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Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype

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At diagnosis, approximately half of myelodysplastic (MDS) patients presents a normal karyotype by conventional cytogenetic analysis (CCA). Fluorescent *in situ* hybridization (FISH) is more sensitive than CCA allowing for the detection of minor clones and of submicroscopic lesions. We have analyzed by FISH 101 MDS patients with normal karyotype for the occurrence of the abnormalities which are most frequently observed in MDS (ie −5/5q−, −7/7q−, +8, 17p−). In 18 patients, 15 to 32% of interphase cells were found to carry one FISH abnormality. Six patients presented trisomy 8, five had del(5)(q31), five del(7)(q31), one monosomy 7 and one del(17)(p13). FISH abnormalities were more frequently observed among patients with an increased percentage of bone marrow blasts (P = 0.001). FISH abnormalities were also associated with a higher rate of progression into AML (13/18 *vs* 12/83, $P < 0.001$) and were predictive for a worse prognosis ($P < 0.001$). Multivariate analysis indicated that FISH positivity and IPSS risk group were independent predictors for a poor survival (P = 0.0057 and 0.0123, respectively) and for leukemic transformation (P = 0.0006 and 0.035, respectively). Leukemic transformation in FISH-positive patients was associated in all cases with an expansion of the abnormal clone. Our data demonstrated that a significant proportion of MDS patients with normal karyotype presented, if analyzed by FISH, clones of cytogenetically abnormal cells which played a determinant role in the progression of the disease. The presence of FISH abnormalities identified a group of MDS patients with normal karyotype characterized by an inferior prognosis. *Leukemia* (2001) **15,** 1841–1847.
Keywords: myelodysplastic syndromes; normal karyotype; FISH analysis; occult chromosome lesions

Introduction

The biologic and clinical relevance of cytogenetic analysis in myelodysplastic syndromes (MDS) is an established acquisition[1–3] and karyotype has been identified by the International Prognostic Scoring System (IPSS) as one of the three variables for the definition of prognosis in MDS.[4] At diagnosis, 40 to 65% of MDS patients present normal karyotypes when assessed by conventional cytogenetic analysis (CCA)[1–8] and these patients are therefore classified, according to IPSS criteria, in the low-risk cytogenetic group.

However, MDS patients with normal karyotype appear quite heterogeneous from a biologic point of view and the outcome in this group of patients may be unpredictable.[5,8] For these reasons, further studies are warranted in order to define biological variables allowing for the identification of MDS patients with normal karyotypes who are at high risk of progression.

In comparison to CCA, fluorescent *in situ* hybridization (FISH) analysis is a more sensitive technique, which allows for the study of non-dividing cells, for the identification of minor abnormal clones and of small chromosome deletions not detectable by CCA.[9]

The goals of this study were: (1) to evaluate by interphase FISH analysis the prevalence of the most frequent cytogenetic abnormalities in a group of MDS patients with normal karyotype; (2) to evaluate, in this subset of MDS patients, the clinical and prognostic significance of the presence of FISH abnormalities.

Materials and methods

Patients

Between January 1990 and December 1999 101 unselected patients with the diagnosis of MDS and normal karyotype were seen at the Section of Hematology of the University of Ferrara.

These patients were derived from a series of 210 consecutive MDS patients and in part have already been presented in previous reports.[5,10,11] All patients were classified in accordance with French–American–British (FAB) group criteria.[12] Patients who had previously received chemotherapy or those with secondary MDS were not included in this analysis. Bone marrow (BM) aspirate was performed in all cases at diagnosis and during the course of the disease and dysplastic features were recognized as previously indicated.[13,14] Patients were reviewed at 1–4 month intervals, depending on their clinical conditions. The patients were treated according to the guidelines in use at our institution during the study period: most patients received supportive treatment while 18 patients were treated with different chemotherapy protocols to control BM failure, hyperleukocytosis and disease progression.

Cytogenetic analysis

Cytogenetic analysis was conducted at the time of diagnosis and at disease evolution by personnel unaware of the clinical data. Cytogenetic studies, using standard techniques, were performed as part of the diagnostic work up.[5] Whenever possible, at least 20 metaphases were analyzed in all patients with normal karyotype. Clonal abnormalities were defined as two or more cells with the same additional whole chromosome or chromosome rearrangements or three or more cells with the same chromosome missing. Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature.[15]

FISH analysis

Interphase FISH analysis: Interphase FISH analysis was performed on the same preparations that were used for cyto-

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genetic investigations at diagnosis and, whenever possible, at AML evolution. The following commercial probes (Vysis, Downers Grove, IL, USA) were used: a probe recognizing sequences at the 5q31 band (conjugated with Spectrum green fluorophore) used simultaneously in dual-color experiments with a chromosome-5-centromeric probe (conjugated with Spectrum orange fluorophore); a chromosome-7-centromeric probe (conjugated with Spectrum green fluorophore) and a probe recognizing sequences at the 7q31 band (conjugated with Spectrum orange fluorophore), a chromosome-8-centromeric probe (conjugated with Spectrum green fluorophore), and a probe recognizing sequences at the 17p13 band (conjugated with Spectrum orange fluorophore). Bone marrow specimens were first incubated for 60 min with RNAase (200 μg/ml; Boehringer Mannheim, Mannheim Germany) at 37°C in a moist chamber, then washed twice in 4 × SSC for 5 min and for 3 min in 1 × PBS at room temperature (RT). Subsequently the specimens were incubated for 20 min in a coplin jar at 37°C with pepsin solution (20 mg pepsin/100 ml 0.01 N HCl) and washed with PBS without intermittent agitation at RT. The slides were then incubated in a coplin jar at RT with 1% formaldehyde (acid-free) in PBS supplemented with 100 mM $MgCl_2$ for 10 min, washed in PBS, dehydrated in ethanol alcohol series (70%, 80%, 90%, 100%) and air dried. The slides were subsequently pre-warmed on a hot plate and then immersed in a 70% formamide 2 × SSC solution at 72°C for 5 min and dehydrated again with ice alcohol series. Fifteen μl of each probe were then added and the slides covered with a cover-lip. Rubber cement was used to seal the edges. The slides were then incubated overnight at 37°C, in a humified chamber. Post-hybridization washes included 50% formamide 2 × SSC for 15 min, 1 × SSC for 10 min, 0.1 × SSC for 5 min baths at 45°C, without intermittent agitation. No antifade solution was applied on the slides. Evaluation of FISH results was performed on a Nikon fluorescence-equipped microscope with couple charged camera device and appropriate hardware and software (Cytovision System, Applied Imaging, distributed by Nikon Italia, Florence, Italy). Signal screening was performed in those slides with high hybridization efficiency as documented by the presence of more than 80% cells with two expected control signals. At least 200 nuclei were counted in each patient; based on the results using these probes in three normal controls, the cut-off point for the identification of trisomy and monosomy/deletion was set at >3% cells with three signals and >5% cells with one signal, respectively.

Metaphase FISH analysis: In those patients with cryptic chromosome deletions (ie a chromosome deletion detected by FISH and not by CCA) a minimum of 10 metaphases was analyzed by FISH at diagnosis and, when material was available, at leukemic transformation, to sort out whether the failure of CCA to detect these chromosome lesions derived from a low mitotic index of the abnormal clone or whether it derived from the sub-microscopic size of the deletion.

Statistical analysis

t-test for paired samples was applied in the comparison of continuous variables while chi-square test was applied in the comparison of categorical variables. Patient survival was estimated by the Kaplan–Meier method from the date of diagnosis until death due to any cause or until the last patient follow-up. The survival curves were statistically compared by the log-rank test. Proportional hazard regression analysis and logistic regression analysis were used to identify the most significant independent prognostic variables on survival or AML progression, respectively. P values <0.05 were considered statistically significant. Data was analyzed in May 2000.

Results

Patient characteristics

One hundred and one consecutive MDS patients with normal karyotype were evaluated in this study. They represent the 48.1% of a series of 210 MDS patients. Patients characteristics at presentation are summarized in Table 1. The median age was 65.1 years (range 22–83) and the male female ratio was 56/45. Of the 101 patients, 46 were refractory anemia (RA), six RA with ringed sideroblasts (RARS), 34 RA with excess of blasts (RAEB), seven RAEB in transformation (RAEB-t), eight chronic myelomonocytic leukemia (CMML). According to the IPSS criteria 29 patients were classified in the low risk group, 57 in the intermediate-1 group, 11 in the intermediate-2 and four in the high risk group. Twenty-five patients progressed to AML. The median survival of this group of patient was 57 months.

FISH analysis

Interphase FISH: Interphase FISH abnormalities were detected in 18 patients with normal karyotype by CCA in 20–25 metaphases (17 cases) and in 18 metaphases (one case, No 4). Trisomy 8 was found in six patients in 17–32% of the cells, a 5q31 deletion in five patients in 16–32% of the cells, a 7q31 deletion in five cases in 18–24% of the cells. Monosomy 7 in 15% of the cells, and a 17p13 deletion in 18% of the cells were observed in one patient each. The results of FISH analysis and the clinical data of the patients with FISH abnormalities are summarized in Tables 2, 3 and 4.

FISH abnormalities were more frequently observed among patients with an increased percentage of bone marrow blasts ($P = 0.001$) while no differences were found between FISH-positive and FISH-negative patients as far as cytopenias are concerned. FISH positivity correlated with FAB diagnosis ($P = 0.001$) and IPSS risk group (0.004). A trend for a higher num-

Table 1 Clinical parameters at presentation in 101 MDS patients with normal karyotype

Variable	*No. of patients*
Age (yrs): ⩽65/>65	48/53
Sex: M/F	56/45
Hb (g/dl): <10.0/⩾10.0	34/67
ANC (× 10^9/l): <1.5/⩾1.5	51/50
Plt (× 10^9/l): <100/⩾100	34/67
BM blasts (%): <5/5–10/11–20/21–30	56/30/8/7
FAB: RA/RARS/RAEB/REAB-t/CMML	46/6/34/8/7
IPSS: Low/INT-1/INT-2/High	28/58/11/4
AML evolution: Yes/No	25/76
Survival (months)	57

M, male; F, female; Hb, hemoglobin level; ANC, absolute neutrophil count; Plt, platelet count; BM, bone marrow; IPSS, International Prognostic Scoring System.

Table 2 Clinical data at presentation in FISH-positive MDS patients with normal karyotype

No.	*Name*	*FISH anomaly*	*Age (yr)*	*FAB*	*Sex*	*ANC 10^9/l*	*Hb g/dl*	*Plt 10^9/l*	*BM blasts %*	*IPSS*	*Survival months*
1	UV	+8	75	RAEB	M	0.9	12.4	152	7	INT-1	36
2	RG	+8	78	RAEB	F	1.9	9.5	60	15	INT-2	1
3	CG	+8	77	RAEB	M	4.2	9.9	303	7	INT-1	2
4	DMG	+8	82	RAEB-T	F	0.8	11.1	166	25	INT-2	2
5	GV	+8	60	CMML	M	1.5	9.1	174	20	INT-2	20
6	MF	+8	36	RAEB-T	F	3.0	10.9	128	28	INT-2	13
7	LB	–7	73	RAEB	F	1.9	11.0	197	6	INT-1	7
8	BE	17p–	73	RA	M	8.3	8.6	115	2	L0W	6+
9	CU	5q–	67	RAEB	M	2.1	13.2	138	6	INT-1	18
10	MS	5q–	71	RAEB	M	3.5	10.9	196	7	INT-1	28
11	PM	5q–	26	RA	F	0.6	5.0	59	2	INT-1	29+
12	PV	5q–	75	RAEB-T	M	1.0	8.5	102	25	HIGH	17+
13	TB	5q–	67	RAEB	F	2.5	12.4	111	6	INT-1	24+
14	BE	7q–	65	RAEB-T	F	0.2	11.1	54	25	HIGH	34
15	CS	7q–	73	RAEB	M	2.0	9.5	289	10	INT-1	34
16	DMV	7q–	55	RAEB	F	1.1	13.0	190	6	INT-1	16
17	PA	7q–	71	RAEB	M	1.0	11.6	132	20	INT-2	63
18	SF	7q–	60	RA	F	1.1	12.4	227	3	LOW	24+

Table 3 Comparison of clinical parameters in FISH-positive and FISH-negative MDS patients with normal karyotype

Variable	*FISH +*	*FISH –*	*P*
Age (yrs): ⩽65/>65	5/13	43/40	0.06
ANC (× 10^9/l): <1.5/⩾1.5	8/10	43/40	NS
Hb (g/dl): <10/⩾10	7/11	27/56	NS
Plt (× 10^9/l): ⩽100/>100	3/15	31/52	NS
Sex: M/F	9/9	47/36	NS
BM blasts (%): <5/5–20/21–30	4/10/4	52/28/3	0.001
FAB: RA + RARS/RAEB/CMML/RAEB-t	3/10/1/4	49/24/7/3	0.001
IPSS: Low/INT-1/INT-2 + High	2/9/7	26/49/8	0.004
AML progression: Yes/No	13/5	12/71	<0.001

NS, not significant.

ber of older patients (>65 years) was observed among FISH-positive cases ($P = 0.06$).

Patients with FISH abnormalities presented a higher risk of progression to AML than patients without FISH abnormalities (13/5 *vs* 12/71, $P < 0.001$). At the time of AML progression, all 13 FISH-positive cases displayed a bone marrow sample with an increased percentage of cells carrying the same FISH abnormality as detected at diagnosis (Table 4). At diagnosis the mean value of FISH-positive cells was 22.9% *vs* 56.7% of FISH-positive cells at AML progression ($P < 0.001$). In three of these 13 cases (Nos 4, 5, 6) CCA showed, at leukemic progression, an abnormal karyotype which was consistent with FISH results, whereas in two cases (Nos 16, 17) a clonal abnormality apparently unrelated to the FISH anomaly was found. In six cases no karyotype anomaly could be detected at leukemic progression while in the remaining two cases karyotype analysis could not be performed.

Metaphase FISH: Detailed results are shown in Table 4. At diagnosis, trisomy 8 was found in few mitotic figures of too poor quality for karyotyping (fuzzy and overlapping chromosomes) in four out of six patients (Nos 1, 2, 3, 4) with apparently normal karyotype and trisomy 8 by FISH interphase analysis.

No mitotic cell with –7 could be visualized by metaphase FISH in patient No. 7. Metaphase FISH analysis showed the presence of a clone carrying a submicroscopic deletion of chromosome 5q31 (Figure 1) in four out of five cases (Nos 9, 10, 12, 13, 14) with an apparently normal chromosome 5 pair. Likewise, a minor clone with submicroscopic 7q31 deletion in metaphase cells was found by metaphase FISH in three out of five patients with 7q–. The patient with 17p– showed a minority of mitotic cells carrying a small 17p13 deletion not detectable by karyotype analysis.

As shown in Table 4, experiments of metaphase FISH analysis in those patients with deletions analyzed at the time of leukemic transformation confirmed the results obtained in the MDS phase with four patients (Nos 9, 12, 13, 14) carrying a submicroscopic deletion and one patient (No 15) carrying a non-dividing clone. Some mitotic figures with +8 were visualized at leukemic progression in three patients (Nos 1, 4, 5).

Outcome

Univariate analysis results are presented in Table 5. IPSS risk group (Figure 2) and FISH positivity (Figure 3) were significantly associated with a worse outcome. Cox's proportional hazard model confirmed the independent role on survival of IPSS risk group ($P = 0.0057$) and of FISH positivity ($P = 0.0123$). Logistic regression analysis demonstrated that FISH positivity ($P = 0.0006$) and IPSS risk group ($P = 0.035$) were also the best predictors for AML progression.

Discussion

In MDS, cytogenetics play an essential role in the identification of distinct clinico-biological entities[4,16–18] and prognostic subgroups[2,3] and is therefore considered a crucial step for the definition of the most appropriate treatment plan.[19–23] In this regard, a recent large multicenter cooperative study has

Table 4 FISH and cytogenetic data at diagnosis and at AML progression in FISH-positive MDS patients with normal karyotype

No.	*Name*	*FISH anomaly*	*FISH+ results at diagnosis*		*AML progression*	*Karyotype at AML progression (No. of metaphases)*	*FISH + results at AML progression*	
			Interphase % of cells	*Metaphase No. of cells*			*Interphase % of cells*	*Metaphase No. of cells*
1	UV	+8	19	3/10*	YES	46, xy [26]	60	3/15[a]
2	RG	+8	20	2/10*	YES	ND	ND	ND
3	CG	+8	22	2/10*	NO	NA	NA	NA
4	DMG	+8	32	2/10*	YES	46, xx [25]/47, xx + 8 [2]	58	4/20
5	GV	+8	18	0/10	YES	46, xy [27]/47, xx + 8 [2]	45	2/15
6	MF	+8	17	0/10	YES	46, xx [8]/47, xx, +8 [8]	70	ND
7	LB	–7	15	0/10	NO	NA	NA	NA
8	BE	17p–	18	2/14	NO	NA	NA	NA
9	CU	5q–	32	2/10	YES	46, xy [20]	55	6/10
10	MS	5q–	26	2/11	YES	no mitoses	48	NA
11	PM	5q–	23	0/15	NO	NA	NA	NA
12	PV	5q–	31	6/9	YES	46, xy [20]	55	6/10
13	TB	5q–	16	6/10	YES	46, xx [20]	60	8/10
14	BE	7q–	22	2/8	YES	46, xx [20]	60	7/10
15	CS	7q–	23	0/10	YES	46, xy [18]	50	0/15
16	DMV	7q–	18	0/10	YES	46, xx [14] 46, xx, t(1;7)(p35;q32) [11]	65	ND
17	PA	7q–	24	2/10	YES	46, xy [14] 47, xy, + mar [2]	55	ND
18	SF	7q–	18	1/15	NO	NA	NA	NA

[a]Trisomy 8 in mitotic figures of too poor quality for karyotyping.
ND, not done; NA, not applicable.

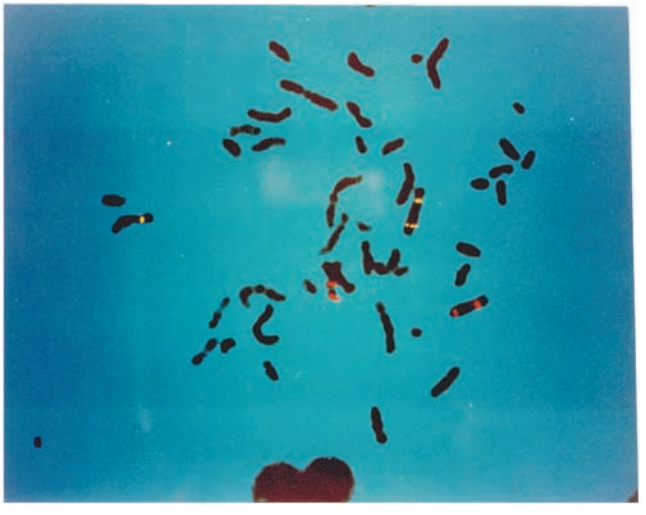

Figure 1 The metaphase FISH image (patient No. 13) shows two chromosomes 5 of apparently identical size, marked by the green centromeric signals. The lack of the 5q31 signal in one of the two chromosomes demonstrates the presence of a small deletion not detectable by conventional karyotyping. Hybridization efficiency was documented by the normal hybridization pattern on the two chromosomes 7 displaying red signals on the centromere and at the 7q31 band. The two red signals on the chromosome 7 in the middle of the figure are close to each other possibly due to crossing with another chromosome.

included cytogenetics along with a number of hematopoietic lineages involved in the cytopenias and bone marrow blasts in a scoring system for the definition of prognosis in MDS.[4] Three main cytogenetic groups have been identified: low risk (normal karyotype, 5q–, 20q– and –y as single abnormalities), high risk (abnormalities involving chromosome 7 and complex karyotypes) and intermediate risk (all other abnormalities). The IPSS not only has further elucidated, on a large series of patients, the role of cytogenetics in the identification of MDS patients with an indolent course from those who are likely to have a more aggressive disease but has also introduced such biological parameter in a reproducible scoring system.

However, despite these observations, the most frequent cytogenetic pattern in MDS is represented by the normal karyotype. Around 40–65% of *de novo* MDS patients in fact present at diagnosis a normal karyotype, whereas among secondary or therapy-related MDS the proportion of patients with normal karyotype is only 20%.[1–8] Cytogenetic data in this analysis confirmed this observation, with a figure of 48.1% of normal karyotypes in a series of 210 MDS patients observed in a 10-year period.

Even though the finding of a normal karyotype has been considered by the IPSS as a low risk prognostic feature, the prognosis of patients with normal karyotype is sometimes unpredictable. Our analysis, for example, showed that only 29 karyotypically normal patients (28.7% of the cases) fulfilled the criteria for the attribution to the low risk IPSS group while most of the patients (57 cases, 56.4%) were classified in the intermediate-1 risk group and 15 patients were instead attributed to the intermediate-2 and high risk groups.

New biological parameters should then be considered in order to better characterize this subgroup of patients. FISH analysis in particular has increased our ability to detect cytogenetic abnormalities using specific DNA probes to identify each of the chromosomes individually.[9] In comparison with conventional cytogenetics, FISH analysis does not depend on cell division and can be performed on interphase nuclei giving the possibility to examine a larger number of cells. The lower threshold of detection allows for the identification of small clones of abnormal cells which, although missed by conven-

Table 5 Univariate analysis of variables related to survival in 101 MDS patients with normal karyotype

Variable	*No. of patients*	*Median survival (months)*	*Standard error (months)*	*P*
Age (yrs)				
⩽65	48	Not reached at 120 months	–	
>65	53	45	12	0.0034
BM blasts (%)				
<5	56	118	29	
5–10	30	45	14	
11–20	8	21	1	
21–30	7	13	1	<0.0001
FAB				
RA + RARS	52	92	16	
CMML	8	45	24	
RAEB	34	36	15	
RAEB-t	7	13	1	<0.0001
HB (g/dl)				
<10	34	34	7	
⩾10	67	80	19	0.0036
ANC (10^9/l)				
<1.5	51	64	14	
⩾1.5	50	53	8	NS
Plt (10^9/l)				
<100	34	33	9	
⩾100	67	64	13	0.041
Sex				
M	56	57	15	
F	45	118	46	NS
IPSS				
Low	28	118	20	
Int-1	58	53	12	
Int-2 + High	15	21	2	0.0001
FISH				
Positive	18	28	9	
Negative	83	80	17	<0.0001

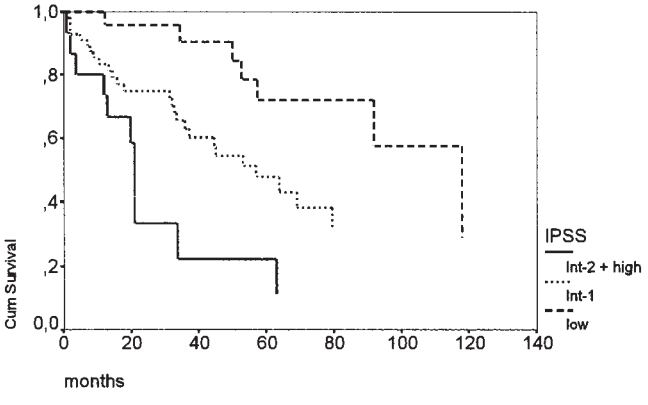

Figure 2 Overall survival of 101 MDS patients with normal karyotype according to IPSS risk group distribution ($P = 0.0001$): low risk (28 patients), intermediate-1 (58 patients) and intermediate-2/high risk (15 patients).

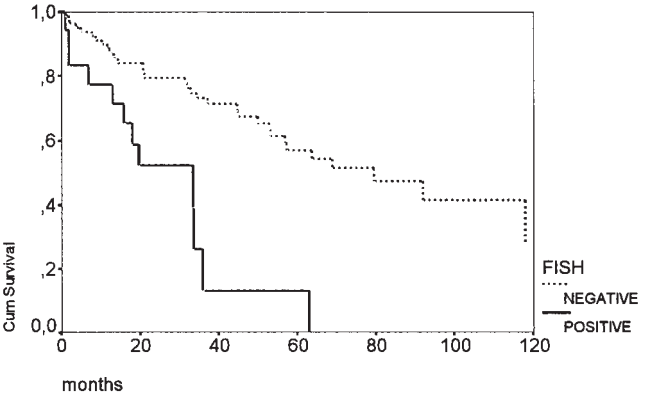

Figure 3 Overall survival of 101 karyotypically normal MDS patients with (18 patients) and without (83 patients) occult chromosome anomalies by FISH ($P < 0.0001$).

tional cytogenetics, could instead have a major role in the progression of the disease.[24] Moreover, FISH analysis is also a suitable technique to detect submicroscopic lesions, especially deletions, which are undetectable by conventional banding analysis.[25,26]

We therefore studied by interphase FISH a large number of consecutive MDS patients with normal karyotype, using a panel of probes detecting the most frequent anomalies in MDS, in an attempt to define a possible role for FISH in risk assessment.

We chose to analyze numerical abnormalities involving chromosome 8, 5 and 7 and deletions involving the segments 5q31, 7q31 and 17p13. These abnormalities were previously shown to have prognostic significance accounting for approximately 25% of chromosome anomalies in all MDS patients.[4,19,27–32]

Our analysis showed that 17.8% of the patients with normal karyotype presented some of these chromosomal abnormalities in 15–32% of the cells when analyzed by FISH. The most frequent FISH numerical abnormality was represented by trisomy 8 which was detected in the 33.3% of these patients. In 4/6 patients with trisomy 8 few mitotic figures of too poor quality for karyotyping were detected by metaphase FISH analysis, a finding reflecting the inability of the abnormal clone to divide properly *in vitro*. This observation is in keeping with previous reports,[24,27,28] which have shown that FISH methodology is an excellent way to detect occult trisomy 8.

Monosomy of chromosomes 5 and 7 were less commonly encountered in this series, a finding in keeping with a recent report.[33] However, while monosomy 5 is relatively rare in MDS and, in fact, it was not found in this series, FISH analysis allowed for the demonstration of one case with a minor non-dividing clone with –7. Interestingly, a previous report showed FISH to significantly increase the ability to detect –7 as compared with conventional cytogenetics.[29] Deletions were observed in the 61.1% of FISH-positive cases and were represented in five cases each by del(7q) and by del(5q). None of the cryptic 5q– cases fulfilled the criteria for the diagnosis of the '5q– syndrome';[16] in fact, four cases had RAEB/RAEB-t and one had RA with multilineage dysplasia. Moreover, the 5q probe we have used in this analysis recognized sequences located in the minimal region of loss observed in AML-associated 5q deletions and not the more telomeric segment which is instead lost in the classical 5q– syndrome.[34] Overall, the frequency of these chromosome abnormalities in MDS

with normal karyotype resembled what is usually found, by conventional cytogenetics, in unselected MDS patients.[3,18,19]

FISH positivity, in this series of patients, was associated with a higher percentage of bone marrow blasts, with high risk FAB subgroups, and with high risk IPSS groups. As a consequence of these findings, FISH-positive MDS patients presented a higher risk of disease progression and a worse outcome in comparison to FISH negative subjects (Figure 3). Multivariate analysis confirmed the prognostic relevance of FISH analysis as an independent prognostic factor along with IPSS risk group.

On the whole, these data showed that FISH analysis may be a useful investigation in MDS patients at diagnosis when a normal karyotype is observed. FISH investigation may therefore be of great clinical relevance in the planning of treatment, particularly in younger patients for whom more aggressive alternative therapeutical strategies are nowadays available.[19–23]

The finding that the detection by FISH analysis of minor clones of abnormal cells is predictive for a worse prognosis suggests some considerations. From a biological point of view, it is well known that, in MDS, normal and abnormal stem cells may function together and both contribute to hematopoieis.[19] In this study we were able to demonstrate that minor clones played instead an important pathogenetic and prognostic role as, with disease progression, they took a proliferative advantage over normal clones. The increase in size of the abnormal clones that was observed by interphase FISH in all patients undergoing leukemic transformation supports this argument.

The reason why CCA failed to detect these numerical and structural rearrangements is unlikely to be represented by a low sensitivity of this method in our laboratories, because the overall incidence of trisomies and deletions in our series fell within the incidence range previously described.[18,19] Moreover, metaphase FISH analysis showed that, in four out of six patients, at diagnosis trisomy 8 was present in mitotic figures of too poor quality for karyotyping. As previously suggested,[24] a low mitotic index of the abnormal clone could be the most likely explanation for the discrepancy between CCA and FISH results in the remaining cases with numerical anomalies in interphase cells (two patients with +8 and one patient with –7). At the time of leukemic transformation the presence of trisomy 8 was demonstrated in three out of four cases by karyotypic analysis of a large number of G-banded metaphases.

In the majority of cases with cryptic deletion (5q–, 7q– and 17p–) metaphase FISH analysis showed that the size of the deletions was beyond the resolution power of conventional banding analysis. In this regard, some cases with myeloid neoplasias and apparently normal karyotype were recently shown to carry, when investigated by FISH, a minor clone with a submicroscopic deletion,[26,35] suggesting that cryptic rearrangements may occur in MDS and AML more frequently than previously thought. The fact that many of our MDS patients had been exposed to myelotoxic agents in the workplace[5] might have played a role in the development of chromosomal deletion. The demonstration that genotoxic drugs may determine the appearance of deletions in various hemopoietic cells further supports this argument.[36]

The delineation of the boundaries of deletions in these patients was beyond the scope of this analysis. Large series were recently published showing that one or more genes in these regions may be involved in the transformation process;[33] furthermore we cannot exclude that molecular alterations involving other regions containing tumor suppressor genes and proto-oncogenes could also play an important role in the definition of prognosis in these MDS patients with normal karyotype.[37]

In conclusion, we have demonstrated that 17.8% of MDS patients with normal karyotype presented, when analyzed by interphase FISH, an occult chromosome lesion, escaping detection because of the low sensitivity of conventional cytogenetics, which may be unable to detect abnormal clones with a low mitotic index or with submicroscopic deletion. These abnormal clones may have a determinant role in the progression of the disease and may identify a subgroup of MDS patients with normal karyotype characterized by an inferior prognosis.

This approach is simple and may be easily used in clinical practice; additional probes might be tested allowing for a further refinement of this heterogeneous group of MDS.

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