Fluorescence *in situ* hybridization improves the detection of 5q31 deletion in myelodysplastic syndromes without cytogenetic evidence of 5q–

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ABSTRACT

Background
More than 50% of patients with myelodysplastic syndromes present cytogenetic aberrations at diagnosis. Partial or complete deletion of the long arm of chromosome 5 is the most frequent abnormality. The aim of this study was to apply fluorescence *in situ* hybridization of 5q31 in patients diagnosed with *de novo* myelodysplastic syndromes in whom conventional banding cytogenetics study had shown a normal karyotype, absence of metaphases or an abnormal karyotype without evidence of del(5q).

Design and Methods
We performed fluorescence *in situ* hybridization of 5q31 in 716 patients, divided into two groups: group A patients (n=637) in whom the 5q deletion had not been detected at diagnosis by conventional banding cytogenetics and group B patients (n=79), in whom cytogenetic analysis had revealed the 5q deletion (positive control group).

Results
In group A (n=637), the 5q deletion was detected by fluorescence *in situ* hybridization in 38 cases (5.96%). The majority of positive cases were diagnosed as having the *5q- syndrome*. The deletion was mainly observed in cases in which the cytogenetics study had shown no metaphases or an aberrant karyotype with chromosome 5 involved. In group B (n=79), the 5q deletion had been observed by cytogenetics and was confirmed to be present in all cases by fluorescence *in situ* hybridization of 5q31.

Conclusions
Fluorescence *in situ* hybridization of 5q31 detected the 5q deletion in 6% of cases without clear evidence of del(5q) by conventional banding cytogenetics. We suggest that fluorescence *in situ* hybridization of 5q31 should be performed in cases of a suspected '5q- syndrome' and/or if the cytogenetic study shows no metaphases or an aberrant karyotype with chromosome 5 involved (no 5q- chromosome).

Key words: myelodysplastic syndromes, karyotype, fluorescence *in situ* hybridization

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Introduction

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell diseases characterized by dysplasia and ineffective hematopoiesis in one or more myeloid cell lines.[1] In 1982, the French-American-British (FAB) group developed a morphological classification that distinguishes five MDS subtypes based on the percentage of bone marrow and peripheral blood blasts, the percentage of bone marrow ringed sideroblasts and the level of circulating monocytes.[2] In 2001, the World Health Organization (WHO) proposed a new classification which represents an extension of the FAB proposal with several modifications taking into account dysplasia, cytopenias, percentage of blasts and cytogenetic and molecular findings. The WHO classification identified the following MDS subtypes: refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), '5q- syndrome' (MDS 5q-), refractory cytopenia with multilineage dysplasia (RCMD), RCMD with ringed sideroblasts (RCMD-RS), refractory anemia with excess of blasts (RAEB) type 1 (RAEB-1), RAEB type 2 (RAEB-2) and MDS, unclassifiable (MDS-U). Two FAB subtypes were reclassified in the WHO classification: chronic myelomonocytic leukemia (CMML) was considered as a myelodysplastic/myeloproliferative disease (MDS/MPD) and refractory anemia with excess of blasts in transformation (RAEB-t) as acute myeloid leukemia (AML). Moreover, the WHO classification considers MDS associated with an isolated del(5q) chromosome abnormality as a new entity within MDS: '5q- syndrome'. This entity was defined as macrocytic anemia, marrow and blood blast cell percentage <5%, an isolated del(5q) cytogenetic abnormality and a favorable clinical course. The bone marrow is usually hypercellular or normocellular with normal or increased megakaryocytes, many of which have hypolobulated nuclei (in most of cases ≥50%). The most common clinical symptoms are usually related to anemia that causes transfusion dependency.[1]

The prognosis and clinical course of MDS vary among patients. Several scoring systems have, therefore, been established in order to predict the prognosis with regards to survival and evolution to AML. These scoring systems are mainly based on multiple prognostic parameters such as the percentage of blasts, age, karyotype, number of cytopenias and transfusion requirements.[3-6] The International Prognostic Scoring System (IPSS), introduced in 1997, became the gold standard for risk assessment in patients with *de novo* MDS.[4] However, subsequent studies revealed some pitfalls of the system; one of the most important was the inclusion of the less frequent single chromosome defects and double defects in the intermediate cytogenetic category.[7-10]

Cytogenetic findings have been demonstrated to play an important role in both the diagnosis and prognosis of MDS and have been given more weight in the WHO classification, which recognizes the '5q- syndrome' as a new subtype of MDS.[1] Conventional banding cytogenetics remains an integral component and standard in the diagnostic work up of patients with suspected MDS. Although MDS are not associated with any specific chromosomal abnormality, there are some frequent alterations: 5q-, -7/7q-, +8, -18/18q-, 20q-, -5, -Y, -17/17p- (including i(17q)).[10]

Partial or complete deletion of the long arm of chromosome 5 is the most recurrent cytogenetic abnormality in MDS patients, being found in 10-15% of all cases of *de novo* MDS.[8-10] Abnormalities of chromosome 5 can present as either a sole karyotypic abnormality or in combination with other chromosomal abnormalities.[11] In clinical practice, the 5q deletion can be detected by cytogenetics or by fluorescence *in situ* hybridization (FISH) with a fluorescently labeled probe that recognizes the 5q31 locus (EGR1).

Recently, lenalidomide (CC-5013, Revlimid®; Celgene) was approved by the USA Food and Drug Administration (FDA) for the treatment of patients with MDS with an interstitial deletion of the long arm of chromosome 5. Patients with del(5q) MDS frequently have symptomatic anemia, and the treatment of this condition has traditionally consisted of red bood cell transfusions and, for some, iron chelation therapy.[12] Clinical trials assessing the efficacy of lenalidomide in MDS showed that this drug can reduce transfusion requirements and reverse cytologic and cytogenetic abnormalities in patients who have MDS with the 5q31 deletion.[13,14]

The aim of the present study was to apply the FISH technique in patients diagnosed with MDS in whom cytogenetic analysis had shown a normal karyotype, absence of metaphases or an abnormal karyotype without evidence of del(5q). FISH would allow the detection of the 5q deletion in those cases in which cytogenetic analysis had not found the deletion. In consequence, these patients might be candidates for treatment with lenalidomide.

Design and Methods

Patients

Seven hundred and sixteen patients diagnosed with MDS were retrospectively included in the study. All of them came from centers affiliated to the Spanish Haematological Cytogenetics Working Group (GCECGH). The patients were divided into two groups: group A consisted of 637 patients who did not present the 5q deletion at diagnosis as determined by cytogenetics, while group B comprised 79 patients in whom cytogenetic analysis had revealed the 5q deletion, who were, therefore, used as positive controls. All cases were diagnosed as having primary MDS: 659 patients according to the FAB classification and among them, 555 could also be classified according to the WHO criteria. For 57 patients no diagnostic data were available to classify them according to either the FAB or WHO criteria. The patients' diagnoses are listed in Table 1.

Among the 637 cases in group A, we had cytologic information for 307 and 14 of them had a morpholog-

ical orientation of '5q- syndrome'.

The study was conducted with the approval of the ethical committee from our institution and in keeping with the guidelines of the Declaration of Helsinki.

Cytogenetic and FISH analyses

Cytogenetic and FISH studies were performed according to the standard methods used in our laboratory.[15] Seven hundred and seventeen samples from 716 patients were analyzed by cytogenetics and FISH with LSI5q31 (EGR1)/D5S23, D5S21 probe (Abbott Molecular Inc, Des Plaines, IL, USA) at the individual centers. In two cases, whole chromosome 5 and 6 painting (Metasystems GmbH, Altlussheim, Germany) was also performed. These studies were carried out on bone marrow cells from 24-hour cultures. For FISH studies, between 100 and 400 nuclei were analyzed in order to detect the 5q deletion and/or monosomy 5. At least ten metaphases were analyzed for the painting study.

The cut-off value established to consider a sample as 5q- positive by FISH varied among the centers, from 3% to 10%. The cut-off was defined as the average plus two or three standard deviations analyzing 10-20 peripheral blood or bone marrow control samples and 200-500 nuclei.

Statistical methods

In order to analyze differences between the proportion of cells with 5q deletion detected by conventional banding cytogenetics and FISH, a statistical analysis called *one-way intraclass correlation coefficient* was applied. This test assesses rating reliability by comparing the variability of different ratings of the same subject to the total variation across all ratings and all subjects; the result is a value between zero and one: zero is indicative of no concordance between FISH and cytogenetic results, while one indicates complete concordance.

Results

Group A: no evidence of 5q- by conventional banding cytogenetics

When FISH for 5q31 was applied in the 637 cases in which the cytogenetic study had not revealed 5q-, the 5q deletion was detected in 38 patients (5.96%). The results are shown in Table 2, which presents the percentage of 5q deletion detected by FISH in different groups divided according to the results of conventional banding cytogenetics: normal karyotype (in 20 or less metaphases) (2.7%), no metaphases or not evaluable (due to the poor morphology of chromosomes)

Table 1. FAB and WHO diagnoses of myelodysplastic syndromes patients included in the present study.

FAB classification	*Group A n=637, n (%)*	*Group B n=79, n (%)*	*WHO classification*	*Group A n=500, n (%)*	*Group B n=55, n (%)*
RA	214 (33.6)	32 (40.5)	RA	69 (13.8)	1 (1.8)
RARS	160 (25.1)	6 (7.6)	RARS	82 (16.4)	0
RAEB	145 (22.8)	20 (25.3)	'5q- syndrome'	16 (3.2)	25 (45.4)
RAEB-t	18 (2.8)	0	RCMD	95 (19)	7 (12.7)
CMML	53 (8.3)	1 (1.2)	RCMD-RS	46 (9.2)	3 (5.4)
MDS-U	10 (1.6)	0	RAEB-1	50 (10.2)	12 (21.8)
Unknown	37 (5.8)	20 (25.3)	RAEB-2	62 (12.4)	6 (10.9)
			MDS-U	14 (2.8)	0
			MDS/MPD CMML	39 (7.8)	1 (1.8)
			MDS/MPD,U-RARS associated with marked thrombocytosis	6 (1.2)	0
			MDS/MPD no CMML	2 (0.4)	0
			AML	18 (3.6)	0

RA: refractory anemia; RARS: refractory anemia with ringed sideroblasts; RAEB: refractory anemia with excess of blasts; RAEB-t: refractory anemia with excess of blasts in transformation; CMML: chronic myelomonocytic leukemia; MDS-U: MDS unclassifiable; RCMD: refractory cytopenia with multilineage dysplasia; RCMD-RS: refractory cytopenia with multilineage dysplasia with ringed sideroblasts; MDS/MPD: myelodysplastic/myeloproliferative disease; AML: acute myeloid leukemia.

Table 2. Cytogenetic and FISH 5q31 data from cases without evidence of 5q- by conventional banding cytogenetics (group A).

			FISH result	
Conventional banding cytogenetics result	*Cases (n=637)*	*5q31-, n (%)*	*-5, n (%)*	*+5, n (%)*
Normal CBC (20 metaphases)	324	9 (2.7)	0	1 (0.3)
Normal CBC (10-19 metaphases)	107	2 (1.9)	0	0
Normal CBC (1-9 metaphases)	43	2 (4.6)	0	0
CBC without metaphases	54	11 (20.4)	0	0
Abnormal CBC with chromosome 5 affected	11	9 (81.8)	0	0
Abnormal CBC without chromosome 5 affected	98	5 (5.1)	1 (1)	0

(20.4%) and abnormal karyotype (with chromosome 5 affected, 81.8%, or not, 5.1%).

Two cases are worth noting. The first one (#39) is a patient diagnosed as having RAEB/RAEB-1 and multiple myeloma, who presented a complex karyotype with no aberration of chromosome 5. FISH analysis revealed monosomy of chromosome 5 (in 18% of nuclei), which had not been observed by cytogenetics. FISH analysis in eight metaphases did not reveal the monosomy 5. The second case (#40) is a patient, diagnosed with RARS/RCMD-RS and multiple myeloma, who presented a trisomy 5 (49% of nuclei); this aberration had not been seen by cytogenetics (46,XY[20]). FISH did not reveal trisomy 5 in either of the two metaphases analyzed (Table 3).

Table 3 shows the diagnostic morphological and cytogenetic data of cases in which the 5q deletion was detected by FISH. A high number of these patients had RA (17/38, 44.7%) or RAEB (10/38, 26.3%), according to the FAB classification. As regards the WHO classification, the majority of the positive cases (16/38, 42.1%) had '5q- syndrome'.

Table 3. Morphological and cytogenetic data from myelodysplastic syndromes patients with a 5q alterations detected only by FISH.

Case	*FAB*	*WHO*	*Karyotype by conventional banding cytogenetics*	*Percentage of aberrant nuclei %*	*Metaphase FISH analysis (altered/ total metaphases analyzed)*	*'5q- syndrome' morphology*
1	RAEB	5q- syndrome	No metaphases	35	NA	Unknown
2	RA	5q- syndrome	No metaphases	42	NA	Unknown
3	RAEB	5q- syndrome	No metaphases	95	NA	Unknown
4[a]	RAEB-t	AML	46,XX,add(2)(p22),-5,-11,del(12)(q13),add(18)(q23), add(19)(q13),+2mar[20]	99	NA	Unknown
5	RARS	RCMD-RS	47,XY,der(5)t(5;17)(q10q10),del(7)(q33),+8,der(12) t(12;13)(p13;q13)[18]/46,XY[2]	90	NA	Unknown
6	RA	5q- syndrome	N20	30	NA	Unknown
7	RAEB	RAEB-2	46,XY,t(11;17)(q24;q21)[3]/46,XY[12]	90	NA	Unknown
8	RA	-	N20	18	NA	Unknown
9	RAEB	-	N20	60	NA	Unknown
10	RA	5q- syndrome	N1-9	20	NA	Unknown
11	RA	5q- syndrome	N10-19	85	NA	Unknown
12	RA	5q- syndrome	N20	54	NA	Unknown
13	RAEB	RAEB-1	46,XX,der(5),t(5;6)(q13;q14),del(6)(q14)[18]/46,XX[2]	90.5	3/5	Unknown
14	RA	5q- syndrome	N20	8	1/20	Unknown
15[a]	RAEB-t	AML	45,XY,del(1)(q11),-5,add(7)(q36),der(21)t(1;21)(q22;q35) [13]/46,XY[4]/ polyploid(id.add12p)[3]	87	10/10	Unknown
16	RA	-	N20	25	1/3	Unknown
17	RA	5q- syndrome	N1-9	66	3/7	Unknown
18	RAEB	-	46,XX,-7,+G[18]/46,XX[2]	25	2/10	Unknown
19	RA	5q- syndrome	46,XY,del(1)(p34)[4]/46,XY[9]	5	0/20	Yes
20	RA	5q- syndrome	Not evaluable	70	NA	Yes
21	RA	5q- syndrome	Not evaluable	62	NA	Yes
22	RA	5q- syndrome	Not evaluable	52	NA	Yes
23	RAEB	RAEB-2	50,XX,+1,+8,+11,add(11)(p13),+mar [15]/46,XX [7]	53.5	NA	No
24	RAEB	-	49,XY,+2,+3,-7,+12,+mar [12]/46,XY[6]	72.5	NA	No
25	RAEB	RAEB-2	No metaphases	44.5	NA	No
26	RA	5q- syndrome	N20	50	3/3	Yes
27	RARS	5q- syndrome	No metaphases	21	NA	Yes
28	RAEB	RAEB-2	N20	14	1/1	Yes
29	RA	5q- syndrome	N10-19	18	6/7	Yes
30	RA	RA	No metaphases	7	NA	No
31	RA	CRDM	No metaphases	30	NA	Yes
32	CMML	MDS/MPD CMML	46,XY,t(3;5)(p21;q14) [11]	50	NA	No
33[a]	Unknown	-	45,X,-Y[21]/42,XY,-5,-7,-8,t(15;?)(p13;?),-16,-17,add(17)(p13), +mar[21]/46,XY[8]	53.5	10/10	Unknown
34[a]	Unknown	-	45,XY,-5,-17,-21,+2mar[21]/46,XY[9]	68	5/7	Unknown
35[a]	Unknown	-	47,X,add(Y)(q12),-5,-15,-17,-18,-21,+22,+5mar[22]/46,XY[8]	35	4/10	Unknown
36[a]	Unknown	-	45,XX,-4,-5,-12,-17,+3mar[4]/46,XX[26]	38.5	3/10	Unknown
37	Unknown	-	No metaphases	40	NA	Unknown
38	Unknown	-	N20	6	2/11	Unknown
39[b]	RAEB+MM	RAEB-1+MM	46,XX,add(11)(q25),del(16)(q22),+19,-21,del(22)(q11)[15]/47, XX,der(7),add(11)(q25),del(16)(q22),+19,-21,del(22)(q11), + r[3]/92,XXXX,id,+r,+r[1]/46,XX[6]	18	0/8	Unknown
40[c]	RARS+MM	RCMD-RS+MM	N20	49	0/2	Unknown

N20: normal karyotype in 20 metaphases; N10-19: normal karyotype in 10-19 metaphases; N1-9: normal karyotype in 1-9 metaphases; NA: not available; MM: multiple myeloma. [a]These patients showed, by conventional banding cytogenetics, a monosomy of chromosome 5 but FISH demonstrated a deletion of 5q.
[b]Case with monosomy 5. [c]Case with trisomy 5.

In FISH 5q- positive cases, the available FISH slides were reassessed in order to analyze the 5q- chromosome in metaphases. In 7 of 13 cases with normal karyotype and 5q- detected by FISH, the FISH slides were reviewed and metaphases with 5q deletion were detected (the results are shown in Table 3). Among five cases showing an abnormal karyotype with no involvement of chromosome 5, two cases were re-evaluated looking for metaphases. In one of them, two out of ten metaphases presented 5q-, whereas, in the other case the 5q- was not detected in any of 20 metaphases. In six of nine cases with an abnormal karyotype and chromosome 5 involved, the reanalyzed metaphases showed 5q-.

Two of these cases are worth particular comment. The first one (#15) is a patient with RAEB-t/AML who had monosomy 5 according to cytogenetic analysis but the FISH study revealed only a deletion of 5q31. The metaphase analysis showed two chromosomes with a similar size, one of them with a 5q31 deletion. Whole chromosome painting for chromosome 5 was also performed; it revealed one normal chromosome 5 and another one with a portion of chromosome 5, this marker chromosome could not be identified. The other interesting case (#13), at the time of diagnosis of RAEB/RAEB-1, had, according to cytogenetic analysis, a translocation involving chromosome 5. The interphase analysis of FISH 5q revealed a deletion of the 5q31 region. The deletion was also observed when analyzing the metaphases. Whole chromosome painting of chromosomes 5 and 6 was carried out in order to confirm the t(5;6). The whole FISH studies enabled the patient's cytogenetic profile to be defined as: 46,XX,der(5)t(5;6)(q13;q14),der(6)(t(5;6)(q33;q14)[18]/ 46,XX[2].

Fourteen cases were referred with a cytologic diagnosis of '5q- syndrome' without evidence of 5q- by cytogenetics; among them, nine (cases #19, 20, 21, 22, 26, 27, 28, 29 and 31) were found to have the 5q deletion by FISH.

Group B: evidence of 5q- by conventional banding cytogenetics (positive controls)

Seventy-nine samples from patients, whose cytogenetic studies had shown 5q deletion were used as positive controls. In all of them, FISH for 5q31 was performed and confirmed the 5q deletion. We compared the proportion of cells with 5q- detected by conventional banding cytogenetics with that detected by FISH applying a statistical analysis called *one-way intraclass correlation coefficient*. We obtained a value of 0.284, showing a lack of significant concordance in the detection of 5q- by FISH and cytogenetics. Furthermore, this statistical test gave an average 5q- detection of about 58.20±26.62% for conventional banding cytogenetics and 53.85±22.73% for the FISH technique.

We also analyzed the distribution of gender among patients with deletion of 5q, although we only had information about sex for 489 patients. Of 38 patients from group A (5q- evidence by cytogenetics), 10 (26.3%) were male, 14 (36.8%) female and in 14 (36.8%) the gender was unknown (Table 3). Regarding cases with a diagnosis of '5q- syndrome', one was male (6.25%), seven were female (43.75%) and the gender was unknown for eight (50%). Among 79 patients with 5q- detected by conventional banding cytogenetics (group B), 28 (35.4%) were male and 51 (64.6%) female. Of the cases with '5q- syndrome' (n=25), five (20%) were male and 20 (80%) female.

Discussion

To our knowledge, this is the largest reported series of primary MDS in which 5q deletion was studied by FISH in cases with no evidence of 5q- in the karyotype. In group A, we found 5q31 deletion by FISH in about 6% of the cases. Deletion 5q was observed more frequently in cases with an abnormal karyotype with chromosome 5 involved (81.8%), and in cases with no mitoses or in those that could not be evaluated because of the poor morphology of the chromosomes (20.4%).

Within group A, among the cases with a normal karyotype (n=474), FISH detected 5q- in 13 cases (2.7%). Our results are in agreement with those of previous studies (Table 4) in which the FISH technique detected the 5q deletion in 0% to 14% of cases.[16-22] The percentage of 5q- detection differed depending whether metaphases or interphase nuclei were studied. This could be related to a different rate of mitoses in cells carrying or not the 5q deletion. We tried to provide support for this hypothesis by analyzing metaphases from the FISH slides (only seven of 13 cases could be assessed): all of them presented some metaphases with the 5q deletion. This might indicate that the finding of the deletion in the FISH analysis, but not in the conventional banding cytogenetic study, could be due to the number of cells analyzed rather than a cryptic deletion. This explanation could be applied to case #40 in which a trisomy 5 was detected by FISH while cytogenetics showed a normal karyotype in 20 metaphases.

It is interesting to note that in our series a high percentage of the cases of 5q- detected by FISH were in those cases without mitoses or which were not evaluable (20.4%). This might be because the 5q- clone could have a low proliferation and a high apoptotic rate. Nevertheless, when Washington *et al.* studied

Table 4. Comparison of FISH findings with those of previous series of myelodysplastic syndromes with normal cytogenetic results.

Author	*N.*	*FISH 5q-/-5, n. (%)*	*Region analyzed*
Rigolin *et al.*[16] (2001)	101	5 (5)	5q31
Shen *et al.*[17] (2001)	48	6 (12.5)	5q31
Ketterling *et al.*[18] (2002)	31	0	5q31 (EGR1)
Cherry *et al.*[19] (2003)	29	0	5q31 (EGR1)
Bernasconi *et al.*[20] (2003)	57	2 (3.5)	5q31 (EGR1)
Beyer *et al.*[21] (2004)	17	0	PAC 144G9 (5q31)
Yilmaz *et al.*[22] (2005)	21	3 (14.3)	5q31 (EGR1)
Present series (2008)	474	13 (2.7)	5q31 (EGR1)

apoptosis in '5q- syndrome' and other RA, they found significantly lower rates of apoptosis in bone marrow cells isolated from patients with '5q- syndrome' than in the cells from patients with other RA.[23] Our hypothesis about the proliferation and the apoptotic rate of cells with 5q- is speculation based on our findings comparing cytogenetics and FISH results and further studies are needed to understand the behavior of cells that carry the 5q deletion.

It is noteworthy that nine cases with an abnormal karyotype involving chromosome 5 were found to have the 5q31 deletion when studied by FISH. Indeed, in our series we have six cases with a complex karyotype that showed monosomy 5. Five (cases #4, 32, 33, 34 and 35) of them had marker chromosomes in the conventional banding cytogenetic studies and were identified by FISH as 5q- chromosomes (see Table 3). The other case (#15) presented a monosomy 5 by conventional banding cytogenetics but no marker chromosomes. The FISH analysis revealed 5q deletion in interphase nuclei and in metaphases as well. Whole chromosome 5 painting showed a normal chromosome 5 and another chromosome with material from both chromosome 5 and of unknown origin.

Several studies have shown the usefulness of molecular cytogenetic techniques, such as spectral karyotyping (SKY) or multicolor FISH (M-FISH) and FISH to define abnormal karyotypes involving chromosome 5 or presenting with monosomy 5.[24-29] These studies demonstrate that FISH analysis can provide additional information about chromosome 5 abnormalities. It would, therefore, be recommendable to use FISH techniques to study those cases with monosomy 5 and/or marker chromosomes in order to identify translocations with a breakpoint in 5q or possible 5q- chromosomes.

Returning to our series, three cases (#5, 13 and 32) showing a translocation involving chromosome 5 by cytogenetics were all found to have 5q31 deletion by FISH. FISH analysis of metaphases was not available for cases #5 and 32, while for patient #13, the FISH analysis revealed 90.5% of deleted nuclei and whole chromosome 5 painting helped to redefine the karyotype. These findings suggest that in cases with an abnormal karyotype involving chromosome 5 (and no evidence of 5q- by cytogenetics) it should be mandatory to apply FISH of the 5q31 region in order to detect interstitial deletions.

In our series, 5% of cases with 5q- by FISH had an abnormal karyotype without involvement of chromosome 5. Among them, two patients (cases #23 and 24) showed a complex karyotype without evidence of 5q- but by conventional banding cytogenetics had marker chromosomes. These could have been 5q- chromosomes, and this hypothesis could have been confirmed by analyzing metaphases from FISH slides but, unfortunately, this was not possible in either of the cases.

Three cases (#7, 18 and 19) had an abnormal karyotype without evidence of 5q-; these findings could suggest the presence of two clones: one with 5q- and another one with an abnormal karyotype. A similar hypothesis could explain the case previously mentioned (#39) which presented with an abnormal karyotype with two normal chromosomes 5 and monosomy 5 by FISH. This might have been confirmed by analyzing more metaphases. In one case (#18), with available fixed material, the conventional banding cytogenetic analysis was performed again and no 5q- chromosome was identified. We could assume that there were two clones, one with '-7, +G' and another one with 5q-. Two groups have studied cytogenetics of unrelated clones in MDS. The most commonly encountered abnormalities in the unrelated clones in patients with RA were del(5q), +8 and -7. Aberrations such as +8 and 5q- could be secondary abnormalities that develop during tumor progression.[30-31]

In the present study, we also used FISH to analyze 79 cases with a karyotype which had presented 5q- (group B). FISH confirmed the deletion in all cases. Regarding the proportion of cells with 5q- detected by cytogenetics and FISH, a previous study affirmed that the percentage of cells with 5q deletion detected by cytogenetics was usually lower than that detected by FISH. Nevertheless, the authors pointed out that FISH cannot be a substitute for conventional banding cytogenetics.[32] According to our experience the 5q deletion can be correctly identified by both techniques, but, due to the small differences in the mean percentage of deletion 5q cells detected by cytogenetics and FISH; we cannot conclude that one technique had a higher sensitivity than the other.

In patients with 5q- detected by FISH (from group A), we were not able to compare the proportion of females and males due to the fact that gender was unknown for 14 of the patients. With regards to patients with the diagnosis of '5q- syndrome', we were able to assume a high predominance of females although there are eight patients with this diagnosis for whom we do not know the gender. We were, however, able to assess the sex ratio within group B because we had gender information for all these patients: there were more females (64.6%) than males. Examining gender distribution in patients with the '5q- syndrome', we found than 80% of these patients were female. This is in agreement with the well-known female predominance of '5q- syndrome'.[33]

In nine cases, which were referred with the cytologic diagnosis of '5q- syndrome' without evidence of 5q- by cytogenetics, the 5q deletion was detected by FISH. In these cases, FISH helped to make the definitive diagnosis, which must be based on the presence of the cytogenetic anomaly.

Conventional banding cytogenetics and FISH techniques are both able to detect del(5q). FISH is a good technique to find the 5q deletion and it has a similar efficacy to cytogenetics. Even so, it has some limitations; it can only detect anomalies that its probes are designed to detect. FISH should not be used alone at diagnosis because of the clinical implication of the karyotype;[4,8,10,34] it is a complementary technique to achieve a more accurate cytogenetic analysis.

In conclusion, taking into account our results in a large series of cases of primary MDS studied by FISH,

we consider that it is mandatory to apply FISH of 5q31 to detect 5q deletion in cases with an abnormal karyotype involving chromosome 5 and in cases without metaphases or that are not evaluable. In cases with a normal karyotype or an abnormal karyotype without evidence of 5q-, it would be recommendable to apply FISH in order to confirm the morphological diagnosis of '5q- syndrome' and to diagnose MDS patients with 5q deletion. Both groups of patients could be candidates for treatment with lenalidomide.

Authorship and Disclosures

MM contributed to the conception and design of the study, acquisition, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content, and gave final approval of the version to be published. LA, BE, MS, JMH, EL, MdR, EA, SR, PF, OG, MR, JC, ES, GFS, EL, CS, MG, MJC, JM, CG-B, VA, RC, IO, FC, EB, AI, LY, MJM, EG-B, RA, PL, VG, ÁS, NC, EM, AA, MLM, CP, SS, and LF: referred cases and revised the final version of the manuscript. The order of the authorship was based on the contribution of each author to the design of the study, data interpretation and writing of the manuscript. FS: contributed to the study design, data interpretation, supervised the whole study and wrote and revised the last version of the manuscript. All authors approved the version to be published. The authors also reported no potential conflicts of interest. Cytogenetics and some clinical information concerning some of the patients included in the present study have been previously reported (ref. #8). Preliminary findings of this study were presented at the 49th Annual Meeting of the Asociación Española de Hematología y Hemoterapia, Pamplona, October 25-27, 2007.

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