Prognostic significance of reproducible immunophenotypic markers of marrow dysplasia

The pathological hallmark of myelodysplastic syndromes (MDS) is marrow dysplasia, which represents the basis of the WHO classification of these disorders.[1] The WHO proposal has raised some concern regarding minimal morphological criteria for formulating the diagnosis of MDS, since morphological abnormalities are also present in patients affected with non-clonal cytopenia.

Several studies have evaluated flow cytometry as a potential diagnostic tool to improve the accuracy of the evaluation of marrow dysplasia.[2,3] Despite a high sensitivity reported by different studies, there is still no consensus as to which diagnostic parameters are the most appropriate, and published protocols are mainly based on a qualitative analysis of cytometric variables, thus limiting widespread clinical implementation.[4] We recently designed a flow cytometric protocol that is widely applicable and verified its diagnostic utility in patients with low-grade MDS.[5] The cardinal parameters are: i) the percentage of $CD34^+$ myeloblasts in all nucleated cells; ii) the percentage of $CD34^+$ B-progenitor-related cells in all $CD34^+$ cells; iii) lymphocyte to myeloblast CD45 ratio (mean fluorescence intensity [MFI] of CD45 on lymphocytes ÷ MFI of CD45 on $CD34^+$ myeloblasts); and iv) granulocyte to lymphocyte SSC peak channel ratio (SSC channel number where the maximum number of $CD10^-$ granulocytic cells occurs ÷ SSC channel number where the maximum number of lymphocytes occurs).[5] These parameters are reproducible in many laboratories when measured by methods ensuring little inter-operator variability, and when combined into a flow cytometric score (FCM-score) are able to differentiate correctly patients with MDS from those with non-clonal cytopenia.[6] The FCM-score may represent a basis to design cytometric protocols for the diagnostic workup of low-grade MDS patients.[7]

In addition to its diagnostic value, the evaluation of the amount of marrow dysplasia in MDS has important prognostic implications and affects the probability of response to disease-modifying treatments.[8] In this multicentric study, we aimed to evaluate the prognostic effect of FCM-score in a cohort of low-grade MDS. The procedures followed were in accordance with the ethical standards of the Institutional Committee on Human Experimentation and the Declaration of Helsinki.

We studied 258 patients from Italy and Japan affected with refractory cytopenia with unilineage dysplasia (n=72, 28%), refractory cytopenia with multilineage dysplasia (n=157, 61%), sideroblastic anemia (n=21, 8%), and MDS with del5q (n=8, 3%). Median age was 71 years (range 27-94). Patients were stratified by the Revised International Prognostic Scoring System (IPSS-R).[9] Accordingly, 25 subjects (10%) had very low risk, 100 (40%) had low risk, 93 (37%) had intermediate risk, 31 (13%) had high risk and 4 (2%) had very high risk. The majority of patients received supportive care or erythroid stimulating agents. A significant difference between the Italian and Japanese cohort was found in age (median age 68 *vs*. 75 years, respectively; $P<0.001$). Moreover, a higher prevalence of MDS with multilineage dysplasia was found in the Italian cohort ($P<0.001$). After adjusting for demographic factors, no significant difference was found in survival between Japanese and Italian patient populations ($P=0.12$).

Table 1. Clinical correlates of FCM-score.

Clinical variable	FCM-Score 0	1	2	3	4	*P*
Marrow dysplasia						
Unilineage	28 (65%)	24 (59%)	41 (44%)	6 (10%)	2 (11%)	<0.001
Multilineage	15 (34%)	17 (41%)	52 (56%)	57 (90%)	16 (89%)	
Number of cytopenias*						
0-1	16 (39%)	20 (49%)	43 (46%)	19 (30%)	4 (22%)	0.026
2-3	25 (61%)	21 (51%)	50 (53%)	44 (70%)	14 (78%)	
Transfusion-dependency**						
Yes	10 (25%)	14 (34%)	33 (37%)	41 (66%)	14 (78%)	<0.001
No	30 (75%)	27 (66%)	56 (61%)	21 (44%)	4 (22%)	
Cytogenetics by MCSS***						
Very good risk	4 (10%)	–	2 (2%)	–	–	<0.001
Good risk	31 (74%)	31 (82%)	63 (68%)	33 (53%)	7 (39%)	
Intermediate risk	6 (14%)	4 (11%)	13 (14%)	21 (34%)	7 (39%)	
Poor risk	1 (2%)	2 (5%)	13 (14%)	7 (11%)	4 (22%)	
Very poor risk	–	1 (2%)	2 (2%)	1 (2%)	–	
IPSS-R risk						<0.001
Very low	7 (17%)	5 (13%)	13 (14%)	–	–	
Low	19 (45%)	21 (55%)	51 (55%)	8 (13%)	1 (6%)	
Intermediate	15 (36%)	9 (24%)	18 (19%)	42 (68%)	9 (50%)	
High	1 (2%)	2 (5%)	10 (11%)	12 (19%)	6 (22%)	
Very high	–	1 (3%)	1 (1%)	–	2 (11%)	
Evolution into acute leukemia						
Yes	1 (2%)	4 (10%)	12 (13%)	19 (30%)	6 (33%)	<0.001
No	42 (98%)	37(90%)	81 (87%)	44 (70%)	12 (67%)	

According to IPSS criteria; **According to WPSS criteria; * MDS Cytogenetic Scoring System.*

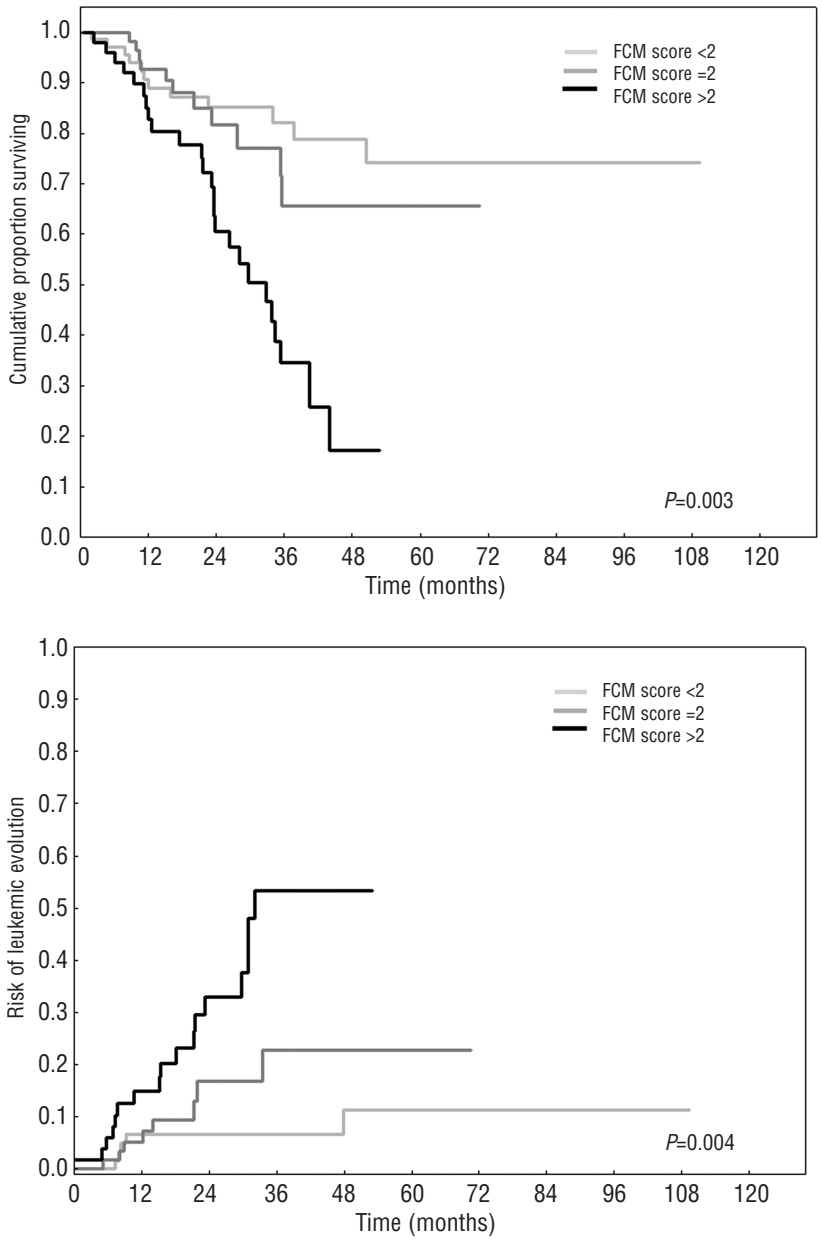

Figure 1. (A) Overall survival and (B) risk of leukemic evolution of low-grade MDS patients stratified according to FCM-score. Five-year overall survival (OS) was 74% in patients with FCM-score <2, 65% in patients with FCM-score of 2 and 17% in patients with FCM-score >2 (*P*=0.003). Five-year risk of leukemic evolution was 11%, 22% and 53%, respectively (*P*=0.004) (B).

In all patients, we examined the four cardinal parameters analyzed from a marrow cell sample stained with the CD10/CD34/CD45 antibody combination. Analytical methods have been described previously.[5] FCM-score was calculated by assigning a value of 1 to each abnormal parameter with respect to reference range defined in control patients affected with non-clonal cytopenia.[6]

FCM-score value was 0 in 43 patients (17%), 1 in 41 patients (16%), 2 in 93 patients (36%), 3 in 63 patients (24%), and 4 in 18 patients (7%). In patients stratified according to WHO criteria, subjects affected with refractory cytopenia with multilineage dysplasia presented higher FCM scores with respect to those with refractory or sideroblastic anemia (*P*=0.001). FCM-score over 2 was significantly associated with multilineage dysplasia (*P*<0.001), severe cytopenias (*P*=0.04), transfusion-dependency (*P*<0.001) and unfavorable cytogenetics according to the MDS Cytogenetic Scoring System[9] (*P*<0.001), leading to a higher IPSS-R risk (*P*<0.001) (Table 1). Five-year overall survival (OS) was 74% in patients with FCM score under 2, 65% in patients with FCM score of 2, and 17% in patients with FCM score over 2 (*P*=0.003) (Figure 1A). Five-year risk of leukemic evolution was 11%, 22% and 53%, respectively (*P*=0.004) (Figure 1B). The significant effect of FCM score on patient outcome was maintained even when Japanese and Italian patients were analyzed separately (*data not shown*).

There was a significant difference in OS between patients with FCM score over 2 and both those with FCM score of 2 and under 2 (*P*=0.002 and *P*=0.001, respectively), while no significant difference was seen between the two latter groups (*P*=0.89). Patients with FCM score over

2 also showed a significantly higher probability of leukemic evolution (P=0.014 and P<0.001, respectively).

In a multivariable analysis including age, gender and IPSS-R risk as covariates, FCM score showed a significant effect on the probability of overall and leukemia-free survival (HR 1.39, P<0.001 and HR 1.51, P<0.001, respectively). Focusing on MDS stratified according to IPSS-R criteria, FCM score significantly affected survival in patients with very low/low risk (5-year probability of survival 73% *vs*. 39% in patients with FCM score ≤2 *vs.* >2, respectively; P<0.001) and intermediate risk (5-year probability of survival 68% *vs*. 22%, respectively; P=0.03).

Finally, in order to verify whether FCM score could improve the prognostic stratification of MDS patients provided by IPSS-R, we fitted two separate multivariable analyses including age, gender and IPSS-R category as covariates, with and without FCM score, respectively, and compared them by Akaike information criterion (AIC).[10] Among a set of candidate models, a lower AIC value indicates a better trade-off between fit and complexity (a difference of 3 or more indicating a substantial difference in favor of the model with the lowest AIC value). AIC were 358 and 362 for multivariable analyses with and without FCM score, respectively, confirming the importance of considering immunophenotypic data in the prognostic model.

These results indicate that immunophenotyping based on FCM score may provide additional survival information in low-grade MDS stratified according to conventional prognostic systems.[1,8]

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