Published in final edited form as:

Int J Lab Hematol. 2010 August 1; 32(4): 443–448. doi:10.1111/j.1751-553X.2009.01210.x.

Myelodysplastic syndrome in elderly patients: correlation of CBC with cytogenetic and FISH analysis

K.-E. T. Codispoti and L. Depalma

Department of Pathology, The George Washington University Medical Center, NW, Washington, DC, USA

Summary

Unexplained anemia in the elderly could represent myelodysplastic syndrome (MDS). We assessed the utility of using a fluorescence in situ hybridization (FISH) panel for common chromosomal abnormalities seen in MDS. A total of 101 elderly outpatients with anemia of unknown etiology were evaluated. Complete blood count, bone marrow biopsy, conventional cytogenetic analysis (CC), and FISH panel were reviewed. A total of 21 (21%) of the 101 patients had MDS. A combination of CC and FISH identified chromosomal abnormalities in 17 (81%) of the patients with MDS. The remaining 4 (19%) were diagnosed with MDS based solely on morphologic criteria. Except in two cases, FISH did not reveal abnormalities not already detected by CC. Furthermore, MDS patients infrequently had isolated anemia (14%) as opposed to those without MDS (75%). A MDS FISH panel is not more sensitive than CC in elderly outpatients with unexplained anemia. MDS is more likely if in addition to anemia, leukopenia and/or thrombocytopenia are also present.

Keywords

MDS; FISH; anemia of elderly

Introduction

A substantial proportion of the American elderly population is anemic and the presence of anemia has been associated with adverse outcomes, including increased mortality (Joosten, 2004; Guralnik *et al.*, 2005). A national survey found the most common causes of anemia in the elderly to be nutritional deficiency and chronic disease/inflammation, which combined represent close to 60% of cases (Guralnik *et al.*, 2004). The diagnosis of anemia of chronic disease is often made when the constellation of low serum iron, low total iron binding capacity (TIBC), low serum transferrin, low iron saturation index, and normal or elevated ferritin are seen in the setting of a chronic disease (Joosten, 2004). Although the standard for iron deficiency is the absence of iron stores in the bone marrow, biochemical markers can suggest the diagnosis (Joosten, 2004). Despite the large number of patients who fit into these two categories, over 1/3 of cases of anemia in the elderly remains unexplained. Potential factors that may play a unique role in the development of anemia in the elderly include blunting of the hypoxia/erythropoietin sensing mechanism, decreased muscle mass, altered stem cell physiology, and decreased levels of sex steroids (Guralnik *et al.*, 2004). Importantly, a significant proportion of these unexplained anemias could represent early or undiagnosed MDS (Steensma & Tefferi, 2007).

Myelodysplastic syndromes are clonal stem cell disorders that result in peripheral blood cytopenias due to defective, dysplastic maturation (Bernasconi *et al.*, 2006; Nimer, 2008). The current WHO classification recognizes seven subtypes of which one, MDS with 5q-, is defined by a cytogenetic abnormality (Bernasconi *et al.*, 2006). Although not currently specific for the remaining categories of MDS, altered cytogenetics not only confirm the clonality of the process, they play a significant role in predicting clinical outcome (Bernasconi *et al.*, 2006; Nimer, 2008). The introduction of fluorescence in situ hybridization (FISH) brought a sensitive technique which can be performed on nondividing cells (Cherry *et al.*, 2003). Altered chromosomes are often only found in a subset of cells in patients with MDS and FISH offers increased sensitivity as many more cells are able to be analyzed when compared with CC (Steensma & List, 2005). At present, CC and FISH are viewed as complementary tests in the evaluation of MDS (Bernasconi *et al.*, 2006; Steensma & List, 2005).

Currently the evaluation of anemia involves a combination of clinical and laboratory findings and often includes bone marrow biopsy. A standard FISH panel for common aberrations seen in MDS is now commonly performed. We hypothesized that unexplained anemia in the elderly could represent early MDS and assessed the sensitivity of a FISH panel as compared to morphology and CC in detecting changes associated with MDS in elderly patients with unexplained anemia who underwent bone marrow biopsy.

Methods

All outpatient bone marrow biopsies submitted to our institution during the years 2006–2008 were reviewed to identify those which were submitted to evaluate anemia (defined according to WHO criteria as <13 g Hb/dl (130 g/l) for men and <12 g Hb/dl (120 g/l) for women) with or without additional cytopenias (Guralnik *et al.*, 2005). Patients under the age of 65 and those who did not have both CC and FISH analysis performed were excluded.

All specimens had an aspirate stained by standard methods with Wright-Geimsa and Prussian blue for iron as well as formalin fixed core biopsy stained separately with hematoxylin and eosin. Concurrent complete blood count, red cell indices, serum vitamin B12, folic acid and erythropoietin were reviewed for each patient. CC analysis and FISH analysis were performed by a standard technique. CC consisted of tissue culture of lymphocytes for 24 h with 20 metaphases evaluated. FISH analysis was performed using probes for 5p15.2 (D5S23, D5S72), 5q31 (EGR1), 7cen (CEP7), 7q31 (D7S486), 8cen (CEP8), and 20q12 (D20S108) (Vysis, Abbott Molecular, Abbott Park, IL, USA). Interphase nuclei were scored to detect gain, loss, or chromosome rearrangements of the probe regions (loss if $\geq 5\%$ abnormal nuclei; gain if $\geq 2\%$ abnormal nuclei). The number of nuclei scored is 200 for normal results (2 techs, 100 each), and 100 for abnormal results (2 techs, 50 each).

Patients were included in the MDS group if they had either moderate dysplasia ($\geq 10\%$ of erythroid, granulocytic or megakaryocytic lineage), and/or a positive CC and/or positive FISH, with normal vitamin B12 and folic acid lab results. Based on a blinded, retrospective re-evaluation of the material, there was no significant inter-observer variability of morphologic findings between the authors and two additional staff hematopathologists. Patients were deemed negative for MDS if they lacked completely morphologic, cytogenetic, and FISH findings diagnostic for MDS. These patients were classified as anemia due to iron deficiency, anemia of chronic disease, or anemia of unknown cause based upon the iron stain evaluation in their bone marrow aspirate, and negative test results for serum vitamin B12, folic acid, and serum erythropoietin.

Results

A total of 101 outpatients met the inclusion criteria (Table 1). Based on our study criteria, there were 21 patients (21%) who were diagnosed with MDS. Thirteen (62%) of these patients were male and 8 (38%) patients were female. The mean age of MDS patients was 78.7 (65–96). There were 80 patients who were negative for MDS criteria. Fifty (62.5%) were male and 30 (37.7%) were female. The mean age of the non-MDS patients was 75.7 (66–91).

The mean hemoglobin (Hgb) for patients with MDS was 10 g/dl (100 g/l) [8–12.9 g/dl (80–129 g/l)]. Three (14%) MDS patients had isolated anemia, eight (38%) had anemia and leukopenia, two (10%) had anemia and thrombocytopenia, and eight (38%) had pancytopenia. The mean Hgb for non-MDS patients was 10.8 g/dl (108 g/l) [7.3–12.9 g/dl (73–129 g/l)]. The mean MCV was not significantly different between the two groups (MDS mean MCV 96.3 ± 13.17 , non-MDS mean MCV 92.15 ± 6.73 ; $P > 0.05$). Sixty (75%) non-MDS patients had isolated anemia, six (7.5%) had anemia and leukopenia, six (7.5%) had anemia and thrombocytopenia, and eight (10%) had pancytopenia (Table 2).

A combination of CC and FISH identified characteristic chromosomal abnormalities in 17 (81%) of the 21 MDS patients (Table 3). The most common cytogenetic abnormality found in patients with MDS was trisomy 8 which was seen in 7 (33%) patients. Of the seven trisomy 8 patients, two patients had isolated anemia, two patients had associated leukopenia, two patients had associated thrombocytopenia, and one patient had pancytopenia. The remaining 4 (19%) MDS patients were diagnosed solely on WHO morphologic criteria. Three (14%) of the patients had total (2) or partial (1) incongruent CC and FISH results (Table 3). The two total disagreements consisted of one patient who's CC revealed a del 20 with a negative FISH, and one patient with FISH showing del 7 with a negative CC. The partial incongruent result was the finding of a del 7 in FISH analysis. However, the remaining abnormalities in the patient (del 5, del 20) were seen in both CC and FISH. Except in two cases, FISH did not reveal abnormalities not already detected by CC.

Of the four MDS patients diagnosed solely by morphology, three had a combination of anemia and leukopenia and were diagnosed with refractory anemia, refractory anemia with ring sideroblasts, and refractory anemia with excess blasts II respectively. The patient who presented with pancytopenia was diagnosed with refractory anemia with ring sideroblasts (Table 4).

Of the 80 patients who did not have morphologic or chromosomal findings of MDS, 62 (78%) had anemia of chronic disease, 7 (9%) had iron deficiency, and 11 (14%) had an unknown cause for their anemia.

Discussion

We determined the incidence of MDS in elderly outpatients presenting with isolated anemia or in association with other cytopenias and for whom initial laboratory tests did not disclose a specific etiology. MDS is characterized by cytopenias due to impaired blood cell production and patients often initially present with symptoms of anemia which leads to clinical evaluation including bone marrow biopsy (Nimer, 2008; Steensma *et al.* 2005). Not surprisingly, our study of 101 elderly outpatients found a majority of patients, 62 of 101 (62%), to have anemia of chronic disease. It is of note that only 9% of our patients had anemia due to iron deficiency which is much less than the expected one-third reported in larger population surveys (Guralnik *et al.*, 2004). This is perhaps due to a referral bias in that most of our cases came from patients who were seen in the outpatient hematology clinic and perhaps those elderly individuals with anemia due to iron deficiency were diagnosed based on laboratory studies alone and were not

referred for a hematology consult. The combined incidence of anemia of chronic disease and iron deficiency in our study is in range with that reported by the above cited national survey.

There does not appear to be a consensus regarding the incidence of MDS in the elderly patient population. However, the second largest cause for anemia in our patients was MDS (21%). Our data indicates an interesting correlation between the number of cytopenias and the likelihood of MDS. The majority (86%) of elderly patients with anemia who were found to have MDS also had the presence of leukopenia, thrombocytopenia, or even overt pancytopenia. In contrast, elderly patients who had anemia due to chronic disease, iron deficiency, or unknown causes overwhelmingly had isolated anemia (75%) with only a minority having additional cytopenias. The hemoglobin level and MCV were not helpful in separating patients with MDS from those without MDS. The number of cytopenias has been a component of the International Prognostic Scoring System (IPSS) (Kao, McMillan & Greenberg, 2008). Our data indicate that evaluating the number of cytopenias may play a role in stratifying patients regarding their likelihood of having MDS. The strength of the association may be limited by small sample size, but suggests a potential important parameter in the initial diagnostic evaluation of elderly patients with anemia. This association is supported by data collected during a recent cross-sectional survey of physicians that found the majority of recently diagnosed MDS patients had mild thrombocytopenia and neutropenia in addition to their anemia (Sekeris *et al.*, 2008).

The current WHO classification scheme for MDS is based on morphologic findings. However, recurrent common chromosomal abnormalities have been identified in MDS and are found in 40–70% of *de novo* cases and 95% of secondary cases (Bernasconi *et al.*, 2006, 2007). These chromosomal aberrations include 5q-, 7q-/monosomy 7, trisomy 8, 20q-, deletion 12p, as well as abnormalities in 17p, 11q23, and chromosome 3 (Sole *et al.*, 2005; Bernasconi *et al.*, 2006; Nimer, 2008). Cytogenetic evaluation is important in the evaluation of MDS as the presence of chromosomal abnormalities is included in the IPSS and specific findings have important prognostic significance (Bernasconi *et al.*, 2006; Nimer, 2008; Steensma *et al.*, 2005; Bernasconi *et al.*, 2007; Romeo *et al.*, 2002). Favorable prognostic markers include a normal karyotype, deletion 5q as an isolated anomaly, deletion 20q as an isolated anomaly, and loss of the Y chromosome. Karyotypic findings associated with poor prognosis include complex karyotypes and abnormalities of chromosome 7. Other CC abnormalities confer an intermediate prognosis (Sole *et al.*, 2005).

In addition to aiding in prognosis, CC plays a role in morphologically ambiguous cases as the finding of a cytogenetic abnormality can support the diagnosis of MDS (Steensma *et al.*, 2005). CC is also instructive in patients with anemia who have a morphologically normal bone marrow biopsy but have chromosomal abnormalities commonly seen in MDS. Data from Steensma *et al.* (2003) support this concept as they found cytogenetic abnormalities in patients without morphologically diagnosable MDS have an increased risk of leukemic transformation as well as mortality due to cytopenias.

FISH analysis has been shown to be important in defining cryptic and occult chromosomal abnormalities in patients with known MDS and normal karyotypes (Bernasconi *et al.*, 2003). FISH is able to identify deletions that are too small to resolve with CC (Rigolin *et al.*, 2001; Ketterling *et al.*, 2002). Rigolin *et al.* (2001) examined 101 patients with MDS and normal karyotypes. They found FISH abnormalities in 17.8% and these abnormalities occurred more frequently in patients with increased bone marrow blasts and ultimately a higher rate of progression to acute myelogenous leukemia and a worse prognosis (Rigolin *et al.*, 2001). However, the incidence of cryptic deletions in MDS patients varies and some studies have found only a single patient who benefited from FISH analysis in addition to CC (Ketterling *et al.*, 2002). Thus the literature is unclear regarding the relative value of CC and FISH. These

two methods may play a complimentary role in the evaluation of MDS in certain patient groups (Steensma *et al.* 2005).

The majority of studies using a FISH panel for common chromosomal abnormalities in MDS examine the ability of FISH to identify cryptic abnormalities in patients with a known diagnosis. Our interest in this study was to determine if FISH could identify clonal abnormalities in the elderly population associated with MDS. Although the majority of these chromosomal aberrations are not currently part of the diagnostic criteria for the majority of MDS subtypes, the information can provide a valuable tool in the continued evaluation and monitoring of these elderly individuals with anemia. However, our results show that a FISH panel for common chromosomal abnormalities in MDS is not more sensitive than CC in the elderly population with unexplained anemia. FISH results confirmed the morphologic findings and CC results in the majority of the patients with MDS and do not appear to add information. Importantly, FISH did not agree with CC in only a minority of cases (3/21 or 14%). Most cases of anemia in patients without positive CC or FISH could be explained as a result of chronic disease or iron deficiency. Our findings suggest that a detailed clinical history and laboratory evaluation in combination with bone marrow biopsy and CC remain the keys to an accurate diagnosis of anemia in the elderly. A minority of patients in our study did not have a definitive cause for their anemia identified after evaluation of their bone marrow and neither CC nor FISH added additional diagnostic information. In this study, CC did not reveal abnormalities also known to be associated with MDS (chromosomes 11, 12, 13, and 17). It is unlikely that utilization of FISH probes for these defects may have significantly increased the diagnosis of MDS with this study. Although our results do not support the utilization of the current FISH panel for evaluation of anemia in the elderly, it may play a role in the future as information regarding the cytogenetic abnormalities in MDS is refined and allows for the incorporation of new probes into the current FISH panel.

References

- Bernasconi P, Cavigliano PM, Boni M, Calatroni S, Klersy C, Giardini I, Rocca B, Crosetto N, Caresana M, Lazzarino M, Bernasconi C. Is FISH a relevant prognostic tool in myelodysplastic syndromes with a normal chromosome pattern on conventional cytogenetics? A study on 57 patients Leukemia 2003;17:2107–2112.
- Bernasconi P, Boni M, Cavigliano PM, Calatroni S, Giardini I, Rocca B, Zappatore R, Dambruoso I, Caresana M. Clinical relevance of cytogenetics in myelodysplastic syndromes. Annals New York Academy of Sciences 2006;1089:395–410.
- Bernasconi P, Klersy C, Boni M, Cavigliano PM, Calatroni S, Giardini I, Rocca B, Zappatore R, Caresana M, Dambruoso I, Lazzarino M, Bernasconi C. World Health Organization classification in combination with cytogenetic markers improves the prognostic stratification of patients with *de novo* primary myelodysplastic syndromes. British Journal of Haematology 2007;137:193–205. [PubMed: 17408458]
- Cherry AM, Brockman SR, Paternoster SF, Hicks GA, Neuberg D, Higgins RR, Bennett JM, Greenberg PL, Miller K, Tallman MS, Rowe J, Dewald GW. Comparison of interphase FISH and metaphase cytogenetics to study myelodysplastic syndrome an Eastern Cooperative Oncology Group (ECOG) study. Leukemia Research 2003;27:1085–1090. [PubMed: 12921944]
- Guralnik JM, Eisenstaedt RS, Ferrucci L, Klein HG, Woodman RC. Prevalence of anemia in persons 65 years and older in the United States: evidence for a high rate of unexplained anemia. Blood 2004;104:2263–2268. [PubMed: 15238427]
- Guralnik JM, Erschler WB, Schrier SL, Picozzi VJ. Anemia in the elderly: a public health crisis in hematology. Hematology American Society of Hematology Education Program 2005:528–532.
- Joosten E. Strategies for the laboratory diagnosis of some common causes of anemia in elderly patients. Gerontology 2004;50:49–56. [PubMed: 14963371]

- Kao J, McMillan A, Greenberg P. International MDS risk analysis workshop (IMRAW)/IPSS reanalyzed: impact of cytopenias on clinical outcomes in myelodysplastic syndromes. *American Journal of Hematology* 2008;83:765–770. [PubMed: 18645988]
- Ketterling RP, Wyatt WA, VanWier SA, Law M, Hodnefield JM, Hanson CA, Dewald GW. Primary myelodysplastic syndrome with normal cytogenetics: utility of 'FISH panel testing' and M-FISH. *Leukemia Research* 2002;26:235–240. [PubMed: 11792411]
- Nimer SD. Myelodysplastic syndromes. *Blood* 2008;111:4841–4851. [PubMed: 18467609]
- Rigolin GM, Bigoni R, Milani R, Cavazzini F, Roberti MG, Bardi A, Agostini P, Della Porta M, Tieghi A, Piva N, Cuneo A, Castoldi G. Clinical importance of interphase cytogenetics detecting occult chromosome lesions in myelodysplastic syndromes with normal karyotype. *Leukemia* 2001;15:1841–1847. [PubMed: 11753603]
- Romeo M, Chauffaille M, Silva M, Bahia DMM, Kerbaui J. Comparison of cytogenetics with FISH in 40 myelodysplastic syndrome patients. *Leukemia Research* 2002;26:993–996. [PubMed: 12363467]
- Sekeres M, Schoonen M, Kantarjian H, List A, Fryzek J, Paquette R, Maciejewski JP. Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. *Journal of the National Cancer Institute* 2008;100:1542–1551. [PubMed: 18957672]
- Sole F, Luño E, Sanzo C, Espinet B, Sanz GF, Cervera J, Calasanz MJ, Cigudosa JC, Milla F, Ribera JM, Bureo E, Martin ML, Arranz E, Florensa L. Identification of novel cytogenetic markers with prognostic significance in a series of 968 patients with primary myelodysplastic syndromes. *Haematologica* 2005;90:1168–1178. [PubMed: 16154839]
- Steensma DP, List AF. Genetic testing in the myelodysplastic syndromes: molecular insights into hematologic diversity. *Mayo Clinic Proceedings* 2005;80:681–698. [PubMed: 15887439]
- Steensma DP, Tefferi A. Anemia in the elderly: how should we define it, when does it matter, and what can be done? *Mayo Clinic Proceedings* 2007;82:958–966. [PubMed: 17673065]
- Steensma DP, Dewald GW, Hodnefield JM, Tefferi A, Hanson CA. Clonal cytogenetic abnormalities in bone marrow specimens without clear morphologic evidence of dysplasia: a form fruste of myelodysplasia? *Leukemia Research* 2003;27:235–242. [PubMed: 12537976]

Table 1

Patient groups

	MDS <i>n</i> = 21	Negative for MDS <i>n</i> = 80
Age	78.7 (65–96)	75.7 (66–91)
Male	13 (62%)	50 (62.5%)
Female	8 (38%)	30 (37.5%)
Hemoglobin (g/dl)*	10 (8–12.9)	10.8 (7.3–12.9)

MDS, myelodysplastic syndrome.

* Values for hemoglobin are given in conventional units; for conversion to Systeme International (SI) units (g/l), multiply by 10.0.

Table 2

Complete blood count findings

	MDS <i>n</i> = 21	Negative for MDS <i>n</i> = 80
Isolated anemia	3/21 (14%)	60/80 (75%)
Anemia/leukopenia	8/21 (38%)	6/80 (7.5%)
Anemia/thrombocytopenia	2/21 (10%)	6/80 (7.5%)
Pancytopenia	8/21 (38%)	8/80 (10%)

MDS, myelodysplastic syndrome.

Table 3

CC/FISH findings in MDS patients vs. Complete blood count findings

	CC+/FISH+ agree	CC-/FISH- agree	CC/FISH disagree [*]	CC/FISH partial disagree [†]
Isolated anemia (3)	3/3	–	–	–
Anemia/leukopenia (8)	3/8	3/8	2/8	–
Anemia/thrombocytopenia (2)	2/2	–	–	–
Pancytopenia (8)	6/8	1/8	–	1/8

^{*} One patient had CC with del 20, but negative FISH; another patient had FISH with del 7, but negative CC.

[†] One patient had FISH with del 7 in addition to the del 5 and del 20 that both CC and FISH identified.

Table 4

Bone marrow findings in patients with negative CC/FISH

Complete blood count	Diagnosis
Anemia/leukopenia (3)	RA, RARS, RAEB II
Pancytopenia (1)	RARS

RA, refractory anemia; RARS, refractory anemia with ring sideroblasts; RAEB II, refractory anemia with excess blasts II.