Funding: this work was supported by the Leukaemia Research Fund of the United Kingdom.

Correspondence: Carrie Fidler, Ph D, Leukaemia Research Fund Molecular Haematology Unit, Nuffield Department of Clinical Laboratory Sciences, John Radcliffe Hospital, Oxford OX3 9DU. E-mail: carrie.fidler@ndcls.ox.ac.uk

References

1. Fenaux P. Chromosome and molecular abnormalities in myelodysplastic syndromes. Int J Hematol 2001;73:429-37.
2. Paquette RL, Landaw EM, Pierre RV, Kahan J, Lubbert M, Lazcano O, et al. N-ras mutations are associated with poor prognosis and increased risk of leukaemia in myelodysplastic syndrome. Blood 1993; 82:590-9.
3. Padua RA, Guinn BA, Al-Sabah AI, Smith M, Taylor C, Pettersson T, et al. RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia 1998;12: 887-92.
4. Kita-Sasai Y, Horiike S, Misawa S, Kaneko H, Kobayashi M, Nakao M, et al. International prognostic scoring system and TP53 mutations are independent prognostic indicators for patients with myelodysplastic syndrome. Br J Haematol 2001; 115:309-12.
5. Horiike S, Yokota S, Nakao M, Iwai T, Sasai Y, Kaneko H, et al. Tandem duplications of the FLT3 receptor gene are associated with leukemic transformation of myelodysplasia. Leukemia 1997;11:1442-6.
6. Nakano Y, Kiyoi H, Miyawaki H, Asou N, Ohno R, Saito H, et al. Molecular evolution of acute myeloid leukaemia in relapse: unstable N-ras and FLT3 genes compared with p53 gene. Br J Haematol 1999;104:659-64.
7. Christiansen DH, Andersen MK, Pedersen-Bjergaard J. Mutations with loss of heterozygosity of p53 are common in therapy-related myelodysplasia and acute myeloid leukemia after exposure to alkylating agents and significantly associated with deletion or loss of 5q, a complex karyotype, and a poor prognosis. J Clin Oncol 2001;19:1405-13.
8. Castro PD, Liang JC, Nagarajan L. Deletions of chromosome 5q13.3 and 17p loci cooperate in myeloid neoplasms. Blood 2000;95:2138-43.
9. Abu-Duhier FM, Goodeve AC, Wilson GA, Peake IR, Reilly JT. c-FMS mutational analysis in acute myeloid leukaemia. Br J Haematol 2003;123:749-50.
10. Crescenzi B, La Starza R, Romoli S, Beacci D, Matteucci C, Barba G, et al. Submicroscopic deletions in 5q- associated malignancies. Haematologica 2004;89:413-6.

Myelodysplastic Syndromes

Prognostic factors in myelodysplastic and myeloproliferative types of chronic myelomonocytic leukemia: a retrospective analysis of 83 patients from a single institution

We analyzed independent prognostic factors associated with survival and risk of evolution to acute leukemia in our series of 83 patients with previously untreated chronic myelomonocytic leukemia (CMML), with the aim of testing the validity of the stratification based on white blood cell (WBC) count, in myeloproliferative and myelodysplastic types of the revisited WHO classification.

haematologica 2004; 89:866-868
(*http://www.haematologica.org/2004/7/866*)

From 1989 to 2001, 643 adults were diagnosed with myelodysplastic syndromes (MDS) at our Institution; 83 (12.4%) of these had primary CMML. According to the limit of WBC count of 13×10^9/L, 46 had a myelodysplastic (MD) type and 37 a myeloproliferative (MP) type. The median follow-up of patients was 38 months. Bone marrow (BM) dysplasia was assessed according to previously established FAB criteria.[1] Cytogenetic studies were available for 48 patients. Different prognostic scores (Bournemouth, modified Bournemouth, Spanish, IPSS, Gonzalez-Medina score, Dusseldorf score, MDAPS)[2-4] were evaluated for risk distribution and relative impact on survival prediction. Clinical and hematologic features, tested for survival and rate of transformation to acute myeloid leukemia (AML), were compared by the χ^2 and Wilcoxon rank sum tests. Univariate analysis was estimated using Cox regression models. p values <0.05 were regarded as statistically significant in two tailed tests. SPSS software (version 10.00, SPSS, Chicago, USA) was used for statistical analyses. Significant independent variables were used to develop a multivariate model by the Cox method in order to identify independent prognostic relationships. The differences between the MD and MP groups were sex ratio, WBC, monocyte, platelet and lymphocyte count and hemoglobin level.

Trilineage dysplasia was evident in 35 MD (76%) and in 17 MP patients (46%) (p=0.005). A normal karyotype was observed in 38 patients (80%) while in 10 patients a +8 (4 MD and 1 MP patients), a del (13) (2 patients), a del (16) (2 patients), a -7 (1 patient) were identified.

Median survival was 20 and 17.4 months for MD and MP patients, respectively (p=0.007). The disease progression rate was higher for the MP type (29.7%) than for the MD type (15.2%, p=0.001). The median duration of the preacute leukemic phase was 16 and 14 months in the MP and MD variants, respectively (p=NS). At the time of our analysis, 12 patients with MD type (26%) and 19 with MP type (51%) had died (p=0.005). Death was related to AML progression (45%) or hemorrhagic (19%) and/or septic complications of BM failure (19%). Other causes of death were heart failure in 7 old patients receiving marked transfusional support (12%), liver failure (3%), and second neoplasia (2%). Survival after evolution into AML was 1.5 and 2 months for patients with MP and MD variants, respectively (p=NS). Twenty-one MP patients received hydroxyurea to control leukocytosis and 5 patients whose disease evolved into AML received intensive induction treatment. Transfusion requirements were higher in MP patients than in MD patients (59% vs 26%; p=0.003); the frequency of infections and hemorrhages was not significantly different in the two groups (p=NS). Only 6/14 patients with hemorrhages required platelet transfusions.

Sex, WBC > 13×10^9/L, lymphocytes >2.5×10^9/L, platelets < 100×10^9/L, and trilineage dysplasia were individually associated with shorter survival (Table 1). These parameters were entered in a multivariate analysis, but only trilineage dysplasia had independent prognostic value for survival (p=0.02).

BM blasts >5%, WBC count, neutrophils and lymphocytes >2.5×10^9/L, and presence of peripheral blood blasts, associated with AML progression in univariate analyses, were tested in a multivariate analysis but only the lymphocyte count had an independent prognostic value (p=0.01).

All scoring systems applied, except IPSS, stratified

Table 1. Univariate analysis of prognostic factors for survival and incidence of transformation to AML.

Variable	*N° pat*	*ms*	*p*
Sex			
M	54	25	0.05
F	29	13	
Age (years)			
< 65	21	21	N.S
> 65	62	18	
WBC			
> 13×10⁹/L	37	17	0.01
< 13×10⁹/L	46	20	
Neutrophil count			
> 2.5×10⁹/L	39	16	N.S
< 2.5×10⁹/L	44	28	
Lynphocyte count			
> 2.5×10⁹/L	47	17	0.02
< 2.5×10⁹/L	36	20	
Hb			
< 9 g/dL	15	16	NS
>9 g/dL	68	19	
Plts			
< 100×10⁹/L	33	13	0.04
> 100 ×10⁹/L	50	26	
Peripheral blood blasts			
No	74	19	0.02
Yes	9	9	
Monocytes			
< 2×10⁹/L	41	20	NS
> 2×10⁹/L	42	16	
IPC			
0%	52	19	
> 0%	31	19	NS
LDH			
Normal	23	20	NS
Elevated	60	19	
Trilinege BM involvement			
No	31	22	0.03
Yes	52	15	
BM blasts			
< 5%	32	26	0.01
> 5%	51	16	

Table 2. Survival of CMML patients according to risk distribution based on various scoring systems.

Score system	*CMML*			*MD*			*MP*		
	%	*ms*	*p*	*%*	*ms*	*p*	*%*	*ms*	*p*
Bournemouth									
Low	52	23		58	28		43	18	
Intermediate	42	13.5	0.06	39	13	0.07	45	14	NS
High	6	4		3	5		12	4	
Mod. Boumemouth									
Low	53	30	0.02	60	32	0.05	43	28	0.05
High	47	11		40	13		57	9	
Spanish									
Low	25	37		21	42		30	32	
Intermediate	64	19	0.04	66	17	0.04	59	21	0.05
High	11	6		13	7		11	5	
IPSS									
Low				67	30				
Intermediate- 1				9	15	NS			
Intermediate- 2				17	13				
High				7	3				
MDAPS									
Low	46	20		72	20		13	21	
Intermediate- 1	30	18	NS	19	17	NS	43	19	NS
Intermediate- 2	18	17		9	24		30	10	
High	5	17		–			14	17	
Gonzales-Medina									
Low	54	22	NS	67	28	0.05	38	17	NS
High	46	17		33	15		62	18	
Düsseldorf score									
Group A	29	30		11	47		8	12	
Group B	46	19	NS	78	18	0.03	62	21	NS
Group C	19	12		11	12		30	13	

patients into distinct categories within the MD variant, but only the Spanish and modified Bournemouth scores identified categories within MP patients (Table 2). Low risk patients with a good prognosis (median survival 29 months) were identified in all systems. The MDAP score in our series was not able to stratify patients, despite the lymphocyte count being significant in our multivariate analysis.

By applying WHO criteria[5] to our series, 55 patients had CMML1 (median WBC 10.7×10⁹/L, AML transformation 21.8%) and 28 patients had CMML 2 (median WBC 12×10⁹/L, AML transformation 17.8%). Univariate analysis showed no significant differences between the two groups.

The utility of subdividing CMML patients into those with MD and MP types is still an open issue and discordant conclusions have been reached regarding the clinical and/or prognostic significance.[6-9] The MDAP score did not find some significant features, but when applied to patients of the Dusseldorf registry and also to our series, failed to identify low risk patients.[10] Our study supports the idea that separating CMML patients into MD and MP groups may identify two distinct categories of patients with different survivals and leukemic transformation rates. However, it remains to be clarified whether the MD and MP forms of CMML represent distinct clinical-biological entities or rather are different stages or expression of the same disease. Molecular investigations are thus warranted to identify specific markers capable of revealing the nature of the disease while suggesting new, possibly targeted, therapeutic strategies.

Massimo Breccia, Roberto Latagliata, Andrea Mengarelli, Francesca Biondo, Franco Mandelli, Giuliana Alimena

Dipartimento di Biotecnologie Cellulari ed Ematologia, University La Sapienza, Rome, Italy

Key words: chronic myelomonocytic leukemia, myeloproliferative type, myelodysplastic type.

Funding: ROMAIL.

Correspondence: Massimo Breccia, Dipartimento di Biotecnologie Cellulari ed Ematologia, La Sapienza University, Rome, Italy. Phone: international +39.06.857951. Fax: international +39.06.44241984. E-mail: breccia@bce.uniroma1.it

References

1. Bennett JM, Catovsky D, Daniel MT, Flandrin G, Galton DA, Gralnick HR, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189-99.
2. Sanz GF, Sanz MA, Greenberg PL. Prognostic factors and scoring systems in myelodysplastic syndromes. Haematologica 1998;83:358-68.
3. Aul C, Giagounidis A, Germing U, Ganser A. Evaluating the prognosis of patients with myelodysplastic syndromes. Ann Haematol 2002;81:485-97.
4. Onida F, Kantarjian HM, Smith TL, Ball G, Keating MJ, Estey EH,, et al. Prognostic factors and scoring systems in chronic myelomonocytic leukemia: a retrospective analysis of 213 patients. Blood 2002; 99:840-9.
5. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, et al. World Health Organization Classification of neoplastic diseases of the hemopoietic and lymphoid tissues: report of the clinical advisory committee meeting. J Clin Oncol 1999; 17: 3835-49.
6. Gonzàlez-Medina I, Bueno J, Torrequebrada A, Lopez A, Vallespì T, Massagué I. Two groups of chronic myelomonocytic leucemia: myelodysplastic and myeloproliferative. Prognostic implications in a series of a single center. Leuk Research 2002;26:821-4.
7. Germing U, Gattermann N, Minning H, Heyll A, Aul C. Problems in the classification of CMML-dysplastic versus proliferative type. Leuk Research 1998;22:871-8.
8. Noesslinger T, Reisner R, Gruner H, Tuchler H, Nowotny H, Pittermann E. Dysplastic versus proliferative CMML. A retrospective analysis of 91 patients from a single institution. Leuk Research 2001;25:741-7.
9. Voglovà J, Chrobak L, Neuwirtovà R, Malaskovà V, Straka L. Myelodysplastic and myeloproliferative type of chronic myelomonocytic leukaemia: distinct subgroups or two stages of the same disease? Leuk Res 2001;25:493-9.
10. Germing U, Strupp C, Aivado M, Gattermann N. New prognostic parameters for CMML? Blood 2002;100:731-2.

Chronic Myeloid Leukemia

TP53 codon 72 polymorphism in patients with chronic myeloid leukemia

A single nucleotide polymorphism at TP53 codon 72 means that two alleles exist: A1 (proline residue, Pro72) and A2 (arginine residue, Arg72). The Pro72 variant of p53 has a lower apoptotic potential. We found that allele A1 was more frequent in patients with chronic myeloid leukemia (CML) than in controls, and among CML patients who had no cytogenetic response than among responders.

haematologica 2004; 89:868-869
(*http://www.haematologica.org/2004/7/868)*

Treatment of CML has recently been revolutionized by the introduction of imatinib mesylate, an inhibitor of the *ABL* tyrosine kinase domain.[1] Acquired resistance to imatinib, defined as a relapse following an initial response, occurs and is often characterized by either *BCR-ABL* gene amplification or point mutations in the kinase domain of the protein. These latter cause amino-acid substitutions and prevent imatinib-induced inhibition of $p210^{BCR/ABL}$.[2] Some CML patients fail to obtain a cytogenetic response to initial treatment with imatinib, a condition that is sometimes described as refractoriness or primary resistance to imatinib and whose mechanisms are unknown.[3]

The *TP53* gene, which encodes the p53 tumor-suppressor protein, is characterized by a single nucleotide polymorphism at codon 72, which results in either proline (Pro72, allele A1) or arginine (Arg72, allele A2) at amino-acid position 72. The Pro72 variant of the p53 protein has a markedly reduced capacity to induce apoptosis.[4]

In a study aimed at identifying factors potentially related to imatinib resistance in CML, we analyzed *TP53* codon 72 polymorphism in CML patients. Peripheral blood samples were obtained from 44 patients with chronic phase CML undergoing routine hematologic evaluation at the Division of Hematology, University of Pavia Medical School and IRCCS Policlinico San Matteo, Pavia, Italy, before starting treatment with imatinib. All these 44 CML patients were treated with imatinib mesylate for at least six months before being classified as either responsive or unresponsive to imatinib (Table 1). Further blood samples were taken from the 10 patients who failed to show a cytogenetic response to therapy, as defined by Druker and co-workers,[1] following their diagnosis of cytogenetic refractoriness. In order to study a large number of CML patients, archive samples from a further 52 individuals in chronic phase who had been followed at our Institution in the past were also analyzed. To determine the prevalence of *TP53* A1 and A2 alleles in a population of similar ethnic composition, 174 umbilical cord blood samples were examined.

RNA samples from patients with cytogenetic resistance to imatinib were analyzed for the presence of mutations in the tyrosine kinase domain encoding region of *BCR-ABL* by means of reverse transcription polymerase chain reaction (RT-PCR) and sequencing of PCR products. Codon 72 polymorphism of *TP53* was analyzed by PCR of genomic DNA and subsequent digestion with the restriction enzyme BstUI.[5]

Table 1. Features of CML patients treated with imatinib classified according to cytogenetic response to treatment

Characteristic	*Cytogenetic responders (n=34)*	*Non responders (n=10)*	*Significance*
Age			
Median, *yr.*	46	54	NS
Range, *yr.*	24-70	29-71	
Sex			
Female, no.	12	5	NS
Male, no.	22	5	
Time since diagnosis			
Median, mo.	6	15	NS.
Range, mo.	1-120	1-156	
Sokal risk group			
High, no.	2	4	*P*=0.012
Intermediate, no.	10	0	
Low, no.	14	4	
Data missing, no.	8	2	
BCR/ABL mutations, no.	ND	0	

ND: not determined; NS: not significant.