

Comparative assessment of conventional chromosomal analysis and fluorescence *in situ* hybridization in the evaluation of suspected myelodysplastic syndromes: a single institution experience

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ABSTRACT

Background: Myelodysplastic syndromes (MDSs) are a heterogeneous group of clonal hematopoietic neoplasms, roughly half of which harbor cytogenetic abnormalities with diagnostic, prognostic, and therapeutic significance. Fluorescence *in situ* hybridization (FISH) for the most commonly seen abnormalities (5/5q, -7/7q, +8, and -20/20q-) is routinely performed alongside conventional cytogenetics (CC) in the evaluation of suspected MDS despite conflicting reports of its relative contribution compared to CC alone. **Objectives:** To assess the additional diagnostic and prognostic value of performing concurrent FISH versus CC alone in cases of suspected MDS. **Materials and Methods:** A total of 127 bone marrow samples submitted to our cytogenetic laboratory with a presumptive diagnosis of MDS were evaluated by concurrent CC and an MDS FISH panel. **Results:** CC was used as the gold standard method with 100% sensitivity in detecting suspected MDS-associated cytogenetic abnormalities. FISH alone had a sensitivity of 76%, whereas CC alone achieved a sensitivity of 97%. The addition of FISH did not change the diagnosis nor change the Revised International Prognostic Scoring System score in any patient. Moreover, in 12 cases identified as positive by both CC and FISH, CC identified multiple chromosomal aberrations of clinical significance not interrogated by the FISH probe panel. **Conclusion:** CC alone is sufficiently sensitive in detecting suspected MDS-associated cytogenetic abnormalities that influence clinical decision-making. Routine FISH testing does not provide a significant increase in test sensitivity when an adequate karyotype is obtained. Therefore, FISH testing is best reserved for suspected MDS cases lacking sufficient metaphases.

Key words: Chromosomal analysis, fluorescence *in situ* hybridization, karyotype, myelodysplastic syndrome

INTRODUCTION

Myelodysplastic syndromes (MDSs) are a group of clonal hematopoietic neoplasms characterized by ineffective hematopoiesis manifested by morphologic dysplasia, cytopenias, and increased risk of acute myeloid

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leukemia (AML). Roughly half of the patients with MDS harbor cytogenetic abnormalities, a feature that carries diagnostic, prognostic, and therapeutic significance.^[1] Cytogenetic abnormalities are disease defining in some cases and are an integral component of the Revised International Prognostic Scoring System (IPSS-R), which is predictive of overall survival and progression to AML [Table 1].^[2,3] In the case of an isolated del(5q), the presence of this abnormality defines a specific MDS subtype and predicts favorable response to lenalidomide.^[2,4] At this time, the only curative therapy is allogeneic stem cell transplantation, in which cytogenetic risk subgroup has been found to be the most important variable in predicting event-free survival.^[5,6]

The importance of accurate cytogenetic data in MDS is well established with conventional cytogenetics (CC) (karyotyping) firmly entrenched as the gold standard methodology. However, CC is a labor-intensive process, which is dependent on the malignant clone's propensity to divide in culture with analysis limited to a small number of cells (usually no more than 25). In addition, adequate interpretation can be limited by inadequate chromosome spreading and poor quality banding. The addition of interphase fluorescence *in situ* hybridization (FISH) has permitted more rapid quantitative detection of specific genetic alterations in large numbers of non-mitotic cells. In particular, the most common chromosomal abnormalities in MDS, -5/5q, -7/7q, +8, and del(20q), are now routinely tested by FISH, often in parallel with CC. FISH, however, can only detect the presence or absence of the specific chromosomal abnormalities for which the probe is intended to detect, whereas CC offers an unbiased analysis of chromosomal number and structure. Several studies evaluating the

added value of FISH to CC have generated mixed results.^[7-17] In this study, we evaluated 127 patients with a presumptive diagnosis of MDS using CC and FISH to assess the additional diagnostic and prognostic yield over conventional karyotyping alone.

MATERIALS AND METHODS

Patient samples and diagnosis

One hundred and thirty-five bone marrow aspirates collected from 63 female and 72 male patients submitted to the cytogenetic laboratory at Detroit Medical Center University Laboratories with a presumptive diagnosis of MDS between 2010 and 2012 were retrospectively identified and selected for this study. The inclusion criteria consisted of patients with at least one persistent cytopenia and clinical suspicion of MDS after exclusion of nutritional and elemental deficiencies, toxins/drugs, autoimmune diseases, congenital disorders, infections, and other neoplastic conditions. Comprehensive evaluation of the electronic medical record, peripheral blood smear, and aspirate/biopsy material was performed by an experienced board-certified hematopathologist. Concurrent, CC analysis and MDS FISH panel were performed on all cases independent of the morphologic assessment.

Conventional cytogenetics

Bone marrow aspirates were cultured without mitogens for 24 h, followed by 48 h in 10% conditioned medium. G-banding was performed using the Giemsa-trypsin-Leishman banding technique. Metaphase cells from unstimulated culture were examined and karyotype was defined in accordance with the International System for Human Cytogenetic Nomenclature (ISCN) on 20 metaphase cells per patient.

FISH

FISH was performed on all cases using the MDS panel DNA probes that included D5S23:D5S721/5p15.2, EGR1/5q31, D7Z1/CEP-7, D7S486/7q31, D8Z2/CEP-8, and D20S108/20q12. All FISH probes were purchased from Abbott Molecular, Downers Grove IL, USA. 200 interphase cells were examined for each probe to detect -5/5q, -7/7q, +8, and del(20q): 5p15.2 (LSI D5S23), 5q31 (LSI EGR1) (6%), CEP 7, 7q31 (LSI D7S486) (4.4%), CEP 8 (2.3%), and 20q12 (LSI D20S108) (5.7%). Cutoff values, as listed in parentheses, were determined using data from at least 10 normal patients to calculate false-positive cells using a binomial statistical formula to project the upper boundary of the 95th percentile (Standard Cumulative Beta Distribution). An experienced cytotechnologist blinded to the karyotypes analyzed 200 interphase nuclei per case

Table 1: Revised International Prognostic Scoring System (IPSS-R) cytogenetic categories

Very good	-Y del(11q)
Good	Normal Del(5q) Del(12p) Del(20q) Double including del(5q)
Intermediate	Del(7q) +8 +19 i(17q) Any other single or double independent clones
Poor	-7 inv(3)/t(3q)/del(3q) Double including -7/del(7q) Complex: 3 abnormalities
Very poor	Complex >3 abnormalities

without computer assistance. Results were reported using 2009 ISCN.

RESULTS

Bone marrow samples from 127 patients with presumptive MDS were analyzed by both metaphase CC and interphase FISH. The patients included 64 males and 63 females with a median age of 64 years (ranging from 4 to 91 years).

FISH and CC results were concordant in 117 patients (117/127; 92%). A tabulation of the concordant/discrepant results is shown in Table 2. Eighty-nine patients (89/127; 70%) showed normal karyotypes with no abnormalities detected by FISH. In 28 patients (28/127; 22%), CC revealed clonal abnormalities and abnormal results for at least one FISH probe. The most common FISH abnormality was deletion 5q observed in 13 patients, followed by monosomy 7 in 9 patients, deletion 20q in 8 patients, trisomy 8 in 6 patients, as well as 2 patients each with monosomy 5 and deletion 7q. All of these FISH abnormalities were also detected by CC. Of the 28 patients with abnormalities detected both by FISH and CC, eight were diagnosed with MDS with multilineage dysplasia (MDS-MLD); seven with MDS with excess blasts (MDS-EB); three each with therapy-related MDS (TR-MDS); and AML with myelodysplasia-related changes (AML-MRC); and two cases each with chronic myelomonocytic leukemia (CMML), MDS with isolated deletion (5q), and refractory cytopenia of childhood. One patient showed no morphologic evidence of myelodysplasia or other diagnostic abnormality on bone marrow morphologic evaluation. Details of these clonal defects are summarized in Table 3. Among the patients with abnormalities detected by both FISH and CC, additional chromosomal abnormalities including rings, deletions, translocations, inversions, and markers were observed in 15 of the 28 samples (54%), including 12 cases in which the additional/complex abnormalities altered the IPSS-R cytogenetic risk category. Three of these twelve cases with complex karyotypes showed only del(5q) by FISH.

Discordant results between CC and FISH were seen in 10 (10/127; 8%) patients. Nine (9/127; 7%) of those patients

were found to have clonal abnormalities by CC with no abnormal findings on FISH. The findings in these patients are summarized in Table 4. The discrepancies included four patients with MDS-MLD, one patient with MDS with single lineage dysplasia (MDS-SLD), one patient with MDS-EB, and three patients who showed no evidence of myelodysplasia on bone marrow morphologic evaluation. Loss of chromosome Y was observed in the three morphologically normal patients including one patient with trisomy 14 in addition to -Y. In one (1/127; 0.8%) case of cytogenetically normal MDS-MLD, FISH detected an additional fragment (marker) of chromosome 8 interpreted as a trisomy. On reviewing the CC data, this fragment was identified *post hoc* and was missed during the initial analysis because of its small size.

DISCUSSION

The identification of cytogenetic abnormalities is of vital importance in the diagnosis, prognosis, and therapeutic decision-making for patients with MDS. The gold standard remains CC, which, under ideal conditions, detects a wide variety of numerical and structural aberrations associated with myeloid malignancy. FISH has emerged as an alternative method of evaluating nondividing (interphase) nuclei for targeted diagnostic and prognostic abnormalities and is particularly useful as a complementary method to detect cryptic genetic abnormalities and small clones that may otherwise fall below the limit of detection of conventional karyotyping. Published studies offer conflicting reports about the added benefit of FISH over CC in patients with MDS. In this study, we compared FISH and CC findings in 127 patients with presumptive MDS, evaluating the concordance/discordance between the two methods and assessing the impact of using both modalities in accurately diagnosing and risk-stratifying patients as opposed to conventional karyotyping alone.

Several studies have suggested a significant role for FISH analysis, especially in chromosomally normal patients with MDS/AML.^[9-11] In a study of 57 chromosomally normal patients with MDS, Bernasconi *et al.*^[9] detected occult chromosomal abnormalities by FISH in 15% of patients, resulting in a change in IPSS for five of the nine patients. Furthermore, FISH positivity was associated with an eightfold increase in progression to advanced MDS or AML. These findings were corroborated by Rigolin *et al.*^[10] whose analysis of 101 consecutive patients of MDS with normal karyotypes found occult chromosomal abnormalities by FISH in 18 patients (17.8%) and were associated with higher risk disease and worse outcomes as compared to FISH-negative patients. Of note, a third of the FISH-positive patients showed trisomy 8—a chromosomal abnormality—that is

Table 2: Summary of fluorescence *in situ* hybridization and conventional chromosomal analysis

FISH	Conventional chromosomal analysis		Total
	Normal	Abnormal	
Normal	89	9	98
Abnormal	1	28	29
Total	90	37	127

FISH = fluorescence *in situ* hybridization

Table 3: Summary of 28 patients with abnormal findings on fluorescence *in situ* hybridization and conventional chromosomal analysis

Sex	Age (years)	Final diagnosis	FISH	Karyotype
M	63	TR-MDS	7q-/-7	46,XY,r(7)(q11.1p22),t(15;21)(q10;q10)[cp2]/45,XY,-7,t(15;21)(q10;q10)[cp2]/46,XY[1]
M	77	MDS-MLD	5q-/20q	45,XY,-1,ins(1;7)(p13p36;p15),add(3)(p25),t(3;11)(q27;q13),del(5)(q13q33),add(12)(p13),der(16)t(1;16)(q21;p13.3),del(20)(q11.2q13.3),+r[17]/46,XY[3]
F	71	CMML	-7	45,XX,-7[18]/46,XX[2]
F	69	MDS w/ isolated del(5q)	5q-	46,XX,del(5)(q13q33)[9]/46,XX[11]
F	71	MDS-EB2	+8	47,XX,+8[6]/46,XX[14]
M	69	MDS-MLD	+8	47,XY,+8[11]/46,XY[9]
M	68	MDS-MLD	-7/+8	45,XY,t(1;7)(q43;q11.2),-3,del(3)(q21q27),del(5)(q13q35),del(7)(q11.2),+8,inv(9)(q31q34),der(12;17)(q10;q10),-16,add(16)(q11.2),-17,add(21)(p11.2),+mar[cp20]
M	70	MDS-EB2	+8	47,XY,+8[3],46,XY[14]
M	74	MDS-MLD	5q-/-7/20q-	45,XY,add(5)(q13),-7,del(18)(q11.1q11.3),add(20)(q11.2),dic(22;7)(p11;7)[cp17]/46,XY[3]
F	50	TR-MDS	5q-/+8	44,XX,add(2)t(2;4)(p23;q25),-4,del(5)(q23q35),+8,add(8)(q13),-13[cp8]/46,XX[cp12]
F	4	RCC w/ fibrosis	-7	45,XX,-7[19]/46,XX[1]
F	76	CMML	+8	47,XX,+8[2]/46,XX[18]
F	73	MDS-EB2	-7	43-45,XX,del(1)(p21),-7,der(9;22)(q10;q10),-9,inv(12)(q21q24),-19,+1-3mar,-10dmin[cp18]/46,XX[2]
M	61	MDS-MLD	5q-	45,XY,del(5)(q31),t(6;19)(q24;p13.3),add(8)(p23),-16[11]/45,XY,der(5;18)(p10;q10)[9]/46,XY[1]
F	89	AML-MRC	5q-	46,XX,del(5)(q13q33)[6]/43-44,XX,der(1)dup(1)(p34p22)t(1;17)(q32;q21),inv(3)(q21q26),-3,der(4)t(3;4)(q21;p16),del(5)(q13q33),der(7)t(7;9)(q10;q10),-17[cp14]
F	61	MDS-MLD	-7/5q-	45,XX,del(5)(q13q33),-7[9]/45,XX,del(5)(q13q35),-7,del(16)(q22)[5]/46,XX[6]
M	61	MDS-EB2	5q-/-7	43-44,XY,del(5)(q13q33),der(12)t(7;12)(p10;p12),-13,-7,-21,+r,+mar[15]/43-44,XY,idem,del(2)(q24q33),add(5)(q23),add(16)(q24)[3]/46,XX[2]
F	65	MDS-EB1	5q-	46,XX,t(3;5)(q27;q31)[10]/46,XX[10]
M	49	MDS-MLD	20q-	46,XY,del(20)(q11.2q13.3)[18]/46,XY[2]
F	80	MDS w/ isolated del(5q)	5q-	46,XX,del(5)(q13q33)[16]/46,XX[4]
F	76	Neg for MDS	20q-	46,XX,del(20)(q11.2)[6]/46,XX[14]
M	86	MDS-EB1	-5/-7/20q-	40-42,XY,-5,-7,t(9;19)(q22;p13.1),-11,der(11)t(11;12)(p15;q13) or der(12)t(12;13)(p13;q12),-13,-16,add(18)(q22),-19,del(20)(q11.2q13),+r,+mar[cp13]/46,XY[7]
F	41	TR-MDS	5q-/-5/20q-	44-45,XX,t(1;4)(p13;p16),der(3)t(3;15)(q21;q15),add(3)(p12),t(4;11)(q21;q23) or del(4)(q21),del(5)(q13q33) or -5,add(13)(q34),-15,-17,-20,+mar[cp19]/46,XX[1]
M	75	AML-MRC	5q-/-7q-	45-47,XY,+4,der(5)t(5;17)(p10;q10),del(7)(q21q32),+13,der(13)t(13;15)(p11;q15),add(16)(q24),-19,+21[cp15]/46,XY[5]
F	69	AML-MRC	20q-	46,XX,del(20)(q11.2q13.3)[13]/46,XX[7]
M	69	MDS-EB2	20q-	46,XY,del(20)(q11.2q13.3)[1]/46,XY[19]
F	4	RCC	-7	45,XX,-7[17]/46,XX,-7,+21[3]
F	61	MDS-MLD	5q-	-44,XX,del(5)(q13q33),add(6)(p22),-7,-8,t(9;15)(p10;q10),add(12)(p12),-16,+r[cp14]/46,XY[6]

AML-MRC = acute myeloid leukemia with myelodysplastic-related changes, CMML = chronic myelomonocytic leukemia, FISH = fluorescence *in situ* hybridization, MDS = myelodysplastic syndrome, MDS-EB1 = myelodysplastic syndrome with excess blasts type 1, MDS-EB2 = myelodysplastic syndrome with excess blasts type 2, MDS-MLD = myelodysplastic syndrome with multilineage dysplasia, MDS w/isolated del(5q) = myelodysplastic syndrome with isolated deletion 5q, Neg = negative, RCC = refractory cytopenia of childhood, TR-MDS = therapy-related myelodysplastic syndrome

Table 4: Summary of nine patients with normal fluorescence *in situ* hybridization and abnormal karyotype

Sex	Age (years)	Final diagnosis	Karyotype
M	61	MDS-MLD	47,XY,+19[16]/46XY[4]
M	69	Neg for MDS	45,X,-Y[cp7]/46,XY[13]
F	88	MDS-MLD	46,XX,t(1;4)(q25;q21)[4]/46,XX[16]
F	42	MDS-MLD	46,XX,t(10;14)(q23;q32)[4]/46,XX,t(6;13)(p25;q33),i(8)(q10),t(10;14)(q23;q32)[6]/51,XX,+3,+4,del(6)(q21q27),i(8)(q10),+10,t(10;14)(q23;q32),+16,+21[11]/46,XX[4]
M	91	MDS-EB1	45,X,-Y[19]/46,XY[1]
M	83	Neg for MDS	45,X,-Y[5]/46,X,-Y,+14[4]/46,XY[11]
M	89	Neg for MDS	45,X,-Y[7]/46,XY[13]
F	68	MDS-SLD	46,XX,t(4;7;15)(p15;q32;q26)[20]
M	64	MDS-MLD	46,XY,t(1;2)(p36.2;q21)[18]/46,XY[2]

MDS = myelodysplastic syndrome, MDS-EB1 = myelodysplastic syndrome with excess blasts type 1, MDS-MLD = myelodysplastic syndrome with multilineage dysplasia, MDS-SLD = myelodysplastic syndrome with single lineage dysplasia, Neg = negative

no longer considered disease defining, though remains part of the IPSS-R cytogenetic risk stratification schema.^[2] Romeo *et al.*^[11] similarly found that FISH testing conferred a benefit to 4/40 patients (10%) by identifying cytogenetic abnormalities in three patients with normal karyotypes and one patient with no harvestable metaphases for CC.

Other studies, however, have found only a modest benefit to using complementary FISH in patients with MDS.^[13] For instance, Beyer *et al.*^[12] investigated 110 patients with AML and high-risk MDS using probes directed at chromosomes 5, 7, and 8 and found discordance between CC and FISH in only 7.3% of patients, concluding that the primary use of FISH in these cases was to increase the resolution of CC by identifying marker chromosomes and breakpoints as well as detecting minor clones with chromosome 8 aneuploidy. Cherry *et al.*^[13] reiterated the superiority of CC over FISH in a study of patients with predominantly low-risk MDS in which CC and FISH results (probes for $-5/5q-$, $-7/7q-$, $+8$, $11q23$ abnormalities, $13q-$, and $20q-$) were concordant in 46 of 48 patients with an occult $11q$ abnormality detected by FISH in just one of the 30 cytogenetically normal patients (3.3%). Similar results were obtained by Ketterling *et al.*^[14] who tested a wider array of probes by both interphase and metaphase FISH and detected an occult abnormality in just one of the 32 cytogenetically normal patients. More recent studies by Pitchford *et al.*^[15] and Costa *et al.*^[16] have found concordance rates of >99% when adequate high-quality karyotypes can be obtained, asserting that FISH does not confer additional value in those cases. Pitchford *et al.*^[15] further concluded that restricting FISH testing to those patients without adequate karyotypes could generate more savings along with 8.7 weeks of cytotechnologist time in their institution alone. This limited approach is endorsed by He *et al.*^[17] after evaluating 505 patients with possible MDS and finding that FISH results had no diagnostic and only minimal prognostic impact when ≥ 20 metaphases were analyzed by CC.

Our findings align with the latter group with a very low rate of occult FISH abnormalities in our cohort (1/127; 0.8%), very similar to that of Pitchford *et al.*^[15] Although the concordance rate was high (92%), CC yielded additional and potentially clinically meaningful results. Among the concordant cases, CC detected a complex karyotype in 12% (14/117). Four of these patients were found to only have $\text{del}(5q)$ or $\text{del}(20q)$ by FISH, which, in the absence of other karyotypic findings, would incorrectly classify these patients into a more favorable prognostic category. Furthermore, CC was able to detect additional chromosomal abnormalities including rings, deletions, translocations, inversion, and

markers in the majority (15/28; 54%) of FISH-positive cases. The superiority of CC was also shown among the 10 discordant cases, 9 of which showed clonal abnormalities by CC but were negative by FISH. A single patient was found to have trisomy 8 by FISH with normal CC accounting for 1% of the cytogenetically normal patient cohort, although this additional finding would not have changed the diagnosis or the IPSS-R in this patient.

The discrepancies seen among studies may be at least partially explained by the variability of cutoff values used to assess FISH positivity. The highest discordance rates appear in studies with the lowest cutoff values.^[16] In addition, the experience and expertise of the individual cytogenetic laboratories and their personnel may vary between institutions. Our experience suggests that cytotechnologist selection and spreading bias of metaphase nuclei may result in missing undetected aneuploidies, complex metaphases with overlapping chromosomes, and small abnormal clones. Finally, our study largely analyzed cases in which at least 20 metaphases were cultured. FISH may have use in cases in which no metaphases or less than 20 metaphases were available for analysis.

CONCLUSION

In conclusion, our data confirm the superiority of CC to FISH in the initial cytogenetic analysis of MDS cases. These findings contribute to the growing body of evidence that FISH should not be used to replace cytogenetic analysis. In addition, FISH does not add significant diagnostic or prognostic value in the setting of an adequate karyotype by CC. FISH can be a valuable complementary tool to identify marker chromosomes and breakpoints in patients with karyotypically abnormal MDS as well as to unmask minor clones with aneuploidy of chromosome 8, once a diagnosis has been established. We recommend that FISH not be routinely performed in the initial diagnostic workup of suspected MDS unless an adequate karyotype cannot be obtained.

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Conflicts of interest

There are no conflicts of interest.

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