The role of apoptosis, proliferation, and the Bcl-2–related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS

Jane E. Parker, Ghulam J. Mufti, Feyroz Rasool, Aleksandar Mijovic, Stephen Devereux, and Antonio Pagliuca

Bone marrow CD34+ cell apoptosis (annexin V), proliferation (Ki-67), and Bcl-2-related protein expression was evaluated by flow cytometry in 102 patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia secondary to MDS (MDS-AML) and in 30 normal donors (NBM). Apoptosis was significantly increased in refractory anemia (RA)/RA with ringed sideroblasts (RARS) (56.9% [20.4%-93.6%]) and refractory anemia with excess blasts (RAEB) (51.2% [25.2%-76.6%]) compared with NBM (16.7% [3.4%-35.3%], $P < .0001$). In RA/RARS, apoptosis always exceeded proliferation (Ki-67-positivity, 26.1% [9.5%-47.8%]; apoptosis: proliferation ratio 2.08 [1.15-3.63]); whereas in RAEB, this ratio equalized (1.14 [0.93-2.08]) due to increased proliferation (40.4% [22%-69.5%]). Progression to RAEB in transformation (RAEB-t)/MDS-AML was associated with a significant reduction in apoptosis (22.3% [2.1%-53.2%]; $P < .0001$) and proliferation (16.8% [1.9%-75.8%]; $P = .04$; ratio 1.69 [0.16-12.21]). Pro-apoptotic (Bax/Bad) versus anti-apoptotic (Bcl-2/Bcl-X) Bcl-2-related protein ratios were increased in RA/RARS compared with NBM (2.57 [1.93-9.42] versus 1.89 [0.65-4.1]; $P = .06$), whereas disease progression was associated with significantly reduced ratios (1.16 [0.06-3.32]; $P < .0001$) due primarily to increased Bcl-2 expression. Apoptosis and Bax/Bad:Bcl-2/Bcl-X ratio were inversely correlated with both International Prognostic Scoring System score and cytogenetic risk group; highest levels observed in patients with low score and/or good risk cytogenetics. There was a trend toward an association between Bcl-2-related protein expression and apoptosis ($P = .07$). This study indicates that MDS progression arises through multiple hits that alter levels of CD34+ cell apoptosis and proliferation. Early disease is associated with excessive apoptosis and elevated ratio of apoptosis to proliferation. Increased proliferative rates are observed in RAEB, whereas leukemic transformation arises through inhibition of apoptosis rather than excessive cell growth. Although disease progression is accompanied by an fall in pro-apoptotic versus anti-apoptotic Bcl-2–related protein ratios, heterogeneity in patterns of protein expression indicates that factors additional to Bcl-2 family members play a role in the deregulated apoptosis in MDS. (Blood. 2000;96:3932-3938)

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Introduction

The myelodysplastic syndromes are clonal stem cell disorders characterized by dysplastic hematopoiesis and an increased risk of leukemic transformation, especially in advanced subtypes: refractory anemia with excess blasts (RAEB) and RAEB in transformation (RAEB-t).1 Despite a normal or hypercellular marrow, most patients present with peripheral blood cytopenias. This paradox has recently been attributed to excessive programmed cell death or apoptosis of hematopoietic progenitors.2,3 Apoptosis in early disease may represent a pathophysiological mechanism whereby the hematopoietic system is able to abrogate defective and/or potentially harmful clones. Alternatively, an “hit” in the multistep pathogenesis of myelodysplastic syndrome (MDS) could give rise to a clone with a proliferative advantage. Increased apoptosis may thus represent a homeostatic process to control cell numbers. In turn, leukemic progression could arise through the acquisition of genetic lesions that either block programmed cell death (PCD) or promote proliferation over and above apoptosis.

PCD can be triggered by a variety of extracellular stimuli and intracellular signals. Major regulators of these apoptotic pathways are members of the Bcl-2 family. These proteins function as homodimers or heterodimers, and it is the ratio of pro-apoptotic versus anti-apoptotic molecules that determines a cell’s susceptibility to death signals.4 Deregulation of Bcl-2 family members has been demonstrated in a variety of human cancers, including hematological malignancies,5,6 and could feasibly underlie aberrant PCD observed in MDS. Certainly, increased expression of the anti-apoptotic protein Bcl-2 compared to pro-apoptotic Bax has been associated with disease progression in chronic lymphocytic leukemia.7 Moreover, in acute myeloid leukemia (AML), Bcl-2 overexpression is linked with CD34 positivity, resistance to chemotherapy, and short survival,6,8 features commonly attributed to disease arising from MDS. Studies demonstrating reduced Bcl-2 expression in CD34+ cells of patients with early MDS subtypes compared to advanced disease and normal controls lends further support for a role of the Bcl-2–related proteins in the pathogenesis of deregulated apoptosis and ineffective hematopoiesis in MDS.9,10 In the present study, the relationship between hematopoietic progenitor cell apoptosis and proliferation at different stages of MDS evolution was investigated by 2-color flow cytometry, using anti-CD34 monoclonal antibody (Mab) to identify progenitor cells,

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annexin V that binds to exposed phosphatidylserine on apoptotic cells\(^1\) and Ki-67 Mab that specifically binds to DNA of cycling cells.\(^2\) Permeabilized anti-CD34-labeled cells were also incubated with conjugated antibodies to 2 pro-apoptotic (Bax and Bad) and 2 anti-apoptotic (Bcl-2 and Bcl-X) Bcl-2 family members to evaluate whether deregulated apoptosis could be attributed to altered expression of the Bcl-2–related proteins.

### Patients, materials, and methods

#### Patient characteristics

Fresh bone marrow (BM) aspirates were obtained from 102 patients with MDS or AML secondary to MDS (MDS-AML) and from 30 normal donors.

A full explanation of the procedure was given prior to BM aspiration, and informed consent was obtained. The majority of aspirates were taken at the time of initial presentation. In remaining cases, patients treated with growth or differentiating agents or cytoreductive therapy within 3 months preceding analysis were excluded from the study. All samples were analyzed within 4 hours of aspiration.

The degree of apoptosis, proliferation, and Bcl-2–related protein expression was evaluated in 117, 56, and 44 cases, respectively. Data regarding Bcl-2–related protein expression has been previously published.\(^3\) According to the French, American, British (FAB) morphologic guidelines for MDS classification,\(^4\) 48 patients had refractory anemia (RA), 4 had RA with ringed sideroblasts (RARS), 15 had RAEB, 7 had RAEB-t, and 28 had MDS-AML. Within this subset of patients, 6 had AML that had progressed from chronic myelomonocytic leukemia (CMLL), and 5 had relapsed following chemotherapy. No patient had received any therapy other than supportive care for at least 1 month prior to BM examination. Median age was 67 years (range 17-92) with no significant differences between diagnostic categories. Hemoglobin (P = .002) as well as white cell (P = .004) and platelet counts (P = .0002), however, differed significantly between the disease subgroups. Among the 50 evaluable patients with early MDS, 38 (76%) had “good risk” cytogenetics according to the International Prognostic Scoring System (IPSS; normal karyotype or isolated deletions of the long arm of chromosome 5 or 20 or Y).\(^5\) In contrast, 17 of 32 (53%) patients with RAEB-t/AML harbored a “poor risk” karyotype (> 2 chromosomal abnormalities and/or abnormalities of chromosome 7) with 14 (44%) demonstrating abnormalities of chromosome 7 (Table 1).

#### Apoptosis quantification

BM mononuclear cells were isolated by density gradient centrifugation over Ficoll-Paque (d = 1.077 g/mL; Pharmacia Biotech) and washed twice in phosphate-buffered saline (PBS) (Sigma). A total of 10\(^6\) cells was used per sample. Cells were incubated with phycoerythrin (PE)-conjugated anti-CD34 Mab (Clone 8G12, Anti-HPCA-2, IgG\(_1\); Becton Dickinson) for 20 minutes at room temperature in the dark and then washed twice in PBS. Pelleted cells were resuspended in 100 mM binding buffer (10 mmol/L Hepes/NaOH, pH 7.4, 140 mmol/L NaCl, 2.5 mmol/L CaCl\(_2\); Bender Medsystems, Boehringer Ingelheim) and incubated with 2 mM fluorescein isothiocyanate (FITC)-conjugated annexin V (Bender Medsystems, Boehringer Ingelheim) for 10 minutes at room temperature in the dark. Cells were then resuspended in 400 mM binding buffer prior to flow cytometric analysis. Negative controls included MNCs incubated with neither CD34-PE Mab nor annexin V-FITC and cells incubated with CD34-PE Mab only.

#### Ki-67 expression

For cell surface and intracellular protein staining, fresh whole BM containing 10\(^6\) white blood cells per sample was used. Cells were incubated with PE-conjugated anti-CD34 Mab (Clone 8G12, Anti-HPCA-2, IgG\(_1\); Becton Dickinson) for 20 minutes at room temperature in the dark and then washed twice in PBS. Samples were fixed in 100 mM “medium A” that contained formaldehyde (Fix & Perm, Caltag Laboratories, TCS Biologicals) and FITC-conjugated Ki-67 Mab (Clone MIB-1, IgG\(_1\); Immunotech, Coulter) for 10 minutes at room temperature in the dark and then washed in PBS. Cells were fixed in 100 mM formaldehyde (Fix & Perm, Caltag Laboratories, TCS Biologicals) and FITC-conjugated Ki-67 Mab (Clone MIB-1, IgG\(_1\); Immunotech, Coulter) for 10 minutes on ice, washed again, and resuspended in ice-cold PBS. After washing in ice-cold PBS, cells were incubated with PE-conjugated RAM (Dako) for 10 minutes on ice. After washing in ice-cold PBS, cells were incubated with PE-conjugated RAM (Dako) for 10 minutes on ice, washed again, and resuspended in ice-cold PBS prior to flow cytometric analysis. Negative controls were performed by incubating cells with isotype-specific antibodies (Sigma) as nonspecific binding of Mabs to Fc receptors.

#### Bcl-2–related protein analysis

BM cells (10\(^6\)) were incubated with FITC-conjugated anti-CD34 Mab (Becton Dickinson) for 20 minutes at room temperature in the dark and then washed twice in PBS. Cells were fixed in 100 mM medium A for 15 minutes at room temperature in the dark and then washed in PBS to which 2.5% fetal calf serum (Sigma) and 0.1% sodium azide (Sigma) had been added (PBSF). MNCs were subsequently incubated with 100 mM permeabilization “medium B” (Fix & Perm, Caltag Laboratories, TCS Biologicals) and FITC-conjugated Ki-67 Mab (Clone MIB-1, IgG\(_1\); Immunotech, Coulter) for 20 minutes at 4°C in the dark and then washed in ice-cold PBSF. Cell pellets were resuspended in ice-cold PBSF prior to flow cytometric analysis. Cells incubated with FITC-conjugated isotype-specific antibodies (Serotec) were used as negative controls.

### Table 1. Clinical and laboratory characteristics of myelodysplastic syndrome (MDS) and acute myeloid leukemia secondary to MDS patients

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>RA/RARS</th>
<th>RAEB</th>
<th>RAEB-t/MDS-AML</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>52</td>
<td>15</td>
<td>35</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>31 (60%)</td>
<td>11 (73%)</td>
<td>17 (49%)</td>
</tr>
<tr>
<td>F</td>
<td>21 (40%)</td>
<td>4 (27%)</td>
<td>18 (51%)</td>
</tr>
<tr>
<td>Age (y) median</td>
<td>62</td>
<td>72</td>
<td>67</td>
</tr>
<tr>
<td>range (19-92)</td>
<td>(37-86)</td>
<td>(17-81)</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>11.6</td>
<td>10.6</td>
<td>9.4</td>
</tr>
<tr>
<td>WBC (10(^9)/L) range</td>
<td>(5.0-15.8)</td>
<td>(6.1-14.0)</td>
<td>(3.8-12.5)</td>
</tr>
<tr>
<td>Neutrophils (10(^9)/L)</td>
<td>4.7</td>
<td>2.5</td>
<td>10.9</td>
</tr>
<tr>
<td>Platelets (10(^9)/L)</td>
<td>120</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>Chromosome 7 abnormalities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good risk</td>
<td>38/50 (76%)</td>
<td>9/14 (64%)</td>
<td>8/32 (25%)</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>8/50 (16%)</td>
<td>3/14 (21%)</td>
<td>7/32 (22%)</td>
</tr>
<tr>
<td>Poor risk</td>
<td>4/50 (8%)</td>
<td>2/14 (15%)</td>
<td>17/32 (53%)</td>
</tr>
<tr>
<td>Chromosome 7 abnormalities</td>
<td>3/50 (6%)</td>
<td>2/14 (15%)</td>
<td>14/32 (44%)</td>
</tr>
<tr>
<td>IPSS score Low</td>
<td>18 (37.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Intermediate risk</td>
<td>27 (56%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Poor risk</td>
<td>3 (6.5%)</td>
<td>4 (31%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>High</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
<td>7 (100%)</td>
</tr>
</tbody>
</table>

RA indicates refractory anemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess blasts; RAEB-t, RAEB in transformation; MDS-AML, acute myeloid leukemia secondary to MDS; WBC, white blood cell count.
Table 2. Median levels of apoptosis, proliferation, apoptosis:proliferation (A:P) ratio and Bax/Bad:Bcl2/BclX ratio in different diagnostic subgroups

<table>
<thead>
<tr>
<th>Disease subgroup</th>
<th>Apoptosis (%) (range)</th>
<th>Proliferation (%) (range)</th>
<th>A:P ratio (range)</th>
<th>Bax/Bad:Bcl2/BclX ratio (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBM</td>
<td>16.7 (3.4-35.3)</td>
<td>18.7 (5.1-59.6)</td>
<td>0.81 (0.48-1.6)</td>
<td>1.89 (0.65-4.12)</td>
</tr>
<tr>
<td>RA/RARS</td>
<td>56.9 (20.4-90.6)</td>
<td>26.1 (9.5-47.8)</td>
<td>2.08 (1.15-3.63)</td>
<td>2.57 (1.93-9.42)</td>
</tr>
<tr>
<td>RAEB</td>
<td>51.2 (25.2-76.6)</td>
<td>40.4 (22.6-95.5)</td>
<td>1.14 (0.93-2.06)</td>
<td>1.19 (1.14-2.08)</td>
</tr>
<tr>
<td>RAEB-t/MDS-AML</td>
<td>22.3 (2.1-53.2)</td>
<td>16.85 (1.9-75.8)</td>
<td>1.69 (0.16-12.2)</td>
<td>0.85 (0.06-3.32)</td>
</tr>
</tbody>
</table>

NBM indicates normal donors; RA, refractory anemia; RARS, RA with ringed sideroblasts; RAEB, RA with excess blasts; RAEB-t, RAEB in transformation; MDS-AML, acute myeloid leukemia secondary to myelodysplastic syndrome.
Apoptosis versus proliferation

CD34+ cell proliferation equaled or exceeded apoptosis in 8 of 10 (80%) normal controls (median A:P ratio 0.81 [range 0.48-1.6]), whereas in FAB subtypes RA/RARS, levels of CD34+ cell apoptosis always exceeded S-phase percentage (median A:P ratio 2.08 [range 1.15-3.63], P < .0001), with 13 of 22 (59.1%) patients having an A:P ratio greater than 2. Progression to RAEB was associated with an increase in Ki-67 positivity such that apoptosis mirrored proliferation in 8 of 10 (80%) cases (median A:P ratio 1.14 [range 0.94-2.06], P = .0004). The relationship between apoptosis and CD34+ cell division in RAEB-t/MDS-AML was highly variable (median A:P ratio 1.69 [range 0.16-12.21]); however in 10 of 14 (71.4%) cases, proliferation rates were lower than or matched levels of PCD (Figure 2C). The ratio of apoptosis to proliferation was significantly different between the 3 disease categories (chi-square 7.59, 2 df, P = .023) (Table 2). When considering the group as a whole, there was a significant inverse correlation between A:P ratio and IPSS score (Spearman r = −0.39 [CI −0.65 to −0.06]), P = .02. However, within disease subgroups, A:P ratio showed no correlation with laboratory parameters.

Disease progression and sequential patient samples

Two patients showed evidence of disease evolution during the period of study. In the first case, BM blast percentage increased from 3% to 9% non-erythroid cells, whereas in patient 2, transformation to RAEB-t was accompanied by karyotypic evolution 46, XX to 46, XX, del16 (q22-q24). MDS progression in both cases was associated with a fall in levels of CD34+ cell apoptosis. In the latter patient, in whom proliferation rates were additionally measured, evolution to RAEB-t was accompanied by a concomitant reduction in CD34+ cell proliferation (Figure 3).

Bcl-2–related protein expression

As shown in Figure 4, patients with RA/RARS had a higher Bax OPI compared to normal controls (median 92.2 [range 12.2-913], n = 14 versus median 51.4 [range 9.2-170.7], n = 10), whereas progression to RAEB or RAEB-t/MDS-AML was associated with a significant increase in Bcl-2 expression (RA/RARS median OPI, 84.1 [range 9.1-681.7]; RAEB/RAEB-t/MDS-AML median OPI, 336.7 [range 1.1-6448], n = 20, P = .02) and a relative reduction in Bad. A higher pro-apoptotic (Bax/Bad) versus anti-apoptotic (Bcl-2/Bcl-X) apoptotic Bcl-2–related protein ratio was demonstrated in RA/RARS compared to normal controls, although this...
finding did not reach statistical significance (median 2.57 [range 1.93-9.42] versus median 1.89 [range 0.65-4.1], \( P = .06 \)). Evolution to RAEB and RAEB-t/MDA-AML, however, was associated with a significant reduction in Bax/Bad versus Bcl-2/Bcl-X ratio (RAEB, median 1.19 [range 1.14-2.08], \( P = .04 \); RAEB-t/MDA-AML, median 0.85 [range 0.06-3.32], \( P = .0002 \)) (Table 2). Of the 14 patients with RA/RARS, 12 (85.7%) had a pro-apoptotic versus anti-apoptotic Bcl-2–related protein ratio greater than 2; conversely, a ratio of 1 was observed in 12 of 20 (60%) patients with more advanced disease. In MDS patients, there was a significant positive correlation between Bax/Bad versus Bcl-2/Bcl-X ratio and platelet count (Spearman \( r = 0.46, 95\% \text{CI} 0.11-0.71, P = .01 \)) and a negative correlation with IPSS score (Spearman \( r = -0.67, 95\% \text{CI} -0.87 \text{to} -0.26, P = .003 \)). There was a trend toward an association between Bcl-2–related protein expression and apoptosis, although this finding failed to reach statistical significance (\( P = .07 \)). Within disease subgroups however, patterns of Bcl-2-related protein expression were highly variable and showed no correlation with the aforementioned laboratory parameters.

**Cytogenetics**

A significant relationship was observed between PCD and cytogenetic risk group; patients with good risk cytogenetics having the highest degrees of apoptosis (good risk, 55.1% [2.1–93.6%]; intermediate risk, 46.1% [9.9%–83.8%]; poor risk, 25.8% [3%–86.5%], \( P = .02 \)). The presence of chromosome 7 abnormalities was particularly associated with reduced CD34+ cell apoptosis (chromosome 7 abnormalities, 23.3% [3%–56.9%] versus other karyotype, 51.4% [9.9%–93.6%], \( P = .0007 \)) (Figure 5A). Proliferation rates and A:P ratios showed no correlation with karyotype. Cytogenetic risk group also significantly influenced Bcl-2–related protein ratios, with the highest ratios observed in patients with a good risk karyotype (good risk, median 2.36 [range 1.17-9.42]; intermediate risk, median 1.14 [range 0.06-5.77]; poor risk, median 1.17 [range 0.4-2.89], \( P = .03 \)). Low ratios in patients with intermediate or poor risk cytogenetics was primarily due to increased Bcl-2 expression (Figure 5B). There was also a trend for lower pro-apoptotic versus anti-apoptotic Bcl-2–related protein ratios in patients harboring chromosome 7 abnormalities (chromosome 7 abnormalities, median ratio, 1.16 [range 0.4-2.89] versus other karyotype, median ratio, 2.08 [range 0.05-9.42], \( P = .08 \)) (Table 3).

**Discussion**

Several authors3,13,16-21 have demonstrated that excessive intramedullary hematopoietic cell apoptosis may contribute toward the ineffective hematopoiesis characteristic of MDS. Studies demonstrating that early MDS is, in addition, a highly proliferative disorder, with the number of S-phase cells being significantly correlated with the degree of PCD,10,22 have prompted speculation that increased cell division in early disease is a compensatory mechanism to neutralize excessive apoptosis. Alternatively, increased PCD may represent a homeostatic process to counteract high proliferative rates. Data are conflicting, however, regarding the stage of MDS at which apoptosis and/or proliferation is most prominent or indeed, the nature of the primary cell involved.3,9 Moreover, alterations in the balance between cell growth and cell death with disease evolution and the nature of underlying lesions responsible for these changes have not been clearly elucidated. Inconsistencies between studies may be partly explained by the variable cellular composition of MDS BM as well as differences in methodology. Certainly, difficulties in discerning the precise cell type undergoing PCD and/or proliferation, using techniques that identify DNA strand breaks (in situ end labeling [ISEL]), terminal

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**Figure 4.** Pro-apoptotic and anti-apoptotic Bcl-2–related protein expression in MDS/MDA-AML and normal controls. BM samples were incubated with FITC-conjugated anti-CD34 Mab and fixed with formaldehyde. To prevent nonspecific binding of Mabs to Fc receptors, cells were pre-incubated with rabbit-anti-mouse (RAM) immunoglobulins, permeabilized, and then incubated with mouse-anti-human Mabs to Bax, Bad, Bcl-2, and Bcl-X on ice. Washing cells were subsequently incubated with PE-conjugated RAM on ice prior to flow cytometric analysis. The mean fluorescence intensity (MFI) value for each Bcl-2–related protein was calculated by dividing the MFI value of the positively labeled cells by that of cells stained with an isotype control antibody. To determine individual Bcl-2–related protein levels, we calculated an Oncoprotein Index (OPI) whereby OPI equals the product of the percentage of positive cells and MFI. Median Bax, Bad, Bcl-2, and Bcl-X OPI are plotted in specific diagnostic subgroups.

**Figure 5.** Influence of cytogenetic risk group on CD34+ cell apoptosis and Bcl-2–related protein expression. The degree of CD34+ cell apoptosis (A) and Bax, Bad, Bcl-2, and Bcl-X expression (B) was compared in MDS/MDA-AML patients with good (normal karyotype or isolated deletions of 5q or 20q), intermediate (=2 cytogenetic abnormalities, excluding isolated 5q or 20q deletions and chromosome 7 abnormalities) and poor risk (> 2 abnormalities and/or abnormalities of chromosome 7). Apoptosis was additionally evaluated in patients harboring aberrations of chromosome 7.
Andreef detected annexin V positivity in 53.4% (mean) BM cells in 28 patients. We have quantified apoptosis through annexin V staining, demonstrating apoptosis in the process of cell death. In the present study, the degree of PCD and proliferation was evaluated by dual-labeling flow cytometric techniques. The positive correlation between PCD, hemoglobin concentration, and platelet counts may be secondary to low levels of apoptosis observed in advanced MDS, a disease stage characterized by worsening cytopenias. Similarly, the inverse correlation with white cell count is probably due to increased numbers of circulating peripheral blasts in RAEB-t/MDS-AML.

The degree of apoptosis in the present study is considerably higher than previously reported in a number of published series. We found that CD34+ cell apoptosis was significantly increased in RA/RARS and RAEB compared to normal controls, whereas leukemic evolution was associated with a marked reduction in PCD. We also demonstrated a significant relationship between apoptosis, IPSS score, and cytogenetic risk group; patients with a low score and/or good risk cytogenetics have the highest degrees of annexin V positivity. Conversely, the presence of chromosome 7 abnormalities was associated with significantly reduced CD34+ cell apoptosis. The positive correlation between PCD, hemoglobin concentration, and platelet counts may be secondary to low levels of apoptosis observed in advanced MDS, a disease stage characterized by worsening cytopenias. Similarly, the inverse correlation with white cell count is probably due to increased numbers of circulating peripheral blasts in RAEB-t/MDS-AML.

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genomic hits that alter both the balance between apoptosis and proliferation and the ratio of pro-apoptotic versus anti-apoptotic Bcl-2–related proteins. As well as providing a model for the multistep pathogenesis of cancer in general, a clearer understanding of the molecular events leading to deregulation of the balance between cell growth and cell death in MDS should permit the identification of therapeutic targets, diagnostic markers, and useful indicators of prognosis.

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