Recent Advances in Myelodysplastic Syndromes
Guest Editors: Miguel Angel Sanz, Guillermo Sanz & Teresa Vallespi

Pathogenesis, etiology and epidemiology of myelodysplastic syndromes

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ABSTRACT

Background and Objective. The myelodysplastic syndromes are common hematological malignancies affecting predominantly elderly people. Patients usually present with chronic cytopenias which gradually worsen due to progressive bone marrow failure or transformation into acute myeloid leukemia. Disease prevention is more cost-effective than therapeutic intervention and the establishment of the etiology and pathogenesis of MDS therefore assumes considerable importance. This review will outline current concepts of the pathobiology of MDS, putative etiological insults and the mechanisms of disease initiation as well as recent contributions to the descriptive epidemiology of these disorders.

Evidence and Information Sources. The authors of the present review have a long-standing interest in the pathogenesis, etiology and epidemiology of MDS. Journal articles covered by the Science Citation Index® and Medline® have been reviewed and personal experience and discussion with international experts collated.

State of the Art and Perspectives. The initiation processes for the development of MDS remain unknown. A poorly defined transforming event affects a pluripotent or multipotent progenitor cell in the bone marrow, conferring a growth advantage upon it and eventually establishing clonal hematopoiesis. An important pathogenetic mechanism in MDS is premature intramedullary cell death via excessive apoptosis, explaining the apparent paradox of a cellular marrow in combination with peripheral cytopenias (ineffective hematopoiesis). Therapy-related MDS/AML following exposure to alkylating agents is the only clear etiological factor thus identified. Increasing evidence for exposure to benzene and radiation and the development of MDS is emerging. Benzene hematotoxicity is mediated via both genotoxic and non-genotoxic mechanisms, leading to aplasia, apoptosis and initiation (via genetic mutation) of clonal disorders such as MDS. Further studies of benzene hematotoxicity and therapy-related MDS should provide models for the elucidation of initiation events in MDS pathogenesis. The importance of such studies is emphasized by the rising frequency of MDS which largely reflects improved diagnostic criteria, increased physician awareness and extended use of diagnostic procedures in the elderly. Demographic changes will lead to a marked increase in MDS over the next few decades.

Key words: myelodysplastic syndromes, clonality, apoptosis, etiology, genetic factors, environmental and occupational toxins, benzene, alkylating agents, incidence, genetic medical care

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of acquired clonal bone marrow disorders characterized by varying degrees of pancytopenia, morphological and functional abnormalities of hematopoietic cells and an increased risk of transformation into acute myeloid leukemia (AML). They typically occur in elderly people, with the median age at diagnosis varying between 60 and 75 years in most series.1 In 1982, the French-American-British (FAB) Cooperative Group proposed a classification of MDS which includes five morphological subtypes: refractory anemia (RA), RA with ring sideroblasts (RARS), RA with excess of blasts (RAEB), RAEB in transformation (RAEB/T), and chronic myelomonocytic leukemia (CMML).2 Most patients with MDS present with a normocellular or hyperplastic bone marrow, but hypoplastic and myelofibrotic variants have recently been described in up to 8-20% of cases.3,4 The heterogeneity of MDS is reflected not only by the variety of morphological manifestations, but also by marked differences in survival between individual patients. About two thirds of patients succumb to complications of their bone marrow disorder, with AML, infections and hemorrhages being the most frequent causes of death.5

MDS provide a clinical model for studying the progression of a relatively benign clonal bone marrow disorder (RA and RARS) into a frankly malignant neoplasm (AML). Although MDS are traditionally defined by morphological criteria, more...
rapid progress in our understanding of these disorders has come from advancement in other techniques, including karyotype analysis, immunophenotyping, in vitro colony growth assays and molecular genetic investigations. Some of these newer tools are already incorporated into the diagnostic work-up and risk assessment of patients with MDS, and they are also increasingly used to define the response of patients to different therapeutic approaches. The present review concentrates on established and recent contributions to our knowledge of the pathogenesis, etiology and epidemiology of MDS. For a comprehensive review of the molecular biology of MDS, the reader is referred to the excellent article by Gallagher et al.8 that recently appeared in this journal.

Pathobiology of myelodysplastic syndromes

Multistep leukemogenesis of MDS

MDS are clonal disorders which progress through the classical three stages of tumorigenesis, namely 1) initiation comprising the first genetic insult(s) on hematopoietic stem cells; 2) tumor promotion: clonal expansion of a growth advantaged aberrant clone which is most likely accompanied by a high rate of cell death (ineffective hematopoiesis); and 3) malignant transformation: increase in leukemic blast cells with final evolution to AML (Figure 1). Each stage is probably accompanied by further genetic insults to which a component of continuing extrinsic insults and spontaneous intrinsic genomic damage contributes. Clonal evolution is associated with increasingly ineffective hematopoiesis, progressive impairment of cellular function and worsening peripheral blood cytopenia (Figure 2). The precise mechanisms responsible for the initiation of MDS are currently unknown. They probably include nuclear and mitochondrial DNA mutations by carcinogen-DNA adducts and oxygen free radical formation, defective DNA repair resulting in genomic instability, as well as immunological abnormalities (dysregulation of immune surveillance) which probably synergize with genomic mutational events to promote the process of leukemogenesis.

The clonal nature of MDS

The conception of MDS as clonal disorders of hematopoietic stem cells is suggested by quantitative and qualitative changes in all the myeloid cell lineages and supported by a variety of different techniques, including karyotyping, glucose-6-phosphate dehydrogenase isoenzyme studies, fluorescent in situ hybridization (FISH) analysis, detection of gene mutations and X-chromosome inactivation studies. No matter what techniques are employed in clonal analysis, we have to recognize certain drawbacks inherent to each method. Karyo-

Figure 1. The multistep model of MDS development.
Abnormalities in hematopoietic cell proliferation and differentiation

The ability of all of the hematopoietic progenitor cells committed to granulocyte-macrophage (CFU-GM), erythroid (BFU-E and CFU-E), megakaryocyte (CFU-Meg) as well as multiple lineages (CFU-GEMM) to form colonies is either reduced or absent (Table 1). The patterns of in vitro growth of CFU-GM from MDS bone marrow have been related to the risk of AML transformation and survival.12,13 Patients whose marrow or peripheral blood cultures at the time of diagnosis show high cluster/colony ratios or undetectable colony growth have a poor prognosis. In addition, a higher than normal proportion of CFU-GM in MDS are of light buoyant density. When serially examined in individual patients, persistent lack of growth was frequently seen in patients who subsequently developed AML. In contrast, progressive decline in the CFU-GM growth was characteristically seen in patients who eventually died of bone marrow failure.14 A high cluster/colony ratio and decreased CFU-GM growth in face of a cellular marrow would be a reflection of ineffective granulopoiesis. Failure of cultures may be partly due to abnormal function of accessory cells. The bone marrow cells from patients with MDS are unable to produce a healthy adherent cell layer in long-term marrow cultures. However, the marrow cells from patients cultured over a normal adherent layer do not grow well either.14 Thus far, MDS marrow stroma has no consistent functional defect when studied for its activity to sustain the growth of normal hematopoietic progenitors.

Both liquid and clonal culture studies indicate that the marrow cells from MDS patients show defective maturation.9,12,13 Phenotypic analysis suggests that MDS marrow CD34+ cells are predominantly committed to non-erythroid lineages with impaired differentiation.15 Available data suggest that a proportion of MDS cells can mature in vitro, although a considerable overlap in growth and differentiation pattern was seen between MDS and AML. While cellular maturation is less than normal in MDS, maturation is clearly greater than that seen in AML. Delayed cell maturation may account for the increased numbers of early myeloid cells seen in the bone marrow of patients with MDS.

Impaired or aberrant responsiveness to hematopoietic growth factors has been reported, but the weight of evidence suggests that MDS CFU-GM have normal sensitivity to growth factors.13 High endogenous levels of erythropoietin (Epo), granulocyte colony-stimulating factor (G-CSF) and CSF-1 have also been reported.9,12,13,16 Serum levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are often elevated in MDS.17 A high expression of TNF-α and transforming growth factor-β (TGF-β) has been suggested in a histochemical study of MDS marrow biopsy samples.18 The elevated levels of growth factors/cytokines most likely result from negative feedback mechanism.

Table 1. Cell biological abnormalities in myelodysplasia.

| 1. Clonal origin of all (but lymphoid) hematopoietic cells |
| 2. Poor progenitor growth in vitro |
| 3. Decreased colony/cluster ratio in semisolid clonal culture |
| 4. Defective maturation in semisolid and suspension culture |
| 5. Hematopoietic inhibitory activities of macrophages |
| 6. Resistance of GM-CFC to the inhibitory effects by macrophages |
| 7. Elevated cytokine levels (Epo, G-CSF, CSF-1, IL-6, TNF-α) |
| 8. Apoptosis responsible for ineffective erythropoiesis |
It is generally assumed that during evolution the aberrant clone in MDS progressively replaces the residual normal hematopoietic stem cells. The precise mechanisms involved in this process are not known. However, macrophage populations from MDS marrow have been suggested to suppress normal hematopoiesis. Two populations of enriched marrow macrophages suppressed the growth of CFU-GM from normal marrow in coculture experiments. The extent of suppression appears to correlate with the progression of the MDS clone. The precise relationship of the suppression of normal CFU-GM by MDS marrow macrophages to other candidate inhibitors of hematopoiesis remains unanswered. Of interest was the finding that CFU-GM from MDS marrow was resistant to these inhibitory effects. This would indicate a participation of the macrophage population from MDS marrow in the progressive expansion of abnormal MDS progenitors at the expense of normal granulocytopoiesis.

**Premature cell death (apoptosis): an important pathogenetic mechanism in MDS**

Clonal CFU-GM assays appear to recapitulate the in vivo hematopoietic defect in that cells may grow initially, but fail to proceed to further proliferation and maturation. Daily in situ observation of individually proliferating cells mapped in methylcellulose culture dishes demonstrated that a significantly greater proportion of cells in MDS proliferated initially, but failed to do so thereafter and disintegrated in culture. Cells with these abortive growth characteristics apparently contributed to ineffective hematopoiesis. This would explain why CFU-GM cultures from most MDS patients end up with poor colony formation and increased cluster formation. Why do MDS cells degenerate and even die in culture? This was certainly not due to culture conditions or CSF concentrations. Although there was no clear evidence for apoptosis in these experiments even after extensive search, dying cells might have been phagocytized by adjacent marrow cells. From these observations, it was postulated that the abortive growth is inherent to MDS progenitors themselves and that ineffective hematopoiesis is due to premature intramedullary cell death via apoptosis.

Ineffective hematopoiesis is a condition in which cellular bone marrow is unable to produce and deliver adequate numbers of mature cells to the peripheral blood. As such, it is a poorly-defined condition, but is likely to involve an excessive intramedullary cell death in the form of apoptosis. Premature apoptotic cell death within the bone marrow may well explain the apparent paradox in MDS of persistent cytopenia in spite of normal or increased bone marrow cellularity. Findings suggestive of excessive apoptosis in MDS include: 1) abortive in vitro growth pattern of marrow CFU-GM; 2) among human myeloid cell lines, the MDS-derived P39 myelomonocytoid leukemia cell line shows the highest sensitivity to apoptotic stimuli such as actinomycin D and Ca ionophore; 3) marrow biopsy specimens from MDS patients have been reported to contain a high proportion of cells with DNA strand breaks or apoptotic nuclei engulfed by macrophages; 4) increased incidence of apoptosis has been reported following short-term culture of MDS bone marrow cells; 5) altered oncoprotein expression has been shown in CD34+ cell populations from MDS patients, and the ratio of expression of cell-death (c-Myc) to cellsurival (Bcl-2) oncoprotein has been correlated with an increased incidence of cells with apoptotic DNA content; and 6) another study evaluated apoptosis in marrow biopsy samples in MDS patients before and after cytokine treatment, and found a significant reduction in apoptosis in patients responding to the combined use of G-CSF and erythropoietin, since non-responding patients did not show such a reduction in apoptotic cells, the cytokine treatment may act through the amelioration of ineffective hematopoiesis.

Ineffective erythropoiesis is a well-known prototype of ineffective hematopoiesis, and the presence of apoptosis in ineffective erythropoiesis has been shown in conditions other than MDS. Purified erythroblasts from β-thalassemia major and folate deficiency showed excessive apoptosis. Inhibition of heme synthesis in vitro also results in excessive apoptosis in purified erythroblasts. The biochemical lesions in these conditions include defective globin chain synthesis, folic acid deficiency and perturbation of heme synthesis. All these events seriously affect essential metabolic processes closely associated with erythroid differentiation. The question to be asked here is the relationship between apoptosis and differentiation.

In the hematopoietic system where rapid turnover of cells occurs, terminal differentiation probably culminates in apoptosis. However, apoptosis and

<table>
<thead>
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<th>Table 2. Findings suggestive of excessive apoptosis in myelodysplasia.</th>
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<tr>
<td>1. Abortive growth pattern of marrow (GM-CFC) progenitors in clonal culture</td>
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<tr>
<td>2. High sensitivity of MDS-derived cell line (P39) to apoptotic stimuli</td>
</tr>
<tr>
<td>3. High proportion of cells with DNA strand breaks in marrow biopsy</td>
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<tr>
<td>4. High incidence of macrophages engulfing apoptotic nuclei in marrow biopsy</td>
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<td>5. Increased apoptosis in short-term culture</td>
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<td>6. Increased apoptotic CD34+ cells with an increased ratio of c-Myc/Bcl-2</td>
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<td>7. Significant reduction of apoptotic cells in patients responding to cytokines</td>
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Mononuclear cells from a group of MDS patients as well as from normal human CD34+ cells. The activity of this type of endonuclease in MDS tended to be higher in advanced MDS and was correlated with blast cell percentages in the bone marrow. In contrast, purified erythroblasts from MDS bone marrow had a high level of another type of endonuclease, i.e. Ca2+-dependent nuclease. A third type of endonuclease the activity of which depends on acidic pH is found in granulocytic cells of varying maturation stages. The activity of Mg2+-dependent, Ca2+-independent, and Ca2+-dependent nucleases meet some criteria for apoptotic endonucleases, such as inhibition by Zn+ and generation of DNA fragments with 3'-OH and 5'-P ends. Although there is as yet no direct evidence for the participation of these endonucleases in apoptosis, the characteristic distribution of these enzymes in hematopoietic cells of different cell lineages suggests that they form a family of proteins which are present in viable cells. This is another piece of evidence in favor of the view that cells are constitutively equipped with mechanisms for apoptosis.

**Leukemic transformation of MDS**

In contrast to excessive apoptosis in ineffective hematopoiesis, leukemia is a disease associated with increased cell survival and proliferation. As in many other human malignancies, acute leukemia cells have a decreased ability to undergo apoptosis in response to at least some physiological stimuli. Most chemotherapeutic agents are now known to operate through induction of apoptosis and insensitivity to apoptosis induction may be one of the mechanisms of drug resistance. The leukemic transformation of MDS imposes a paradox in that apoptosis, once excessive in preleukemic stage, is no longer in operation to keep cells from uncontrolled growth. One of the final steps along the multistep carcinogenesis is the process of neoplastic transformation of the preneoplastic clone during which the clone has undergone additional genetic alterations, resulting in loss of tumor suppressor gene function and/or activation of growth promoting genes. Clearly, the concept of malignancy strongly implies that deranged apoptosis may be a crucial pathogenetic factor.

Cancer develops when cells accumulate in an uncontrolled manner because malignant cells proliferate at a faster rate than normal, or they live longer than normal. Cancer may result from the combination of both. These growth and survival advantages are considered to arise from somatic DNA mutations due to carcinogenic stimuli such as irradiation or genotoxic agents. Normal cells can detect DNA damage, which will be repaired. If not repaired, cells may commit suicide by apoptosis, thereby sacrificing themselves to eliminate danger-
ous genetic information and safeguard the host. Many genes implicated as inducers or repressors of apoptosis (Table 3) are originally known to be involved in the development of malignancy. A well known example is the induction of apoptosis by wild-type p53 and the inability to induce apoptosis by mutated p53 gene. Mutated p53 gene occasionally found in advanced stage MDS and post-myelodysplastic leukemia is consistent with this notion. The p53 –/- knockout mouse provides a good model to indicate impaired apoptosis in a variety of malignancies. Cells from these mice are resistant to apoptosis induced by irradiation or genotoxic agents, although apoptosis by other stimuli is not prevented. The loss of tumor suppressor gene function not only relates to inactivation of apoptosis, but also correlates with tumor aggressiveness. The interferon regulatory factor-1 gene (IRF-1) mapped to 5q31.1 has been shown to be deleted at one or both alleles in patients with MDS or AML with 5q chromosomal aberrations. As a transcription factor, IRF-1 activates the expression of interferon, leading to negative effects on cell growth. Thus, it is a candidate tumor suppressor gene.

At the heart of issue is the question when and how default apoptosis occurs in the progression of MDS clone. Of relevance is the observation that cells labeled by the in situ end-labeling method in advanced stage MDS were mostly immature erythroid, myeloid and megakaryocytic cells, sparing the most immature blast cells. These findings have been confirmed by other authors (Yoshida Y, unpublished observations, 1997). In addition, the Mg2+-dependent, Ca2+-independent endonuclease is present in blast cells from advanced stage MDS, AML evolving from MDS as well as from de novo AML. This would indicate that blast cells from advanced MDS is comparable to AML both in terms of type and activity levels of endonucleases.

Table 3. Genes/proteins related to apoptosis.

<table>
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<tr>
<th>Genes</th>
<th>Intracellular localization</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>bcl-2</td>
<td>mitochondria, lysosomes, nuclear membrane</td>
<td>prevent</td>
</tr>
<tr>
<td>caspase</td>
<td>cytoplasm</td>
<td>induce</td>
</tr>
<tr>
<td>myc</td>
<td>nucleus</td>
<td>increase sensitivity</td>
</tr>
<tr>
<td>p53</td>
<td>nucleus</td>
<td>induce</td>
</tr>
<tr>
<td>jun/fos</td>
<td>nucleus</td>
<td>related to apoptosis</td>
</tr>
<tr>
<td>Fas/Apo-1</td>
<td>cytoplasmic membrane</td>
<td>induce</td>
</tr>
<tr>
<td>ras</td>
<td>cytoplasmic membrane</td>
<td>evade apoptosis?</td>
</tr>
<tr>
<td>bax</td>
<td>same as bcl-2</td>
<td>dimerize with bcl-2</td>
</tr>
<tr>
<td>bcl-x</td>
<td>same as bcl-2</td>
<td>$X_c$ prevents</td>
</tr>
</tbody>
</table>

Taken together, these findings are consistent with the notion that leukemic transformation from MDS is associated with a reduction in cell death, and that blast cells in advanced MDS probably survived DNA-damaging insults. Is escape from apoptotic control strong enough for leukemic transformation of MDS? Probably, overriding the apoptotic control mechanism is a very critical step, but cells that have escaped from normal controls on cell survival or death may not necessarily be dangerous by themselves, unless coupled with signals or events that stimulate such cells to proliferate. Oncogene transformation may lead to dysregulation of genes that control cell survival or death (Table 3). We propose that two steps, escape from apoptotic control and growth promotion, represent key biological processes involved in the paradigm of leukemic evolution of MDS. Whether the two processes occur in an orderly and successive fashion or whether they are separate processes independent from each other is currently unknown.

Etiology of myelodysplasia

Familial myelodysplasia

Several reports have described familial cases of MDS presenting in adulthood. Numerically this represents a very low proportion of MDS diagnoses. Familial childhood onset of MDS is usually associated with clear hereditary disorders predisposing to myeloid malignancy. Defects of DNA repair underlie Fanconi’s anemia and Bloom’s syndrome while RAS activation via deletion of the tumour suppressor gene NF-1 is thought to be associated with MDS/leukemia predisposition in neurofibromatosis. The mechanism of MDS/leukemia risk for Shwachman-Diamond syndrome and Down’s syndrome is unknown. A recent Danish study failed to demonstrate an increased risk of leukemia or cancer in other family members associated with familial MDS. Except where a clear molecular defect can be demonstrated as causative, it will be difficult to separate the effects of common environmental factors responsible for MDS occurring in the same family versus a true inherited cause.

Aplastic anemia, paroxysmal nocturnal hemoglobinuria and MDS

The evolution of aplastic anemia (AA) and paroxysmal nocturnal hemoglobinuria (PNH) to MDS is now well recognized. Aplastic anemia and PNH may share a component of common etiological factors with further genetic/immunological modulation required for evolution initially from AA to PNH and then to MDS/AML. In one recent study, 30% of cases of PNH had evolved from AA and in turn 5% evolved further into MDS, though another well characterized cohort of PNH cases showed no MDS evolution. Several cases of reappearance of
GPI-linked cell membrane proteins upon evolution of MDS are now described in patients in whom an absence of these proteins characterizes the PNH clone.\textsuperscript{49,50} Evolution of aplastic anemia to PNH/MDS usually follows immunosuppression with antithymocyte globulin, with a 10-year cumulative incidence for MDS evolution of 9.6%.\textsuperscript{51} Etiological agents cited in aplastic anemia such as drugs and hepatitis infection are not known to be associated with the initiation of de novo MDS.

**Ionizing radiation**

Ionizing radiation has mutagenic and leukemogenic effects which have been established through animal studies, follow-up studies on atomic-bomb survivors and other epidemiological investigations.\textsuperscript{52} Leukemogenic effects of radiation are dependent upon dose and duration of exposure. Low dose high linear energy transfer \(\alpha\)-particle ionizing radiation of human bone marrow in vitro is associated with induction of chromosome aberrations which in turn is transmissible to daughter progeny.\textsuperscript{53} In contrast, high dose radiotherapy alone such as is used in the treatment of Hodgkin’s disease does not appear to be leukemogenic.

Since the FAB classification of myelodysplastic syndromes was published,\textsuperscript{2} several cohorts of leukemia occurring in subjects exposed to radiation have been retrospectively examined. Seven out of 35 cases of leukemia following spinal irradiation for ankylosing spondylitis\textsuperscript{52} and 12 out of 190 cases of leukemia in atomic-bomb survivors\textsuperscript{54} have been reclassified as MDS. A recent case-control study identified several weak associations between the MDS group and radiation exposure.\textsuperscript{55} Statistically significantly elevated odds ratios were found for previous radiotherapy treatment and dental X-rays, and a non-statistically significant elevated odds ratio was shown for domicile for > 6 months near (< 8 km) a nuclear power station. Pedersen-Bjer-gaard et al.\textsuperscript{56} reported that in cancer patients previously treated with radiotherapy there was an excess of leukemia with deletions of chromosomes 5 or 7 as compared to de novo AML, but otherwise no significant associations could be observed between previous radiotherapy and the occurrence of specific chromosome aberrations.

**Chemotherapeutic agents**

With increasingly successful treatment of primary malignancies by chemotherapeutic regimens attention has focussed on the lon-term complications of antineoplastic therapy. Distinct clinical and biological forms of therapy-related acute myeloid leukemia (t-AML) have now been recognized in relation to three different groups of therapeutic agents (Table 4). Of these, only previous exposure to alkylating substances frequently leads to t-AML via an MDS phase (50% cases). Therapy-related MDS (t-MDS) differs from de novo MDS in many respects. Clinically there is a younger age of onset, an increased frequency and severity of thrombocytopenia and a greater proportion of patients presenting with RAEB or RAEB/T. Morphologically, bone marrow cellularity is more often reduced, with an increased frequency of moderate fibrosis.\textsuperscript{57} Transformation to AML occurs on 52% of patients compared with 24% in one series, and survival is considerably shorter than for de novo MDS (median, 3 months versus 20 months).\textsuperscript{1}

Secondary MDS/AML following Hodgkin’s disease therapy has been most studied and is directly proportional to the total dose exposure to alkylating agents, particularly mechlorethamine (mustine), rising from 6.4% cumulative incidence at 10 years following 6 courses of MOPP chemotherapy to 37.5% following 12 or more courses.\textsuperscript{58} Newer regimens such as ABVD lacking alkylating agents and hybrid regimens with lower total exposure of mechlorethamine have a much lower cumulative incidence of t-MDS/AML.\textsuperscript{57} The additional risk of t-MDS/AML with chemotherapy plus radiotherapy is controversial.

A higher incidence of t-MDS/AML following chemotherapy for non-Hodgkin’s lymphoma (5-100-fold) has been identified with the widely used alkylating agent cyclophosphamide strikingly exempted, while another alkylating drug chlorambucil was implicated at total exposure doses greater than 1300 mg.\textsuperscript{59} Autologous bone marrow transplantation for Hodgkin’s disease and non-Hodgkin’s lymphoma is associated with an increased risk of t-MDS/AML,\textsuperscript{60} though how much of this risk is transplant-related versus the effects of prior chemotherapy-induced DNA damage is uncertain.

The development of myeloid neoplasias has also been described after chemotherapy for other malignancies. t-MDS/AML following chemoradiotherapy for childhood acute lymphoblastic leukemia is rare except in association with twice weekly epipodophyllotoxin containing regimens.

### Table 4. Therapy-related myelodysplastic syndrome/acute myeloid leukemia.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Peak latency of onset of MDS/AML</th>
<th>AML via MDS</th>
<th>Cytogenetic abnormalities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkylating agents</td>
<td>5-10 yrs</td>
<td>50%</td>
<td>monosomy 5/7 complex</td>
</tr>
<tr>
<td>Epipodophyllotoxins/anthracyclines</td>
<td>6 mos./5 yrs</td>
<td>rare</td>
<td>t(11q23)/t(21q22)</td>
</tr>
<tr>
<td>Bimolane</td>
<td>2-3 yrs</td>
<td>rare</td>
<td>t(15;17)</td>
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The development of myeloid neoplasias has also been described after chemotherapy for other malignancies. t-MDS/AML following chemoradiotherapy for childhood acute lymphoblastic leukemia is rare except in association with twice weekly epipodophyllotoxin containing regimens.
This type of secondary AML frequently exhibits a karyotype anomaly that involves the chromosome 11q23 locus. t-AML has only occurred in 14/4100 UK patients treated for ALL in MRC studies between 1980 and 1995. Alkylating agent-based chemotherapy for ovarian cancer produced a 12-fold increased risk of t-AML compared with surgery alone in one large study, while etoposide-associated t-AML risk was first identified in a cohort of patients treated for testicular cancer. Platinum-based chemotherapy is not usually associated with secondary hematological malignancy. The peak latency period for t-MDS/AML post chemotherapy is 5-10 years. Thereafter, the incidence declines and becomes similar to the one that is found in the general population. In the individual patient, the latency period may be extremely variable. A single case followed after lymphoma therapy acquired RAS mutation and 7q- at 4 years post-chemotherapy and subsequently impaired progenitor growth in vitro at 5 years while hematologically normal and with polyclonal hematopoiesis throughout.

While the leukemogenic effect of alkylating agents and other chemotherapeutic drugs must be due to genetic damage to hematopoietic stem cells (chromosome aberrations and point mutations in critical tumorigenic genes), the molecular mechanisms involved are still imprecisely defined. Recent studies have found a high incidence of mutator phenotype (microsatellite instability) and p53 mutation (with instability at the p53 locus) in patients with t-MDS/AML. This is consistent with defective DNA mismatch repair producing genomic instability either as a risk factor for the development of therapy-related myeloid malignancy or occurring as a result of chemotherapy-induced DNA damage. Microsatellite instability appears to be infrequent in de novo MDS.

**Occupational and environmental carcinogens: case-control studies of patients with MDS/AML**

Several modest sized case-control studies of MDS patients and appropriate control subjects have now been reported. Most have involved a self-completed or assisted questionnaire exploring potential occupational, recreational and environmental factors associated with an increased odds ratio for MDS patients compared with controls. In the largest and most detailed study, 400 cases and controls (individually matched) were compared. Exposure histories including intensity assessment were obtained for 70 chemicals, other hazards or radiation. An increased or possibly increased odds ratio was found for MDS patients exposed to radiation, halogenated organics and metals. Elevated odds ratios at higher exposure thresholds were found for copper, arc welding fumes and hydrogen peroxide, with borderline associations for degreas-
excess of MDS over the expected frequency though numbers are small. A reduction in benzene exposure at one petrochemical manufacturing complex appears to have reduced the frequency of leukemia to background levels, while an increase in MDS and myeloproliferative disease is seen. No increased frequency of hematological disease was associated with a cohort of shipyard painters exposed to ethylene glycol although some biochemical differences consistent with early MDS were suggested in the exposed group.

Benzene-associated MDS/AML: an emerging model

Insight into the mechanisms of extrinsic genomic damage by toxic benzene metabolites is provided by in vitro studies of their effects on hematopoietic cells. Benzene is metabolized initially by cytochrome P450 2E1 (predominantly hepatic) to the phenolic derivatives catechol and hydroquinone. Benzene metabolic intermediated such as muconid acid and benzoquinone are present in proportionately higher quantities in urine following low human occupational in vivo exposure compared with high exposure. Myeloperoxidase (MPO), present in CD34+ hematopoietic cells, is capable of generating the genotoxic p-benzoquinone from hydroquinone and the enzyme NAD(P)H quinone oxidoreductase (NQO1) deactivates p-benzoquinone back to hydroquinone (Figure 3). Human CD34+ cells possess a high MPO/NQO1 ratio. MPO is present at highest concentrations in myeloid precursors while NQO1 is most concentrated in bone marrow stromal cells. A higher frequency of NQO1 inactivating polymorphisms associated with rapid chlorzoxazone excretion (a marker of CYP2E1 activity) has been identified in subjects developing hematotoxicity following benzene exposure. This certainly implies a role for hematopoietic carcinogen detoxification (and defects thereof) at the target site of damage. Carcinogenic properties of benzene are mediated via genotoxic and non-genotoxic mechanisms. Benzene metabolites form DNA adducts and also generate mutagenic oxygen free radicals (OFR). Apoptosis is another consequence of benzene-induced OFR production. Non-genotoxic benzene effects may be mediated via altered bone marrow microenvironment, activation of protein kinase C and immune modulation (Figure 3). In vivo biomarkers of the genotoxicity of benzene include: 1) chromosome aberrations; 2) RAS oncogene mutations; and 3) increased somatic mutation frequency. These biomarkers provide insight into the array of genotoxic events which may initiate the MDS process, but also represent components of disease evolution. An increased frequency of chromosomal aberrations following benzene exposure is reported by several groups. Recent work suggests an increased frequency of leukemia/MDS-associated chromosomal aneuploidy, deletions and translocations [trisomy 9, monosomy 5 and 7,......

Figure 3. Mechanism of benzene-induced hematotoxicity.
CYP2E1: cytochrome P450 2E1; Fe/OH: Fenton reaction catalyzed by iron producing the hydroxyl radical OH; MPO: myeloperoxidase; NQO1: NAD(P)H quinone oxidoreductase 1.
t(8;21) in peripheral blood of healthy benzene-exposed subjects.\(^8\) RAS oncogene mutations were found more commonly in AML patients with a history of chemical exposures, although this study used crude exposure data on relatively small patient numbers.\(^7\) In contrast, a separate study found mutant RAS to be more frequent in benzene-exposed leukemia than in de novo leukemia.\(^8\) Increased somatic mutation frequency can be estimated using hypoxanthine phosphoribyl transferase (HPRT) mutational frequency in blood lymphocytes, and also at the glycophorin A (GPA) MN locus in red cells. These assays probably reflect the mutational rate of multipotent progenitor/stem cells. An increased background mutational frequency occurs with age. Benzene exposure produces an increased frequency of gene duplicating (mitotic recombination; NN) GPA mutants\(^9\) while post-chemotherapy and post-ionizing radiation patients show an increased frequency of deletional mutants (N0) as well as gene duplicating mutants.\(^10,\)^11 It can be expected that exposure to genotoxic agents in adulthood when spontaneous somatic mutation frequency is increasing, is more likely to produce critical genomic damage leading to clonal expansion than if exposed in childhood when spontaneous mutant frequency is lower. This is supported by the low incidence of post-chemotherapy t-MDS/AML in children treated for acute lymphoblastic leukemia and the low frequency of clonal disorders following aplastic anemia in childhood compared with adulthood.

**Studies of DNA damage in heavily polluted areas and other potential etiological agents**

Air pollution remains a major source of environmental carcinogens, the most prevalent of which are polyaromatic hydrocarbons (PAH). An increase in leukocyte PAH-DNA adducts, which are potentially mutagenic genomic lesions, has been associated with high environmental pollution in the Czech Republic and Poland.\(^12,\)^13 These were also associated with an increased frequency of chromosome aberrations in the Polish study.\(^13\) No study has looked systematically at air pollution in relation to MDS.

Dietary exposure to carcinogens has been implicated in many human cancers though this has not been studied in MDS. Presently, there is no evidence for a viral etiological factor in human MDS.

**Epidemiology of myelodysplastic syndromes**

**Age distribution**

It is well known that different hematological malignancies display striking differences in their age pattern. MDS are geriatric disorders, with more than 80% of patients being older than 60 years at diagnosis.\(^1\) This characteristic age distribution possibly reflects the gradual accumulation of random genomic damage from endogenous and exogenous carcinogens during lifetime. However, it should be emphasized that the small numbers of younger adults, adolescents and even children who are diagnosed as having MDS appear to be rising in recent years.\(^2\) In the Düsseldorf bone marrow registry which now includes 1,200 cases of MDS, 9% of patients were younger than 50 years. Goasguen and Bennett have estimated that MDS account for 1-3% of all childhood malignancies.\(^3\) A recent Danish survey found that MDS constituted 9% of all hematological neoplasias in children less than 15 years old.\(^4\) According to a recent French study,\(^5\) younger patients with MDS tend to have more advanced, prognostically unfavourable morphological subtypes (particularly RAEB/T), an increased risk of transformation to AML, and a higher rate of adverse karyotype anomalies (monosomy 7). These findings could partly be confirmed in the Düsseldorf series (Table 6). More than 30% of younger patients belonged to the RAEB/T group, whereas the respective percentage for patients over age 50 was only 15%. Paradoxically, despite a higher risk of AML transformation, the younger patients had a significantly improved prognosis. Their 5-year cumulative survival was 33%, as compared to 24% in patients older than 50 years. This is simply due to the fact that this category of patients usually received intensive chemotherapy in our department rather than being treated with supportive care only.

**Sex ratio**

Men have a slightly higher MDS risk than women. A meta-analysis of 13 studies including a total of

| Table 5. Common themes between epidemiological case-control studies. |
|---------------------------------|-------------------|
|                                | Cigarette smoking | Hair-dye use |
| West et al.\(^6\)              | OR elevated (2.03) | elevated OR (2.38) |
| Nisse et al.\(^7\)             | OR=1.83 (p=0.03)  | NS            |
| Pasqualetti et al.\(^12\)      | OR=2.33 (p<0.03)  | NA            |
| Mele et al.\(^8\)             | no elevated OR,   | probable increased risk |
|                                | but OR for        | also elevated OR |
|                                | >10 pack-years    | for female     |
|                                | greater than      | hairdressers  |
|                                | for 1-10 pack-years | (trend test: p<0.05) |
| Ido et al.\(^10\)             | OR=1.8 (NS)       | OR=1.77 (NS)  |

OR: odds ratio; NS: not significant; BS: borderline significance; NA: not assessed.
2,579 patients yielded a male-to-female ratio of 1.2. A similar sex ratio was found for patients with AML and may be explained by an increased occupational exposure of men to leukemogenic substances. Chronic myelomonocytic leukemia (CMML) stands out by its high male-to-female ratio which varies between 1.5 and 3 in different series. On the contrary, the 5q− syndrome is more common in women than in men. On reviewing 43 patients with refractory anemia and 5q− as the sole chromosome anomaly, Van den Berghe et al. reported a sex ratio of 0.5. These data reflect the heterogeneity of MDS and point to different etiological factors involved in the process of leukemogenesis.

Problems and limitations of incidence studies in MDS

Although MDS have been actively investigated in recent years, there is still a lack of reliable incidence data. For many years, changing definitions and classifications have impeded large-scale studies that are required for obtaining truly representative data on the incidence and prevalence of MDS. MDS is not listed in the ninth version of the ICD coding system for hematological malignancies. Therefore, cases must be assigned to various other diagnoses which are not always well defined. For example, CMML is grouped with the chronic myeloid leukemias rather than with MDS. A recent Swedish study found that cases of MDS were recorded under five different diagnoses in cancer registries. Due to these shortcomings, current morbidity and mortality statistics are inadequate tools for characterizing the magnitude of the MDS problem. At present, accuracy of diagnosis and completeness of case registration seem to be confined to specialized registers such as regional cancer surveys or hospital-based statistics. It should be recognized that the usefulness of such registers is also restricted. Limiting factors include small and ill-defined reference populations, inability to detect regional variations in the incidence of MDS, and bias due to patient referral patterns which are influenced by special diagnostic expertise of the participating institutions.

Crude and age-specific incidences of MDS

Data from recent epidemiological studies (Table 7) suggest that MDS are relatively common hematological disorders. Crude incidences varied between 3.5 and 12.6 per 100,000 population per year in different studies. Considering the characteristic age distribution of MDS, it is appropriate to determine age-specific incidence rates rather than crude incidences. Only age-specific incidences make comparisons between different populations meaningful, because crude rates are strongly influenced by the age structure of each given population. Concerning the age groups which are mainly affected (> 70 years), we are now faced with incidence rates of 15-50 per 100,000 per year, suggesting that in older persons MDS are at least as common as chronic lymphocytic leukemia or multiple myeloma. The incidence of MDS continues to rise into very old age. For example, the study of Williamson et al. reported an annual average incidence of 49/100,000 for the age group 70-79 years, whereas it climbed to 89/100,000 for the over 80-year-old people. These are preliminary data, which must eventually be confirmed by truly representative population-based statistics.

It should be emphasized that even the highest incidence figures that can be calculated from present epidemiological studies may still be an underestimate of the true incidence of MDS. Because of the paucity of clinical symptoms in early stage MDS, there will always be a considerable number of undetected patients. Furthermore, in at least some cases the morphological features of myelodysplasia on the peripheral blood and bone marrow smear are so marginal as to defy diagnosis even by an experienced hematologist. Thus, a reliable estimate of the true incidence of MDS is difficult to obtain. It would require screening for abnormal blood counts in a sizeable population and performing bone marrow biopsies in all cases of unexplained cytopenia. However, as long as therapeutic options are lacking, invasive diagnostic procedures are not easily justifiable.

Several etiological surveys as well as cytogenetic studies have pointed to a link between MDS development and certain occupational hazards. It this
respect, it is interesting to note that current incidence studies have failed to demonstrate a difference in incidence figures between rural parts of the population and people living in heavily industrialized areas.98,103

Real or apparent increase of MDS

Until 1973 there were only 143 patients with MDS reported worldwide.107 Nowadays, referral centres collect similar case numbers within 2-3 years. A substantial increase in MDS cases diagnosed was particularly noted between 1980 and 1990, whereas more recent incidence studies failed to document a significant change in the frequency of these disorders. According to an international Delphi study performed by Reizenstein and Dabrowsky in 1991,102 about 90% of hematologists shared the impression that the incidence and prevalence of MDS had increased by at least 100% during the past 10 or 20 years. Principally, this rise in MDS cases may be attributed to several factors, including demographical changes, better or more widely available laboratory facilities, expansion of invasive diagnostic procedures (bone marrow biopsy), agreement on diagnostic criteria of MDS (FAB classification), increased exposure to known or new leukemogenic agents, or a combination of these factors.

In our opinion, much of the increase in MDS reflects more accurate diagnosis and case registration, partly as a result of improvement in geriatric medical care. The increased case finding seen in many centers coincided with the publication of the FAB classification which for the first time provided practicable morphological criteria for the diagnosis and categorization of MDS.2 Whereas patients with RAEB or RAEB/T can be easily identified on routine bone marrow smears, the FAB proposals and the increasing availability of cytogenetic and molecular genetic investigations have facilitated the diagnosis of patients with early stage MDS and chronic myelomonocytic leukemia. On the other hand, the dramatic gain in life expectancy in the general population has demanded adaptation of Medicine and Public Health. The development of geriatrics is part of this response to current demographic trends. Demographical, epidemiological and socioeconomic issues have forced attention upon research into ageing and encouraged intensification of health service delivery for elderly people. Hematologists have participated in this development, as reflected by more aggressive diagnostic and therapeutic efforts in older persons. In this respect, the increasing frequency of MDS cases diagnosed is the result of more careful and invasive hematological diagnostics in geriatric patients. This assumption is supported by data from the Düsseldorf bone marrow registry. Referring to our bone marrow register as a whole (not just MDS cases), we noticed a change in the age distribution of patients undergoing bone marrow biopsy. The proportion of patients over age 60 among new entries to the register rose from 42% in 1975 to 54% in 1990. This is an increase of nearly 30%. Over the same time period, the percentage of over 80-year-old patients even climbed from 2.5% to 9%. Not surprisingly, there was a strong correlation between the proportion of elderly patients and the relative frequency of MDS cases. Therefore, we think that the rising incidence of MDS largely reflects increased physician awareness and extended use of diagnostic procedures in the elderly.

Contrary to the assumptions of hematologists in Reizenstein’s opinion poll,102 we do not think that medicinal measures (increased use of cytotoxic agents and other myelosuppressive drugs) sufficiently explain the rising incidence of MDS. Although therapy-related MDS/AML are becoming more frequent as a consequence of intensified chemotherapeutic regimens, it is clear that this group of patients cannot account for the substantial rise in the overall incidence of MDS. It has been estimated that t-MDS contribute 10-15% of cases at the most.108 According to several studies, this percentage has not increased in recent years. For example, the proportion of t-MDS in the Düsseldorf bone marrow registry was 7% between 1976 and 1880, and 5.8% between 1986 and 1990. It thus appears that the rise in MDS cannot be explained by an increased use of antineoplastic agents.

**Conclusions and future directions**

For many decades, uncontrolled growth of cells has been regarded as the result of defects in genes

### Table 7. Crude and age-specific incidences of MDS.98,103,105

<table>
<thead>
<tr>
<th>Geographic area</th>
<th>Aul et al.</th>
<th>Radlund et al.</th>
<th>Williamson et al.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Düsseldorf (Germany)</td>
<td>4.1</td>
<td>3.5</td>
<td>12.6</td>
</tr>
<tr>
<td>Jönköping (Sweden)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Dorset (England)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;49 yr</td>
<td>0.2</td>
<td>0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>50-59 yr</td>
<td>4.9</td>
<td>1.6</td>
<td>5.3</td>
</tr>
<tr>
<td>60-69 yr</td>
<td></td>
<td></td>
<td>15.0</td>
</tr>
<tr>
<td>70-79 yr</td>
<td>22.8</td>
<td>15.0</td>
<td>49.0</td>
</tr>
<tr>
<td>≥80 yr</td>
<td></td>
<td></td>
<td>89.0</td>
</tr>
<tr>
<td>All ages</td>
<td>4.1</td>
<td>3.5</td>
<td>12.6</td>
</tr>
</tbody>
</table>

Incidence figures per 100,000 population per year.
controlling either cell proliferation or differentiation. There is now accumulating evidence that the other side of the coin, the rate of cell death, can be an equally important factor in controlling the size of a cell population.\(^{53}\) As detailed in this review, apoptosis is an important pathogenetic mechanism in MDS. Moreover, apoptosis is a cell biological process conserved throughout evolution. However, our knowledge of apoptosis and its genetic control and signal transduction in human cell systems is still sparse. Future studies on apoptosis and escape from apoptotic control should be directed to molecular biological approach to identify 1) genes the activation of which promotes apoptosis; 2) signals the accumulation of which either induces apoptosis or decreases the threshold at which such signals induce apoptosis; and 3) causative lesions leading to abnormalities in the apoptotic control in MDS. In addition, we need to develop more sensitive techniques for the detection of pre-apoptotic cells that are about to die. It is hoped that with further understanding of these central questions, we may be able to define the molecular sequence of events in MDS from excessive apoptosis in preleukemic stages to default apoptosis in the final leukemic transformation and to establish new treatment modalities.

A single etiological insult leading to the initiation of MDS remains unlikely. Epidemiological studies are beginning to identify some of the more likely etiological factors such as alkylating agents, radiation (X- and \(\alpha\)-particle), benzene, cigarette smoke and possibly hair-dye. Many more factors are suggested from these epidemiological studies, though with weaker associations. The molecular initiation and clonal evolution of hematotoxicity associated with benzene exposure is increasingly understood and provides a model for mechanisms by which initiation of MDS could follow other genotoxic insults. Case-control and cohort epidemiological studies in tandem with molecular and cellular studies of the mechanism of carcinogen-induced cellular damage continue to be the route to identify the MDS initiation process(es).

Our knowledge of the descriptive epidemiology of MDS is still based on a few regional studies. Data of these studies suggest that MDS are rather common hematological malignancies. While the overall incidence is 3-13/100,000/year, incidence figures rise steeply with age. In people over age 70, we are now faced with incidence rates of 15-50/100,000/year. Large-scale epidemiological studies are required for obtaining truly representative statistics on the incidence and prevalence of MDS. As a prerequisite, future revisions of disease classification systems must incorporate MDS as a separate group of disorders. With regard to the greying of the population in developed countries, we can be almost certain that there will be a substantial increase in MDS over the next few decades.

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The three authors equally contributed to the conception of this review and to the writing of the paper. Many of the initial studies and much of the early interest in this field were inspired by the late Professor Allan Jacobs whose contribution to this area of MDS research cannot be underestimated. The authors wish to thank Professor Martyn T. Smith for his critical review of this manuscript.

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