Subjects homozygous for C282Y or Y250X were significantly younger (36.5 and 34.0 years vs 47.5 and 54.0, respectively, p <0.001) and had significantly higher ferritin levels and tranferrin saturation (1134.5 μg/L [range 68-3823 μg/L], 70.4% [range 12.6-96%] for individuals homozygous for C282Y and 900.0 μg/L [range 649-1151 μg/L], 90.0% [range 81.3-98.6%] for individuals homozygous for Y250X vs 597.6 μg/L [range 9-2737 μg/L], 46.2% [range 2.8-94.0%] for individuals with other mutations, p <0.001) than those showing other mutations. We did not find any difference in the frequency of HH gene mutations in the two groups of patients who were screened for 2 (82/148, 55.4%) or 12 (102/191, 53.4%) mutations; in fact, the analysis of 12 HH mutations identified only 4 HH gene mutations (C282Y, H63D, S65C in the HFE gene and Y250X in TfR2 gene) in our population, selected for having an abnormal iron status. This evidence raises the question about the utility and costs of this kind of screening in the general population. As regards the 151 subjects lacking HH mutations, in most cases (86.8%) iron overload was associated with other diseases, such as hepatitis C virus infection, alcoholism or systemic diseases which are known to have a role in iron accumulation. Although we did not find any correlation between the presence of liver diseases or solid cancers and HH gene mutations, we observed a high frequency of β-thalassemic trait in heterozygous subjects for H63D mutation (7/97, 7.2%). Increased iron turnover can be easily explained in patients with intermediate thalassemia because of the presence of chronic hemolysis, but is more difficult to explain in β-thalassemia gene carriers, unless it is assumed that H63D mutation may have a modulating effect on iron absorption in these subjects.[7,8] In conclusion, our data confirm the high frequency (55.5%) of HH gene mutations in subjects with abnormal iron status.

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References

1. Piperno A. Classification and diagnosis of iron overload. Haematologica 1998;83:447-55.
2. Pietrangelo A, Camaschella C. Molecular genetics and control of iron metabolism in hemochromatosis. Haematologica 1998;83:456-61.
3. Burke W, Thomson E, Khoury MJ, McDonnel SM, Press N, Adams PC, et al. Hereditary hemochromatosis. Gene discovery and its implications for population-based screening. JAMA 1998;280:172-8.
4. Cazzola M. Novel genes, protein, and inherited disorders of iron overload: iron metabolism is less boring than thought. Haematologica 2002;87:115-6.
5. Camaschella C, Roetto A, De Gobbi M. Genetic haemochromatosis: genes and mutations associated with iron loading. Best Pract Res Clin Haematol 2002;15:261-76.
6. Pietrangelo A, Montosi G, Totaro A, Garuti C, Conte D, Cassanelli S, et al. Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. N Engl J Med 1999;341:725-32.
7. Piperno A, Mariani R, Arosio C, Vergani A, Bosio S, Fargion S, et al. Hemochromatosis in patients with β-thalassemia trait. Br J Haematol 2000;111:908-14.
8. Melis MA, Cau M, Deidda F, Barella S, Cao A, Galanello R. H63D mutation in the HFE gene increases iron overload in β-thalassemia carriers. Haematologica 2002; 87:242-5.

Possible association between reactive oxygen metabolites and karyotypic abnormalities in myelodysplastic syndromes

We demonstrated a significant increase in reactive oxygen metabolites (ROMs), the direct mediators of oxidative damage, in a panel of 50 patients with myelodysplastic syndrome (MDS). Interestingly, increased ROMs were associated with karyotype abnormalities. ROM concentrations may, therefore, represent an easily assessable indicator of potential oxidation in MDS.

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Oxidative damage is caused by an imbalance in pro- and antioxidant ratio. Oxygen metabolites (ROMs) represent direct mediators of oxidation. The anti-oxidant system, defined by total antioxidant activity (TAT), comprises antioxidants and repair enzymes, which limit tissue damage.[1-3] Myelodysplastic syndromes (MDS), clonal disorders with peripheral blood cytopenias and normal or hypercellular dysplastic bone marrow (BM), may progress to acute leukemia. Oxidative stress may play a pathogenetic role in MDS,[4-6] particularly in the genesis of cytogenetic lesions.[7] We investigated the association of MDS and oxidative status by measuring ROMs, homocysteine (acting through ROM production),[3] and TAT in a series of 50 MDS patients.

Thirty males, 20 females (M:F=1.5; aged 26-100 years, median age 75), were observed. According to French-American-British (FAB) classification and the International Prognostic Score System (IPSS), patients were distributed as follows: 16 had refractory anemia (RA), 8 had RA with ringed sideroblasts (RARS), 18 had RA with excess blasts (RAEB), 5 had RAEB in transformation (RAEB-t) and 2 had chronic myelomonocytic leukemia (CMML), 1 hypoplastic MDS; 15 LOW, 19 INT-1, 8 INT-2 and 6 HIGH risk (in 2 patients IPSS was not applicable because of unsuccessful karyotyping). Karyotype was normal in 31 patients and complex in 6; in the remaining cases single chromosome abnormalities were present (chromosomes 5, 7, 8, 20 or Y; Table 1). No significant co-morbidity was present. Fourteen healthy volunteers (M:F=1.3), aged 30-96 years, median 72, represented controls. Plasma total homocysteine (cut-off 10 μmol/L), serum vitamins B12, folate (plus erythrocyte), ROM concentrations, measuring hydroperoxides, and TAT, measuring antioxidant species, including GSH, GSH peroxidase, thiol groups, superoxide dismutase, and catalase, were determined as previously described.[8-10] The ROM cut-off (300 U.Carr) and TAT reference interval (1.5-2 nmol/L) were calculated in controls. Vitamin B12 and folate concentrations were within the relevant reference intervals both in patients and controls (*data not shown*). MDS patients had higher mean plasma homocysteine levels than did controls (10.12 μmol/L vs 8.4 μmol/L; p=0.016; t-test) (Table 1). Increased ROM concentrations were present in 44% (22/50) of the MDS patients and in 21% (3/14) of the controls. MDS patients showed significantly higher mean serum ROM levels than controls (335.54 U.Carr. vs 259.57 U.Carr.;

Table 1. Clinical and laboratory characteristics of the MDS patients included in the study.

#	Sex	Age	FU	IPSS	FAB	Karytotype	ROMs	Hcy	TAT
1	M	66	A	LOW	RA	46,XY	270	11.43	3
2	F	79	A	INT-1	RA	46,XX>46,XX,del(5q)/46,XX	495	6.96	2.3
3	F	80	A	INT-2	RAEB	46,XX	126	7.76	3.4
4	M	54	A	INT-1	RAEB	46,XY	462	–	–
5	F	81	A	INT-2	RAEB	45,XX,-7/46,XX	229	10.27	2.7
6	F	30	D	HIGH	RAEB-t	complex	792	8.69	–
7	F	60	A	INT-1	RA	complex	502	2.59	2.5
8	M	60	A	LOW	RAEB	46,XY	289	8.34	2.6
9	F	63	A	INT-1	RARS	46,XX	294	6,02	2.6
10	M	65	A	LOW	RARS	46,XY	359	11.99	2.7
11	F	75	A	LOW	RARS	46,XX	418	–	–
12	M	53	A	INT-1	RA	46, XY	260	5.22	2.8
13	M	75	A	–	RA	–	301	12.87	3.4
14	F	58	A	INT-1	RAEB	46,XX	268	4.2	1.9
15	M	87	A	INT-1	RAEB	complex	469	8.81	2.6
16	M	56	D	INT-1	MDS hypo	46,XY	355	13.11	3.3
17	M	81	A	HIGH	RA	46,XY	342	11.98	3.6
18	M	78	A	INT-2	RAEB	46,XY	164	11.52	2.4
19	M	63	A	INT-2	RAEB-t	complex	398	9.49	2.7
20	M	76	A	LOW	RARS	46,XY	309	11.39	2.5
21	M	81	A	HIGH	RAEB	46, XY del20(q11q12)	348	14.29	2.2
22	M	75	A	LOW	RARS	46,XY	292	18.56	18.56
23	M	75	A	INT-1	RAEB	46,XY	296	8.4	3
24	M	87	A	INT-1	RAEB	46, XY	334	8.86	3.4
25	F	72	A	INT-2	RAEB	47,XX,+8	377	11.38	2.9
26	F	74	A	INT-2	RA	complex	341	5.05	2
27	M	95	A	LOW	RARS	46,XY	413	–	–
28	M	82	A	HIGH	RAEB-t	46,XY	279	9.24	1.9
29	M	84	D	INT-1	RAEB	46,XY	337	10.96	19
30	M	77	A	LOW	RA	46,XY/45,X,-Y	378	14.52	2.5
31	M	78	A	INT-1	RAEB	46,XY	408	–	-
32	F	72	D	HIGH	RAEB-t	46,XX	652	17.88	–
33	F	78	A	INT-2	RAEB	complex	522	–	–
34	F	59	A	LOW	RA	46,XX,del(5q)/46,XX	363	–	–
35	M	74	A	HIGH	RAEB-t	47,XY+8/46,XY	175	10.14	2.1
36	M	88	A	INT-1	CMML	46,XY	444	–	-
37	F	70	A	LOW	RARS	46,XX	231	9.45	3
38	F	77	A	LOW	RA	46,XX	197	–	-
39	M	84	A	LOW	RARS	46,XY	196	6.24	2.2
40	F	84	A	LOW	RA	46,XX	566	6.25	2.5
41	F	100	A	INT-2	RA	46,XX,del(5)(q13q33)/46XX	340	18.47	–
42	M	59	D	INT-1	RAEB	46,XY	260	10.46	2.1
43	M	80	A	LOW	RA	46,XY	221	17.4	2.8
44	M	78	A	LOW	RA	46,XY	253	8.08	2.2
45	M	80	D	INT-1	RAEB	47,XY,+8	169	–	–
46	F	51	A	–	RAEB	–	405	10.56	2.6
47	M	71	D	INT-1	CMML	46,XY	169	–	–
48	F	26	A	INT-1	RA	47,XX,+8/46,XX	165	7.85	–
49	F	69	D	INT-1	RA	45,XX,-7	350	10.93	3
50	M	70	A	INT-1	RAEB	46,XY	194	7.29	2.8

–: not done; FU: follow-up; A: alive; D: dead; hypo: hypoplastic.

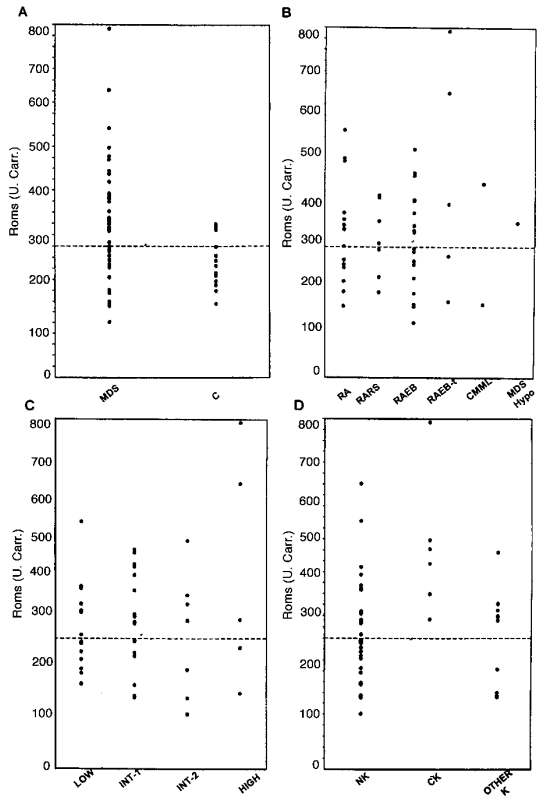

Figure 1. Univariate distribution of ROMs levels according to clinicopathological variables. Panel A: ROMs levels in MDS vs control group. Panel B: ROMs levels according to FAB classification. The case of hypoplastic MDS has been indicated separatedly. Panel C: ROMs levels according to IPSS. Panel D: ROMs levels according to karyotype. NK: normal karyotype; CK: complex karyotype; OTHER K: other karyotype abnormalities. Dashed line: cut-off value.

p=0.002; t-test) (Figure 1 and Table 1). Increased ROM levels were associated with karyotype abnormalities: 13/17 patients with abnormal vs 4/31 with normal karyotype (76% vs 13%; p<0.001; Fisher's exact test). Increased ROM levels were present in all 6 patients with complex karyotype, but only in 20/44 of the remaining patients (100% vs 45%; p=0.02; Fisher's exact test) (Figure 1 and Table 1). In MDS patients, the presence of hyperhomocysteinemia was associated, with borderline statistical significance, with an increase of ROM (p=0.05; Fisher's exact test), but no association was found with chromosomal abnormalities (Table 1). The TAT level was higher than the cut-off in 32/35 MDS patients and in 4/10 controls (91% vs 40%). Its mean plasma levels were higher in patients than controls (3.12 nmol/L vs. 1.94 nmol/L; p=0.020; t-test) (Table 1). TAT did not correlate with ROM levels, nor was it associated with cytogenetic abnormalities, but elevated TAT was associated with hyperhomocysteinemia (p=0.009; Fisher's exact test). No association was found between increased ROMs, homocysteine or TAT and FAB, IPSS subtype, white blood cells, hemoglobin, platelets, age, sex or vital status (Figure 1, Table 1 and *data not shown*). In conclusion, we demonstrated a significant increase in direct (ROMs) and indirect (homocysteine) mediators of oxidative damage in patients with MDS. Increased ROMs and homocysteine levels were spread among both the FAB and the IPSS subclasses; therefore their pathogenetic role could be associated with the early phases of the disease. In our patients we observed that elevated ROM concentrations were not associated with an increase in TAT. This may indicate that TAT did not neutralize the potentially harmful ROM oxidation, as already described in chronic alcohol abuse.[9] This condition may damage tissues and DNA, consistent with our observation that increased ROMs were associated with cytogenetic lesions. Our results are in agreement with the reported increase in DNA oxidation of MDS stem cells.[7] On the other hand, hyperhomocysteine was associated with increased TAT, but not with cytogenetic lesions: TAT, therefore, seemed to neutralize hyperhomocysteinemia-mediated oxidation, preventing DNA damage. In conclusion, serum ROM levels may represent an indicator of potentially harmful oxidation. A simple and reliable *in vitro* method can be applied promptly to study oxidative stress in the heterogeneous field of deregulated hematopoiesis, such as MDS. We are currently investigating the role of monitoring ROM levels as a possible additional prognostic factor in MDS patients.

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References

1. Rikans LE, Hornbrook KR. Lipid peroxidation, antioxidant protection and aging. Biochim Biophys Acta 1997; 1362: 116-21.
2. Toyokuni F. Reactive oxygen species-induced molecular damage and its application in pathology. Pathol Int 1999; 49:91-7.
3. Welch G, Loscalzo J. Homocysteine and atherothrombosis. N Eng J Med 1998; 338:1042-50.
4. Tauro S, Hepburn M D, Bowen DT, Pippard MJ. Assessment of stromal function, and its potential contribution to deregulation of hematopoiesis in the myelodysplastic syndromes. Haematologica 2001;86:1038-45.
5. Cortelezzi A, Fracchiolla NS, Bamonti-Catena F, Motta M, Cighetti G, Carrabba M, et al. High prevalence of hyperhomocysteinemia in myelodysplastic syndromes: specific association with autoimmunity and cardiovascular disease. Leuk Lymphoma 2001;41:147-50.
6. Bowen D, Peddie C. Mitochondrial oxygen consumption and ineffective haematopoiesis in patients with myelodysplastic syndromes. Br J Haematol 2002;118:345-6.
7. Peddie CM, Wolf CR, McLellan LI, Collins AR, Bowen DT. Oxidative DNA damage in CD34+ myelodysplastic cells is

associated with intracellular redox changes and elevated plasma tumor necrosis factor-α concentration. Br J Haematol 1997;99:625-31.
8. G, Finazzi S, Maiolo AT. Folate measurement in patients on regular hemodialysis treatment. Am J Kidney Dis 1999; 33:492-7.
9. Trotti R, Carratelli M, Barbieri M, Micieli G, Bosone D, Rondanelli M, et al. Oxidative stress and a thrombophilic condition in alcoholics without severe liver disease. Haematologica 2001;86:85-91.
10. Lantos J, Roth E, Czopf L, Nemes J, Gal I. Monitoring of plasma total antioxidant status in different diseases. Acta Chir Hung 1997;36:188-9.

Thalidomide before autologous stem cell transplantation in VAD-refractory multiple myeloma patients

We used thalidomide to treat 10 patients with advanced stage multiple myeloma who had failed to obtain at least a partial response after a VAD-based induction therapy. Seven out 10 cases achieved clinical and histologic responses and proceeded to collection of peripheral blood stem cells and transplantation and ASCT.

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Sensitivity of multiple myeloma (MM) to induction chemotherapy has been recognized as a powerful prognostic factor of a favorable outcome after high-dose therapy.[1-3] Recent papers have underlined the importance of a rapid response to induction therapy in candidates for autologous stem cell transplantation (ASCT), since those who had a M-component which halved in <0.5 months[4] or after the first 2 cycles of VAD[5] were more likely to achieve a complete response after ASCT. Moreover, patients who had a refractory disease after conventional therapy could fail to mobilize a sufficient number of peripheral blood stem cells (PBSC), due to the persistence of a massive bone marrow plasma cell (BMPC) infiltration.

For these reasons, we considered patients with a less than 50% decline of the M-component and the persistence of a massive BMPC infiltration after conventional chemotherapy as poor candidates for ASCT and treated them with thalidomide with the aim of reducing the BMPC infiltration before stem cell collection and of leading them to ASCT with the minimum amount of disease.

The patients' clinical characteristics and induction therapy are described in Table 1. Thalidomide was started at 100 mg daily and escalated up to a maximum of 400 mg daily.

PBSC were collected after 7 g/m^2 cyclophosphamide plus granulocyte colony-stimulating factor. The conditioning regimen consisted of 12 mg/kg busulfan and 120 mg/m^{2}melphalan. Responses were evaluated with EBMT, IBMT and ABMTR criteria. After thalidomide administration, 7 patients demonstrated a partial response, whereas the other 3 cases presented refractory or progressive disease (Table 2). An initial reduction of M-component that exceeded 25% was evident by at least 8 weeks, but the maximum decrease of M-component of 60% (median value, with range 57-70%) was reached after a median of 16 weeks of thalidomide therapy (range 8-28). In all the 7 responsive patients, bone pain disappeared, $\beta_2$microglobulin serum concentrations dropped, hemoglobin levels increased and 2 transfusion-dependent patients no longer needed any more packed red cell support. Simultaneously, BMPC infiltration markedly decreased, with a median reduction of 84% (range 40-95%) from pre-treatment values and all the responsive patients

Table 1. Clinical characteristics of the patients.

Patient no. (age/sex)	*Ig isotype*	*Stage*	*Therapy prior to thalidomide*	*Median dose of thalidomide*	*Response*	*Outcome*
1 (60/M)	IgGλ	III A	VAD×4, MP×3	400 mg	partial 3 months after ASCT	Continuing response
2 (61/M)	IgAκ	III A	VAD×4, CTX×3	400 mg	partial 4 months after ASCT	Continuing response
3(61/M)	IgAλ	III A	VAD×4, MP×4	400 mg	partial 5 months after ASCT	Continuing response
4 (59/F)	IgGκ	III A	VAD×4	400 mg	partial 8 months after ASCT	Continuing response
5 (49/M)	IgGκ	III A	VAD×4	200 mg	partial 9 months after ASCT	Continuing response
6 (62/M)	IgGκ	II A	VADx4	400 mg	partial 8 months after ASCT	Continuing response
7 (46/M)	IgGκ	III A	VAD×4	300 mg	partial 3 months after ASCT	Continuing response
8 (44/M)	IgGκ	III B	VAD×4	400 mg	progression TMO	Sibling allogeneic
9 (50/F)	Bence Jones	IIIA	VAD×4	400 mg	progression	Waiting for MUD TMO
10 (59/F)	Bence Jones	IIIA	VAD×4	400 mg	no response	Waiting for sibling allogeneic TMO

M:male, F:female, VAD: vincristine, doxorubicin, dexamethasone, MP:melphalan, prednisone, CTX: cyclophosphamide, MUD: marrow unrelated donor, thal:thalidomide.