Background and Objective. In the present study we analyzed the incidence of nulisomy Y by fluorescence in situ hybridization in a group of 24 males diagnosed with myelodysplastic syndromes (MDS). We explored the relationship between this chromosome abnormality and other clinical and biological disease characteristics.

Methods. Loss of chromosome Y was present in 7 out of the 24 males analyzed (29%); the number of cells carrying this chromosome aberration ranged between 19% and 90%. From the clinico-biological point of view, the group of patients with nulisomy Y showed a higher incidence of RA and RAS FAB subtypes (p=0.04), a lower WBC count (p=0.04), a lower proportion of blast cells both in PB (p=0.009) and BM (p=0.06) associated with a decreased myeloid/erythroid ratio (p=0.01).

Results. No clear association was detected between loss of chromosome Y and other numerical chromosome abnormalities involving chromosomes 7 and 8. In contrast, 2 out of the 7 cases with loss of chromosome Y also displayed monosomy 1 by FISH. However, the use of appropriate dual stainings showed that these two abnormalities were present in different cell populations (that is, they never coexisted in the same cell population), which supports the notion of the existence of clonal heterogeneity in MDS patients.

Interpretation and Conclusions. From the prognostic point of view, MDS patients with loss of chromosome Y displayed a higher survival rate, although these differences did not reach statistical significance.

©1997, Ferrata Storti Foundation

Key words: cytogenetics, FISH, myelodysplastic syndromes, nulisomy Y

Previous studies have shown that the presence of nulisomy Y can be detected in all subtypes of myelodysplastic syndromes (MDS). The overall incidence of this chromosome aberration as measured by conventional cytogenetic techniques ranges between 2% and 5% of all MDS.1-4 Although it has been indicated that MDS patients with nulisomy Y display a relatively good prognosis, no clear associations between this chromosome abnormality and the clinical and biological disease characteristics have been found. On the other hand, loss of chromosome Y has also been found in healthy elderly males, with a similar frequency to that observed in MDS.2-4,5 which makes the clinical significance of this aberration in MDS patients uncertain, at least for the moment. For this purpose, appropriate controls are essential.

In recent years, it has been shown that the use of fluorescence in situ hybridization (FISH) is more sensitive than conventional cytogenetics in the detection of numerical chromosome aberrations,6,7 especially for hematological malignancies in which neoplastic cells display a low proliferative activity.
participating in this study, 20 patients were in the chronic phase and 4 individuals displayed acute leukemia following a primary MDS.

Controls

Bone marrow samples from 8 healthy males undergoing orthopedic surgery were studied as controls. The mean age of this group was 62±16 years, ranging from 32 to 84 years. In all cases, samples were obtained following the recommendations of the Ethics Committee of the University Hospital of Salamanca, Spain.

Conventional cytogenetic analysis and FISH studies

Conventional cytogenetic studies were successfully performed in 13 MDS patients according to previously described techniques. In all cases, FISH analysis was performed on cells from BM samples prepared according to conventional cytogenetic techniques. The slides containing fixed cells were sequentially incubated with 0.1 mg/mL of RNase A (Boehringer Mannheim, Mannheim, Germany) in 2×SSC buffer – NaCl 0.3M, sodium citrate 0.03 M in distilled water, pH adjusted to 7.0 – for 1h at 37°C and with 0.1 mg/mL of pepsin (Sigma) in HCl 10 mM (10 min at 37°C). Cells were then fixed in 1% paraformaldehyde (Merck, Darmstadt, Germany) in PBS containing 50 mM of sodium phosphate, pH adjusted to 7.0 – containing 10 ng of a biotinylated DNA probe, specific for the heterochromatin region of the q arm of human chromosome Y (DYZ1, Oncor, Gaithersburg, MD, USA) was placed on each slide under a coverslip. Subsequently, the slides containing both cell DNA and probe DNA were denaturated at 80°C for 100 seconds. Upon denaturation, slides were placed at 37°C and hybridized overnight in a humidified chamber.

The immunological detection of the hybridized probe was performed upon immunological blocking incubation with 4T buffer [4×SSC buffer containing 0.5% (vol/vol) between 20 (Sigma)] for 30 minutes at 37°C, followed by another incubation (30 min at 37°C) with avidin conjugated with FITC (Vector Laboratories, Burlingame, CA, USA). Immunological amplification of the signals was performed whenever the fluorescence intensity of the hybridization signals was not strong enough to be clearly identified with the microscope. For that purpose a biotinylated anti-avidin monoclonal antibody (Vector Laboratories) and avidin-FITC were used, incubation steps being performed in the same conditions as those mentioned above. Cells were counterstained with 35 µL/slide of a mounting medium containing 75 ng/mL of DAPI (Sigma) and 20 mg/mL of 1,4-diazobicyclo-2,2,2-octane (Sigma) used as antifading agent. The number of hybridization spots per nuclei was evaluated using a Leitz DMRB fluorescence microscope (Leitz, Wetzlar, Germany) that counted at least 200 cells/sample. In all slides measured, only those spots with similar size, intensity and shape were counted.

In those cases displaying an abnormal number of hybridization spots, a simultaneous hybridization for chromosome 7 (D7Z1, Oncor), 8 (D8Z1, Oncor) and 1 (D1Z5, Oncor) was performed according to previously-described methods. For this purpose, simultaneous hybridization was performed for the chromosome Y biotinylated probe and for the digoxigenin-labelled probes directed at chromosomes 7, 8 or 1. The immunological detection of the hybridization of the three digoxigenin-labeled probes was performed using a monoclonal mouse antibody conjugated with TRITC (Boehringer Mannheim) according to previously-described methods.

Statistical methods

Mann-Whitney U or Kruskal-Wallis tests were used (BMDP 35 program) in order to estimate the statistical significance of the differences observed between groups. The χ² test was employed for dichotomic variables (presence or absence) (BMDP 4F program). Survival curves were plotted according to the Kaplan and Meier method, and a comparison of the curves was performed using the Breslow and Mantel-Cox test (BMDP 1L software program). Statistical significance was considered present for p values lower than 0.05.

Results

In all control cases, most nuclei showed one hybridization spot (mean 97.7±1.6%) ranging between 95 and 100%. The mean percentage of nuclei from control samples in which no hybridization signals for chromosome Y were found was of 1.4±1.7% (range: 0 to 5%). The control male showing 5% of cells without hybridization signals was an 84-year-old man.

Of the 24 males diagnosed with MDS, 7 (29%) showed a significant percentage of nuclei displaying no hybridization spots (mean value of 44±31%; range: from 19 to 90%). Results from conventional cytogenetic studies for those MDS patients where they were available (n=13) are displayed in Table 1 according to the presence or absence of the loss of chromosome Y by FISH. As shown, conventional cytogenetics confirmed the presence of nullisomy Y in 3 out of 4 patients who displayed loss of chromosome Y by FISH; these patients were studied in parallel with both techniques. Regarding the incidence of other cytogenetic alterations, no signifi-
cant differences were found between cases with and without nulisomy Y by FISH.

The distribution of MDS patients with loss of chromosome Y by FISH according to the FAB diagnosis is shown in Table 2. As is reported, the incidence of nulisomy Y was significantly higher within the RA/RAS subgroup (58%) than within RAEB/RAEB-t/CMMML patients (17%) (p=0.04). Interestingly, all patients displaying a loss of chromosome Y were in the chronic phase.

Table 3 shows the clinical-biological characteristics of MDS patients with loss of chromosome Y by FISH. As seen here, males with an MDS with nulisomy Y displayed similar characteristics to those individuals who did not show this abnormality except for a significantly lower peripheral blood leukocyte level (p=0.04) and a lower percentage of PB blast cells (p=0.009).

Upon analyzing the features of the BM aspirate, it was found that MDS patients with nulisomy Y displayed a lower myeloid/erythroid ratio (p=0.01) and a lower percentage of BM blast cells (p=0.06) as compared to MDS patients without nulisomy Y. In addition, among RA and RAS patients, those displaying nulisomy Y showed a higher proportion of both sideroblasts (50±40% versus 9±22%, p=0.06) and ring sideroblasts (12±26% versus 1±4%, p=0.04) (Table 4).

Analysis by FISH of the possible existence of other numerical chromosome aberrations involving chromosomes 7 and 8 detected no alterations among those patients with nulisomy Y. In contrast, presence of monosomy 1 by FISH was observed in 2 out of the 7 patients displaying nulisomy Y showed a higher proportion of both sideroblasts and ring sideroblasts (50±40% versus 9±22%, p=0.06) and ring sideroblasts (12±26% versus 1±4%, p=0.04) (Table 4).

Concerning the outcome, patients with nulisomy Y showed a higher median of survival as compared to the remaining individuals (median of survival not reached vs. a median survival of 15 months), although these differences did not reach statistical significance (p=0.45) (Figure 1).

Table 1. Conventional cytogenetic analysis of MDS according to the presence of nulisomy Y by FISH.

| Patients with nulisomy Y (case n./FAB subtype) | 4/CMMoL | 46XY | 45X, -Y |
| Patients without nulisomy Y (case n./FAB subtype) | 2/RAEB-t | Polyploid |
| 3/CMMoL | 46XY |
| 6/RAEB-t | Polyploid |
| 10/CMMoL | 46XY |
| 11/RAEB | 46XY |
| 14/RA | 46XY |
| 18/RAEB | 46XY |
| 21/CMMoL | 46XY, der(9)t(3;9) |
| 24/RAEB | 46XY |

Table 2. MDS with nulisomy Y by FISH: distribution of male patients according to the FAB classification.

| Nulisomy Y n=7 | No Nulisomy Y n=17 |
| RA + RAS | 4 (58%) | 3 (18%) |
| RAEB + RAEB-t + CMMML | 3 (44%) | 14 (72%) |

Table 3. MDS: Clinical and biological characteristics of male patients according to the presence of nulisomy Y by FISH.

| Age (years)* | 48±11 | 66±14 | 0.82 |
| Lymph node involvement | 4 (57%) | 4 (25%) | 0.31 |
| Hepatomegaly | 5 (71%) | 5 (31%) | 0.18 |
| Splenomegaly | 2 (29%) | 6 (38%) | 0.91 |
| Hemoglobin (g/L)* | 92±19 | 82±29 | 0.42 |
| Platelets (x 10^9/L)* | 104±125 | 195±252 | 0.35 |
| Leukocytes (x 10^9/L)* | 9.5±12.7 | 45.6±115.5 | 0.04 |
| % PB blast cells* | 0.0±0.0 | 6.9±10 | 0.009 |

*p Results expressed as mean ± DS.

Discussion

Previous reports have suggested that loss of chromosome Y could represent a physiological event when present in elderly males since this chromosome aberration has been found in healthy individuals of advanced age.11-13 The incidence of nulisomy Y in males diagnosed with MDS has been reported to be similar to that observed using conventional cytogenetic techniques in age-matched control subjects, ranging between 2% and 5% of the cases.1-4 Based on these findings the presence of nulisomy Y should not be considered as a malignant marker in MDS patients.4,15-15 In the present paper, the inci-
The incidence of nulisomy Y in MDS male patients was 25%. This is clearly higher than the incidence observed for a group of age- and gender-matched control subjects, where loss of chromosome Y was detected in less than 2% of the nuclei from these individuals in all except one case, where they represented 5%. By contrast, in the seven MDS patients with nulisomy Y, the number of aberrant nuclei ranged between 19% and 90%. Therefore, the high incidence of nulisomy Y observed in the present series of MDS patients suggests an association between this cytogenetic abnormality and MDS. Moreover, the younger age of patients with nulisomy Y, compared to the age of patients without this abnormality, indicates that the incidence of nulisomy Y in MDS patients does not depend on the putative physiological age-related loss of this chromosome.

Although the mechanisms involved in the loss of chromosome Y in healthy males are still not fully understood, the presence of this aberration in the cells of MDS patients suggests that the loss of chromosome Y in elderly males could be a marker of genetic instability in individuals who are at a high risk for neoplasia. The higher incidence of nulisomy Y detected in the present study as compared to previous reports could be due to the higher sensitivity of FISH compared to conventional cytogenetics. This would support previous studies in which both techniques are compared as regards their sensitivity for the detection of different numerical chromosomal aberrations in almost all types of hematological malignancies. In most of these situations, the advantage of FISH over conventional cytogenetics in the detection of numerical chromosome changes may be related to the low proliferative ability of some neoplastic cells, which makes the observation of metaphases from the neoplastic clones particularly difficult. This may be especially true for MDS patients, in which the number of S-phase cells is usually very low. In fact, in one out of the four patients displaying nulisomy Y by FISH for whom conventional cytogenetic studies were available, this chromosome aberration was not detected, while it was confirmed in the remaining 3 patients.

In the present series, loss of chromosome Y was observed in all FAB subtypes except RAEB, in accordance with previous reports. Interestingly, we found a higher incidence of nulisomy Y among RA and RAS as compared to the other FAB subtypes. From the karyotypic point of view, RAS is one of the FAB subtypes of MDS that displays the lowest incidence of chromosome abnormalities as analyzed by conventional cytogenetic techniques. It should be emphasized that RA and RAS are the two MDS subtypes with the lowest proliferative activity; both findings may contribute, as we have already mentioned, to an increased difficulty in the induction of metaphases from the neoplastic clone in these FAB subtypes. Accordingly, interphase cytogenetics using FISH allows the detection of this abnormality which would otherwise go undetected with conventional cytogenetic techniques, as is shown for one ARS patient in the present group. Therefore, this could help explain the higher incidence of nulisomy Y found for AR and ARS patients as compared to the other FAB subtypes of MDS. However, we should not consider these findings definitive since the number of cases analyzed in the present study is relatively low. Further studies with a higher number of males with RAS and RA analyzed by FISH will be necessary to fully understand this question.

A detailed analysis of the clinical and biological characteristics of MDS patients with nulisomy Y shows that their only differential features as compared to other MDS patients are related to lower WBC counts, lower levels of both peripheral blood and bone marrow blast cells, and a decreased myeloid/erythroid ratio. In any case these differen-

<table>
<thead>
<tr>
<th>Nulisomy Y</th>
<th>No nulisomy Y</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=7</td>
<td>n=17</td>
<td></td>
</tr>
<tr>
<td>Myeloid/erythroid ratio (%)</td>
<td>60±22/40±22</td>
<td>85±14/15±14</td>
</tr>
<tr>
<td>% of BM blast cells</td>
<td>3.3±6.2</td>
<td>12.6±15</td>
</tr>
</tbody>
</table>

Results expressed as mean ± SD. *Mean value exclusively in RA and RAS cases.

Figure 1. Survival analysis according to nulisomy Y in MDS.
tial characteristics could be due to the inclusion of a higher proportion of RA and RAS cases among patients with nulisomy Y. In a similar way, the higher proportion of sideroblasts and ring sideroblasts detected among cases with nulisomy Y would reflect the greater incidence of RA and RAS cases.

It is well-known that there is a correlation between loss of chromosome Y and good prognosis. Accordingly, in the present study, patients with nulisomy Y displayed a higher median survival rate as compared to the remaining MDS patients, although differences did not reach statistical significance. Theoretically speaking, these differences cannot be directly related to other chromosome aberrations since no significant differences were found among those patients where conventional cytogenetics were available according to the presence or absence of nulisomy Y.

Interestingly, in 2 out of the 7 MDS patients carrying nulisomy Y, the presence of monosomy 1 by FISH was observed. Dual stainings showed that these aberrations were not present in the same cell population, but they represented two different clones. The finding that loss of chromosomes Y and 1 never coexisted in the same cell population may reflect the well-known clonal heterogeneity associated with MDS. Unfortunately, conventional cytogenetic studies were not available in these two patients in order to exclude structural abnormalities of chromosome 1 that lead to partial monosomy.

References