrow (BM) aspirate was hypercellular with trilineal dysplasia and 12% myeloblasts. Cytogenetics: 46,XX (45% metaphases)/46, XX, t(5;13)(q13; q14) (35%)/47,XX,+8 (20%). She started low-dose ara-C (10 mg/m^2/d) and GM-CSF (150 mg/d), days 1 to 14, every month. After the fourth cycle she did not need further transfusions. Data in August 1996: normal karyotype; less than 1% of blasts in BM; WBC count, 3.3×10^9/L; hemoglobin (Hb), 143 g/L; 124×10^9 platelets/L. Side-effects were mild (except for flu-like syndrome related to GM-CSF), thus allowing us to administer up to 20 cycles of this protocol. The patient remains stable without complications 24 months after the onset of treatment (Figure 1).

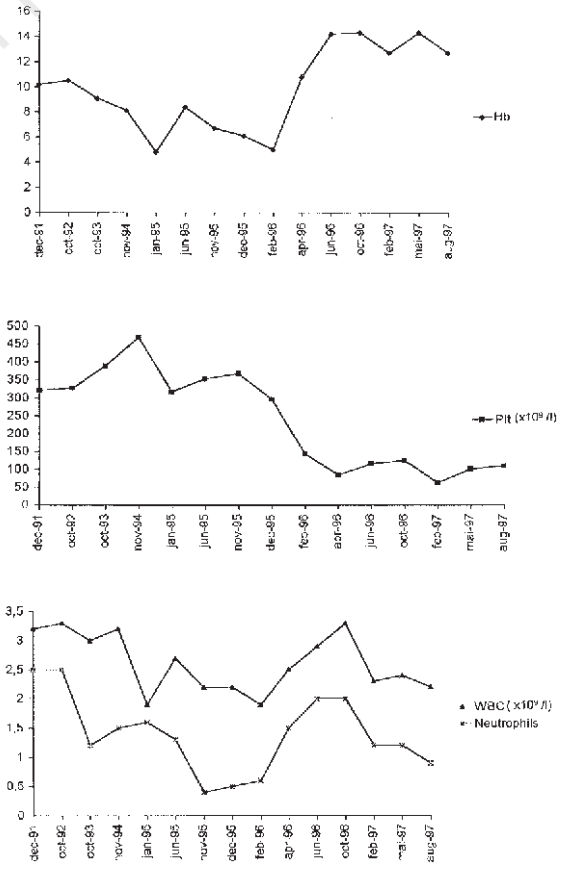

Figure 1. Case #1.

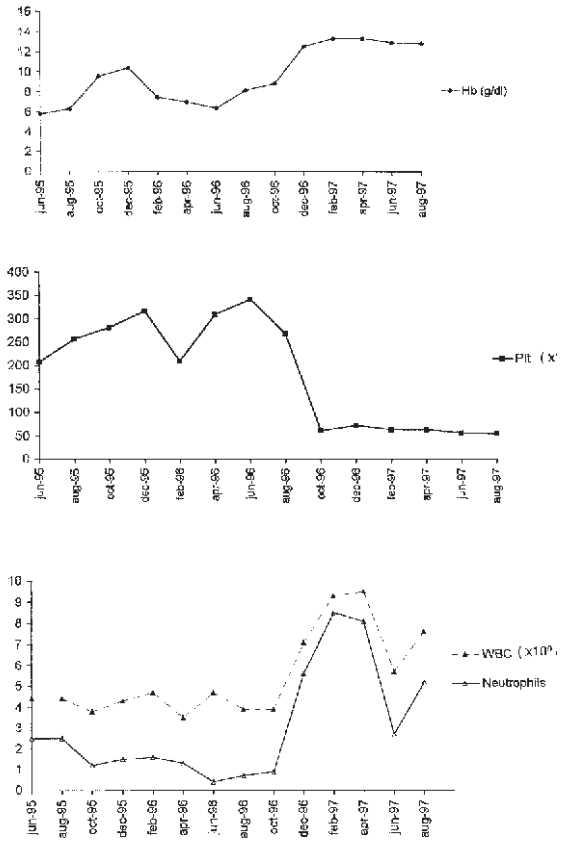

Figure 2. Case #2.

Case #2. A 68-year-old woman was diagnosed as having refractory anemia with ring sideroblasts . Folic acid, vitamin B6 and danazol did not prevent a progressive worsening in blood counts. By August 1996, she needed weekly transfusions and her neutrophil count was 0.7×10^9/L. BM aspirate revealed severe trilineal dysplasia, 4% myeloblasts, and occasional Auer rods; karyotype: 46, XX, del(5)(q13; q33). At this moment the protocol was initiated. Data after the fifth cycle: normal karyotype; less than 1% myeloblasts in BM; WBC 12.3×10^9/L (76% neutrophils); Hb 134 g/L without need for further transfusions. By the 17th month of treatment she maintains a complete response (Figure 2).

The outlook of MDS patients with excess of blasts, pancytopenia and chromosomal abnormalities is ominous.[2] Therapy in older individuals usually aims at merely prolonging survival. Intensive chemotherapy attains variable CR rates of short duration with important morbidity.[1,3] For these reasons milder therapies have been tried: results with low-dose ara-C are similar to intensive protocols, sharing their lack of effect on prolongation of survival;[4,5] GM-CSF increases neutrophil counts and decreases infection rate in these patients. Wadhan-Raj reported the suppression of the myelodysplastic clone and stimulation of polyclonal hematopoiesis after GM-CSF.[6] In a EORTC series of 82 patients given ara-C and GM-CSF, response rate was significant enough (63%) to suggest a role for this combination.[7]

We report two cases whose originality lies in the fact that previous chromosomal abnormalities disappeared under prolonged therapy. Up till now, it is not clear how many cycles should be delivered; besides, most available data come from assays with few courses. In contrast, our patients are kept indefinitely under treatment, provided that side effects are not unbearable or disease progresses overtly.[8] The uncertainty about the relationship between cytogenetic response and cure[9] sustains our long-term policy.

To sum up, we agree with other investigators that ara-C and GM-CSF[10] therapy is useful in selected MDS patients.[7] The dosage and minimum number of cycles remain unclear.

Key words

Myelodysplastic syndromes, cytosine arabinoside, granulocyte/macrophage-colony stimulating factor, complete remission

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Alloimmunization against human platelet antigen 2 (HPA2) in a series of multitransfused β-thalassemia patients

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In our study we investigated the presence of anti-human platelet antigen (HPA) alloantibodies in a series of 10 β-thalassemia *major* patients submitted for more than 10 years to periodic blood transfusions (every 2-3 weeks). We found that 2 out of the 10 patients developed anti-HPA2a+HPA1b and anti-HPA2b antibodies. Our results highlight that HPA alloimmunization in multitransfused patients is a real possibility.

Patients affected by β-thalassemia *major* are usually submitted to many transfusions of packed red blood cells during their life. Multitransfused patients are exposed to different immunogens due to the presence of leukocytes and platelets in packed red blood cells.[1,2] Antibodies raised against the latter components are responsible for some of the febrile non-hemolytic transfusions reactions (FNHTR) and filtered packed red cells are then needed to avoid this kind of reactions. Little is known about possible alloimmunization against human platelets antigens (HPA); this is more difficult to study and to characterize than HLA alloimmunization.[3,4] In this context we analyzed our series of β-thalassemia patients to find alloimmunization against red blood cells, leukocyte and platelets.

Ten patients affected by β-thalassemia *major* (clinical characteristics summarized in Table 1) were submitted to periodic tests for the presence of alloantibodies. Tests were also performed in the case of transfusion reactions.

Antibodies against red cell antigens were detected by standard indirect tests using rabbit anti-human immunoglobulin antisera. Antibodies against HLA were analyzed by a standard cytotoxicity test on HLA-typed donors.

The search for alloantibodies against HPA was performed by a standard indirect immunofluorescence test on random and HPA-typed donor platelets.[5] The samples were also run on a monoclonal antibody-immobilized platelet antigen (MAIPA) test[6] to define the specificity of the recognized antigen. Patients found positive for anti-HPA antibodies were genotyped for HPA-1,-2-,3,-5 genes with SSP-PCR.

Clinical records (Table 1) showed that 6 patients had suffered from FNHTR, indicating a possible alloimmunization against platelets and/or leukocytes. Two patients showed alloimmunization against red blood cell (one anti-Kell and the other anti-Kp^a), while 5 out of 10 had HLA antibodies with a very wide specificity (>80% of positive donors). One patient (#4) had anti- HLA-B35+51 antibodies.

Concerning HPA alloantibodies, patient #1 had HPA2b alloantibodies and patient #2 HPA2a+HPA1b alloantibodies. It should be underlined that HLA antibodies with wide reactivity were found in sera from both patients, and patient #1 had also anti-Kell antibodies. HPA gene typing showed that patient n.1 (anti-HPA2b) was HPA1a/a, HPA2a/a, HPA3b/b, HPA5a/b. Patient #2 (anti-HPA2a) showed the following typing: HPA1a/a, HPA2b/b, HPA3a/a, HPA5a/a.

We analyzed sera from 10 multitransfused patients suffering from β-thalassemia major, looking for both HLA and HPA specificities. HLA antibodies were found in 6 out of the 10 patients. Our investigation also showed that HPA alloimmunization is a real possibility since two patients developed HPA antibodies (anti HPA2b and anti HPA2a+HPA1b).

In multitranfused patients alloimmunization is usually regarded as strictly related to the presence of HLA alloantibodies, since these are responsible for most of the FNHTRs. In addition HPA alloantibodies may be implicated. A retrospective analysis showed that patient #2 also suffered from FNHTR also when receiving blood from HLA-matched donors, positive for the HPA2a antigen. These results suggest that HPA may have been responsible for the FNHTRs.

Table 1. Characteristics of β-thalassemia patients.

N.	Pts.	Age/Sex	No. of transfused RBC units (non-filtered U until 1990)	No. of transfused RBC units (filtered U beginning from 1991)	Transfusion reactions
1	C.M.	26/M	464	215	chills-hyperthermia
2	C.A.	24/F	568	160	chills-hyperthermia
3	D.F.M.	34/F	760	212	chills-hyperthermia
4	D.L	21/F	423	202	chills-hyperthermia
5	F.L	31/M	804	240	chills-hyperthermia
6	F.A.	23/F	420	184	none
7	F.M.	19/M	260	162	none
8	I.I.	27/F	740	187	chills-hyperthermia
9	R.A.	21/F	324	164	none
10	S.F	26/M	580	215	none

M= male; F=female; U=units.