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Integrating WHO 2001–2008 criteria for the diagnosis of Myelodysplastic Syndrome (MDS): A case–case analysis of benzene exposure

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ABSTRACT

We characterized the prevalence of hematopoietic and lymphoid disease for 2923 consecutive patients presenting at 29 hospitals from August 2003 to June 2007. Diagnoses were made in our laboratory using WHO criteria based on morphologic, immunophenotypic, cytogenetic, FISH and molecular data. A total of 611 subjects (322 males/289 females) were prospectively diagnosed with MDS using WHO (2001) criteria. Update and re-evaluation of cases using MDS (2008) criteria resulted in 649 MDS cases. Using WHO (2008) criteria, refractory cytopenia with multilineage dysplasia (RCMD) accounted for 68% of total cases, refractory anemia with excess blasts (RAEB), 16.3%; refractory anemia (RA), 6.5%; refractory cytopenia with unilineage dysplasia (RCUD), 4%; and MDS-unclassifiable (MDS-U), 4.5%. Subjects were administered questionnaires and information on previous disease, work histories and exposures to potential etiologic agents such as benzene (BZ) was obtained. A total of 80/649 (13.2%) were determined to have some BZ exposure. The frequency of clonal cytogenetic abnormalities in all MDS was 30%, the most common being +8 > del(20)q > del(7q) > del(5q), while the analogous frequency in BZ-exposed cases was only 24%. To further investigate the characteristics of MDS associated with BZ, we identified a subset of cases with high BZ exposure. These BZ signal cases were each matched by age and gender to two cases with no known BZ exposure. When contrasting BZ signal cases vs matched cases with no BZ exposure, we found a high odds ratio (OR) for the WHO subtype MDS-U (OR = 11.1), followed by RAEB and RCUD (OR = 1), RA (OR = 0.7) and RCMD (OR = 0.6). Multilineage dysplasia with abnormal eosinophils (MDS-Eo) was strongly associated with BZ exposure, whereas the relative risk of clonal cytogenetic abnormalities was reduced for high BZ-exposed cases (OR = 0.5). These findings are strongly indicative that MDS subtypes are influenced by BZ exposure, and taken together with previous studies, the features of MDS-Eo suggest that altered immune regulation plays a major role in the pathogenesis of MDS following chronic exposure to BZ.

1. Introduction

During the 1960s and 1970s there was increasing appreciation of a group of relatively obscure hematologic disorders that were associated with progressive bone marrow failure, were not characterized by increases in the number of circulating cells found in myeloproliferative or leukemic diseases and that shared certain common features of abnormal maturation and development of hematopoietic precursor cells in the bone marrow. Precision in diagnosis and prognosis has developed considerably for these debilitating, often fatal and sometimes "preleukemic" conditions now known as myelodysplastic syndromes (MDS). Understanding the nature and pathogenesis of MDS remains a work in progress. However, considerable advances have been made over the last decade in standardizing criteria for the diagnosis of the disease, with the widespread adoption of the WHO (2001) – and the recent introduction of WHO (2008) – classification criteria [1–3].

Today, MDS are recognized as a heterogeneous group of hematopoietic malignancies that are characterized by ineffective blood cell production, or hematopoiesis, that is accompanied by abnormal maturation and dysplasia in one or more blood cell lineages in the bone marrow (BM) [1]. Although MDS is usually

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progressive, the prognosis is highly variable. Some patients with MDS will undergo transformation to acute myeloid leukemia (AML) while others may become transfusion-dependent and eventually succumb to bleeding or infection. In still other cases the patient's clinical condition can remain relatively stable for long periods of time. The heterogeneity and clinical variability of MDS poses major challenges for prognosis as well as the rational development of effective therapeutic strategies. Principal differences between WHO (2001) and WHO (2008) criteria focus on the introduction of a new category in WHO (2008), refractory cytopenia with unilineage dysplasia (RCUD), which extends MDS to include BM failure syndromes with single lineage dysplasias in the granulocytic and megakaryocytic lineages, and some refinement in the definitions applied to MDS-unclassifiable (MDS-U) [1].

Regional differences in the prevalence of individual MDS subtypes have been reported, primarily between Asian and Western patients [4–6]. We previously characterized a group of 176 MDS patients diagnosed in Shanghai according to WHO (2001) [7] and more recently reported on the prevalence, clinical and cytogenetic characteristics and survival of 435 patients diagnosed with *de novo* MDS [8]. Our results reveal major differences in the age at onset and prevalence of individual subtypes of MDS between Asian and Western patients with a median age of approximately 55 years and a predominance of refractory cytopenia with multilineage dysplasia (RCMD) that approaches 70% of all cases. Although previous studies have suggested MDS as an outcome in workers exposed to BZ [9,10], until recently identification of MDS or its subtypes associated with BZ exposure using modern criteria has been lacking [11]. Herein we extend our characterization of MDS in Shanghai in order to evaluate the impact of WHO (2008) criteria on the analysis of disease prevalence and to assess the influence of chronic benzene (BZ) exposure on the development of MDS.

2. Materials and methods

2.1. Patients

All patients, 18 years of age, presenting at 29 Shanghai hospitals with initial clinical findings consistent with a hematopoietic abnormality between July 2003 and July 2007 were candidates for inclusion in this study. Informed consent was obtained according to the Declaration of Helsinki, 2004 and the NIH Common Rule (45CFR46), and together with the protocol, were approved by the Combined Institutional Review Board of the University of Colorado Health Sciences Center in Denver, CO and the Internal Review Board at Fudan University in Shanghai, China. Peripheral blood, bone marrow aspirates and core biopsies were obtained on all individuals using standardized procedures and evaluated in our laboratory using morphologic, immunophenotypic, molecular and cytogenetic techniques. Cases initially were diagnosed according to WHO 2001 criteria [2]. Patients presenting with concomitant nutritional deficiencies (Vitamin B12, folate or iron), congenital anemias, viral (including HCV or HIV), recent bacterial infections, or receiving concomitant cytotoxic therapy with alkylating or anti-metabolic agents were excluded in this analysis. Follow-up evaluation was routinely provided. Diagnoses were updated at 6–12-month intervals when possible, and all cases were re-evaluated in 2009 using WHO 2008 criteria [1].

2.2. Sample collection and clinical laboratory analysis

Peripheral blood, bone marrow aspirates, tissue and core biopsies were collected in conjunction with diagnostic procedures. Peripheral blood smears were obtained by finger stick. Blood samples were collected by veinpuncture and processed for routine CBC (Cell Dyne 3700, Abbott, Abbott Park, IL) and viral serology (HCV and HIV) (Imx, Abbott). Serum vitamin B12 and folate were measured by chemical luminescence (Beckman Coulter Dxi800), and total iron binding capacity (TIBC) was determined using a Beckman Coulter LX20.

Bone marrow aspirates and core biopsies were obtained by needle extraction (Jamshidi) from the posterior iliac crest. Aspirate cell suspensions were stained with fluorochrome-conjugated antibodies for flow cytometric analysis of bone marrow cellular subsets. Multiparameter analysis was performed using a dual laser flow cytometer (FC-500, Beckman Coulter, Hialeah, FL; Immunotech, Miami, FL) equipped with compensation software (Software CXP, Beckman Coulter). A broad panel of antibodies was used for immunophenotyping of BM cells (Beckman Coulter, Immunotech).

2.3. Morphology

Morphology and immunophenotype analysis were conducted on both bone marrow aspirate (flow cytometry) and core biopsy (immunohistochemistry) material. Bone marrow aspirate and peripheral blood smears were prepared from fresh tissue and evaluated using Wright–Giemsa stained preparations and special stains, including an iron stain. Core biopsy sections were evaluated using Hematoxylin–Eosin (H&E), Gomori trichrome, iron and immunoperoxidase-immunohistochemistry stains for selected markers. Morphology was independently evaluated by two of us (R.D.I., J.R.). Microscopic analysis was performed using Olympus BX51 bright field microscopes (Olympus Optical Ltd., Tokyo). Standardized criteria for determining dysplastic changes and lineage involvement have been described in detail elsewhere [7].

2.4. Cytogenetic and fluorescence in situ hybridization (FISH) analysis

Cytogenetic studies were performed on either bone marrow or peripheral blood collected at diagnosis. Metaphases were prepared from unstimulated, short-term culture preparations (24- and 48-h) and G-banded with trypsin–Giemsa staining. A minimum of 20 metaphases were analyzed in each case. FISH analysis was performed on short-term cultures of bone marrow or blood cells. Systematic screening for −5/5q−, −7/7q−, +8, del(20q) and 11q23/*MLL* rearrangements was performed on each patient. In some cases, additional FISH studies were used to either characterize chromosome abnormalities (CA) observed in banded chromosome studies or to confirm the presence of cytogenetic aberrations suggested by other diagnostic work-up. A minimum of 500 nuclei and 10 metaphases were analyzed in interphase and metaphase analysis, respectively.

2.5. Questionnaire description

Questionnaire administration and data collection procedures are described elsewhere in this issue [12]. Briefly, all subjects were interviewed by trained personnel in the hospital setting. In a few cases, subjects were interviewed at home if they had left the hospital prior to interview. The questionnaires used in this study were designed in English, translated into Chinese and then administered in the native Chinese language. Information obtained in the questionnaire included patient demographics, family history of disease, patient medical history (diseases, medications), patient occupational history and patient non-occupational exposure history (e.g. hobbies, smoking, alcohol use). Clerical staff entered the data in duplicate from the questionnaires into a database that was verified via an external quality assurance audit. Any disagreements between questionnaire entries were resolved quickly by referring to the

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original paper record or the questionnaire administrator. A summary file was generated from the questionnaire data, which did not include any of the clinical information, such as clinical diagnoses, that could compromise blinding. This summary file was assessed and edited via a comprehensive series of logic and consistency checks to assure data integrity.

Information from different fields on the questionnaire was combined to define an enhanced and more inclusive variable for analysis. These instances included: (a) information on diabetes and diabetes treatments (i.e. tolbutamide) for a diabetes indicator, (b) information on tuberculosis (TB) and TB treatments for a tuberculosis indicator, (c) growing crops indicator which used information on fertilizers, insecticides, pesticides, and growing crops, and (d) an inflammation indicator that combined information on infections and inflammatory diseases such as arthritis, hepatitis and TB.

The questionnaire that solicited exposure information for subjects consisted of a detailed assessment of each patient's work history. This work history queried jobs held over each patient's entire career. Subsequent exposure assessment steps involved at least one, but often several, of the following steps: (a) location of exposure measurement records for the job and location where the subject worked through the Shanghai Institute of Public Health Supervision (IPHS) database, (b) location of records for surrogate factories, industries or jobs in the IPHS database, (c) assessment of exposures detailed in the Chinese literature for relevant industries, (d) assessment of regulatory and technology changes for key industries, (e) monitoring data from nearby facilities, and/or (f) conducting task simulations. An expert panel assessed exposures using the above data. Additional independent checks with source data further refined the expert panel assessment.

2.6. Exposure assessment

Five ordinal categories of exposure: 0, <1 mg/m^3, 1 ≤ 10 mg/m^3, 10 ≤ 100 mg/m^3, and 100+ mg/m^3 were formed. Each job/location scenario was placed into one of these five categories using the procedures summarized above. Category 1 was assigned a 'score' of 1, category 2 a score of 2, etc. We then further categorized the third category (10–100 mg/m^3) into 3.1 (10–32 mg/m^3), 3.2 (33–66 mg/m^3) and 3.3 (67–100 mg/m^3) and calculated the months exposed at each estimated concentration level. We believe the scores represent an accurate assessment of relative BZ exposure in diverse Chinese industries, and should be considered one of the strongest exposure assessments for BZ in the literature to date. While we are confident that the relative degree of BZ exposure is represented in the scoring scheme used, we are more cautious regarding precise exposure concentrations assigned to each score. Therefore, we have chosen to report exposure grades rather than assigning a midpoint concentration to each category in order to calculate a measure analogous to 'ppm' or 'ppm-months'.

2.7. Case–case selection

An objective of this study was to compare both the demographic and clinical characteristics of MDS cases highly exposed to BZ with a similar population of MDS cases with no documented BZ exposure. Although it is impossible to determine the etiology of any individual case within a group, we hypothesized that BZ exposure is most likely to have a role in the development of MDS for the most highly exposed cases in our series. Therefore, our rationale was to establish a clear contrast between background cases (unexposed) and those that could reasonably be attributed to BZ. Consequently, for some of the analyses performed in this study we defined "signal cases" as individuals who were exposed to >67 mg/m^3 (i.e. categories 3.3 or 4) for at least 6 months with a mean exposure duration of 144 months (N = 29). We matched these cases by gender and exact age to two cases from a randomized group of MDS cases with no documented BZ exposure (i.e. score category 0).

Table 1
Comparison of WHO 2001/2008 criteria MDS subtypes in Shanghai, China.

WHO 2001	Total cases	WHO 2008	Total cases
RA	38 (6.2%)	RA	38 (5.9%)
RARS	7 (1.1%)	RARS	7 (1.1%)
RAEB	106 (17.3%)	RAEB	106 (16.3%)
RCMD	432 (70.7%)	RCMD	441 (68%)
MDS-U	26 (4.3%)	MDS-U	29 (4.5%)
MDS 5q−	2 (0.3%)	MDS 5q−	2 (0.3%)
		RCUD	26 (4.0%)
Total	611	Total	649

2.8. Blinding

All study personnel involved in the clinical laboratory diagnosis of disease, as well as members of the expert exposure assessment panel, were blinded to the exposure status of subjects throughout the data acquisition phase of the study.

2.9. Statistical analyses

t-Tests (pooled variance) or tests for independent proportions, where appropriate, were used to examine differences between clinical features for MDS subtypes. Kaplan–Meier survival analysis was used to estimate overall survival (OS), and conditional logistic regression analysis was used to calculate odds ratios (OR), along with 95% confidence intervals, for matched case–case analyses, both employing STATA 9.0 software (StataCorp, College Station, TX). All *p*-values were two-sided and values less than 0.05 were considered statistically significant.

3. Results

3.1. Prevalence and age distribution of MDS in Shanghai

The total number of cases of MDS initially diagnosed according to WHO 2001 was 611, including 322 males (52.7%) and 289 females (47.3%). A comparison of the prevalence of MDS subtypes diagnosed according to 2001 and 2008 WHO revisions is provided in Table 1. Diagnosis using 2008 criteria resulted in a 6% increase in the total number of MDS cases (N = 649). This included 9 cases which were not initially diagnosed with MDS, but met criteria for a diagnosis of RCMD with subsequent follow-up evaluation. An additional 26 cases with single lineage dysplasia that initially were not diagnosed with MDS using 2001 criteria were reassessed according to 2008 criteria and diagnosed with RCUD. The age and gender distribution of MDS cases diagnosed according to WHO 2008 is presented in Fig. 1a. The ratio of males to females (341:308) diagnosed using 2008 criteria remained unchanged from WHO 2001 as did the median age at diagnosis: WHO (2001) males/females: 56/52 years; WHO (2008) males/females: 57/52 years. Based on a median age of 70 years typically reported for MDS in the West, these results confirm and extend major differences in the age distribution and prevalence of individual subtypes of MDS between Asian and Western patients [6–8]. Asian patients tend to present with MDS at a much earlier age and develop predominantly RCMD.

3.2. Frequency of cytogenetic abnormalities in MDS in Shanghai

The overall frequency of clonal cytogenetic abnormalities in MDS diagnosed according to WHO (2008) was 30.2% (196/649).

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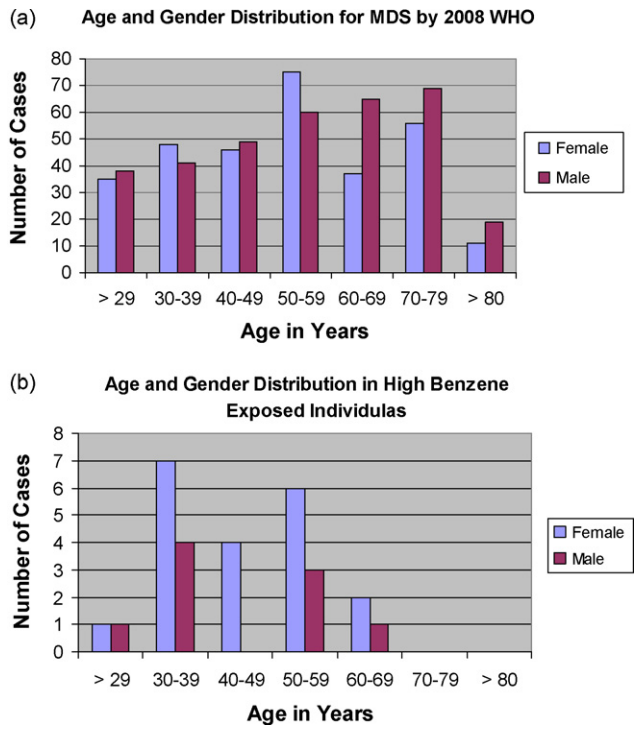

Fig. 1. (a) Age and gender distribution of WHO (2008) cases grouped by decade. (b) Age and gender distribution of benzene signal cases grouped by decade.

Table 2
Clonal cytogenetic abnormalities by MDS subtype.

MDS subtypes	Total cases	Clonal abnormality
RA + RARS	45	4 (8.9%)
RAEB	106	64 (60.4%)
RCMD	441	112 (25.4%)
RCUD	26	2 (7.7%)
MDS-U	29	12 (41.4%)

Although both banded chromosome analysis and FISH were routinely employed in diagnosis, only 10 cases presenting with a normal karyotype were found to have a clonal abnormality using FISH alone. The majority of these involved del(20q). The overall frequency of clonal abnormalities was somewhat less than reported in Western studies and is consistent with the predominance of RCMD (Table 2). Trisomy 8 was the most frequent abnormality encountered in our MDS cases, followed by del(20q) > del(7q) > del(5q) abnormalities (Table 3). Chromosome abnormalities involving both

Table 3
Clonal cytogenetic abnormalities MDS (2008) $N = 649$.

Clonal abnormality	*N*	%
Total	196	30.2
+8	53	8.2
del(20q)	26	4.0
7q−/−7	22	3.4
5q−/−5	20	3.1
−5/−7[a]	19	2.9
−Y	7	1.1
del(9q)	4	0.6
11q23	5	0.8
Ch 3	5	0.8
del(13q)	3	0.5
−X	3	0.5
Other	29	4.5
Complex karyotype ≥ 3 abnormality	46	7.1

[a] Exclusively in complex karyotypes.

del(7q) and del(5q) in combination were invariably associated with complex karyotypes involving three or more abnormalities.

3.3. Benzene exposure and MDS

A total of 80 out of 649 subjects (13.2%) diagnosed with MDS were determined to have some BZ exposure (i.e. BZ exposure grade ≥ 1). Our exposure assessment for BZ was derived from questionnaire responses, along with a detailed assessment of the presence and degree of BZ exposure in the jobs and industries observed from our work history data. This led to the development of the ordinal exposure categories that were further assessed by independent scientists using various ranking schemes. The frequency of CA in the BZ "ever-exposed" group was lower than that observed in the total population of MDS cases (i.e. 24% vs 30%). The median age of the subset of 29 BZ highly exposed cases, which were defined as "signal cases," was compared to 569 cases which were determined to have no BZ exposure (i.e. exposure grade = 0). BZ-exposed signal cases (median age = 49) were significantly younger at diagnosis than this comparison group (median = 55, $p < .001$) (Fig. 1b). Gender differences between BZ signal cases and the total population of MDS cases were observed as well, with the proportion of females in the BZ signal group (20/29) significantly higher than for female MDS cases not in the BZ signal group (288/620) ($p < .05$).

3.4. Case–case comparisons: clinical and cytogenetic features

Comparison of the prevalence of individual MDS subtypes between BZ signal cases and matched unexposed cases indicated a marked increase in MDS-U, no differences in refractory anemia

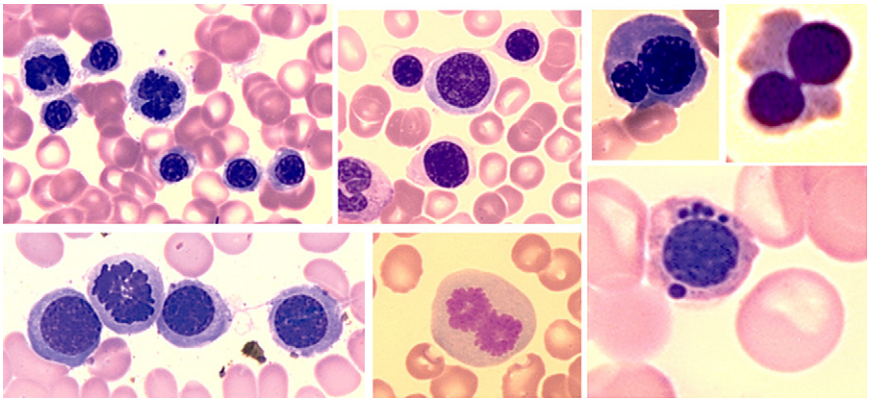

Fig. 2. Dyserythropoiesis with megaloblastic changes and nuclear bridging in MDS associated with previous high BZ exposure. Bone marrow aspirate with Wright–Giemsa stain (original magnification 1000×). Reproduced with permission [11].

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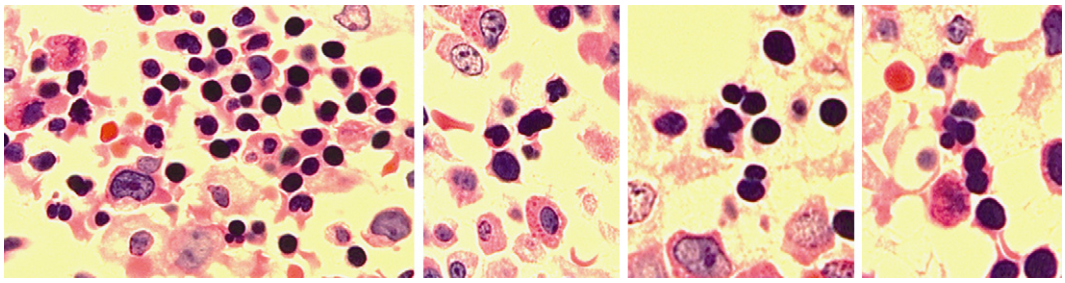

Fig. 3. Dyserythropoiesis and stromal degeneration accompanying MDS-Eo associated with previous high BZ exposure. Core biopsy section. Hematoxylin–Eosin stain (original magnification 1000×). Reproduced with permission [11].

with excess blasts (RAEB) or RCUD, and relative deficits of RCMD and refractory anemia (RA) in BZ signal cases compared to cases with no BZ exposure (Table 4). The increased number of MDS-U in BZ signal cases reflects a frequently observed imbalance between cytopenias found in peripheral blood and lineage-specific dysplasia in the bone marrow of these patients (primarily normal or near-normal hemoglobin (Hg) values in the presence of marked bone marrow dyserythropoiesis).

A comparison of the clinical features between BZ signal cases and matching cases for which there is no documented exposure to BZ is presented in Table 5. Features that frequently characterized BZ signal cases included marked erythroid dysplasia in the bone marrow (Figs. 2 and 3), no evidence of increased ring sideroblasts, near-normal hemoglobin values (Hb) in the face of marked BM dyserythropoiesis, a tendency toward low-to-normal BM cellularity, and a striking increase in multilineage dysplasia with abnormal

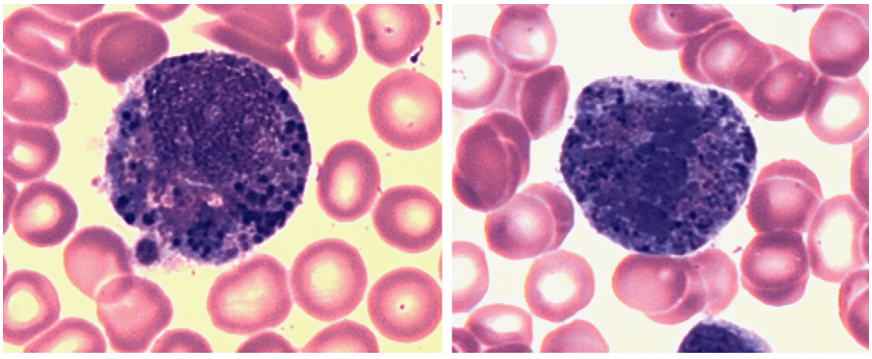

Fig. 4. Abnormal eosinophilic precursor cells in MDS-Eo associated with previous high BZ exposure. Cells exhibit megaloblastic nuclear abnormalities, nuclear hypersegmentation and atypical giant cytoplasmic granulation. Bone marrow aspirate with Wright–Giemsa stain (original magnification 1000×). Reproduced with permission [11].

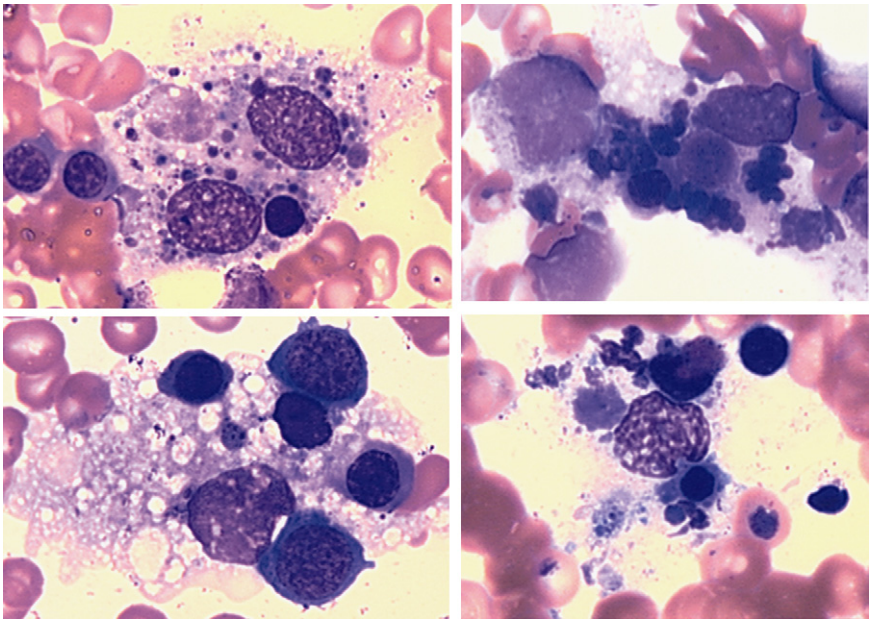

Fig. 5. Hematophagocytosis accompanying MDS-Eo associated with previous high BZ exposure. Activated histiocytic cells exhibit prominent phagocytosis of degenerating erythroid granulocytic cells typical of an immune-mediated inflammatory process. Bone marrow aspirate with Wright–Giemsa stain (original magnification 1000×). Reproduced with permission [11].

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Table 4
Odds ratios for MDS subtypes in benzene signal cases.

Subtype	*N*	OR	CI
RCMD	16	0.58	0.24–1.4
RAEB	2	1.4	0.19–11.1
MDS-U	7	11.12[a]	1.34–92.4
RCUD	2	1.0	0.18–5.46
RA	2	0.67	0.13–3.3

[a] $p < 0.05$.

eosinophils (MDS-Eo) (Fig. 4), hematophagocytosis (Fig. 5) and stromal degeneration (Fig. 3). Quantitative case–case comparison of clinical features in BZ signal cases are presented in Table 6. Hb levels >10 g/dL were increased and the prevalence of MDS-Eo between BZ signal cases and matched unexposed cases revealed an infinite OR. No cases of peripheral eosinophilia were observed in any of these subjects. The prevalence of clonal cytogenetic abnormalities was lower in BZ signal cases than matched unexposed cases with relative deficits observed for trisomy 8 as well as del(7q) abnormalities. Clonal CA involving del(5q) alone were not found in BZ signal cases. Other CA observed in the BZ signal cases were del(9q22) and +X, which each occurred once. Abnormalities involving inv(16)(p13.1q22) were not observed. These findings indicate that CA in MDS cases developing secondary to high BZ exposure are not consistent with the paradigm involving unbalanced loss of chromosomes 5 and/or 7 that is characteristic of t-MDS/t-AML secondary to treatment with alkylating chemotherapeutic agents.

3.5. Survival

Analysis of overall survival (OS) between the BZ signal group and matched unexposed cases revealed differences in the pattern of OS between these two groups (Fig. 6). Initial survival (<10 months) after diagnosis was lower in BZ signal cases than in matched unexposed cases. The reasons for this are not known. However, long term OS was markedly increased in BZ signal cases. One possible explanation for the latter observation is that we have found Hb, which is higher in BZ signal cases relative to unexposed cases, is a significant favorable independent variable in predicting OS in MDS [8]. These disparate outcomes suggest biologically significant differences in the pathogenesis of MDS between BZ highly exposed and unexposed (i.e. *de novo*) cases. Comparison of OS between matched unexposed cases and a larger subset of *de novo* MDS cases for which follow-up data was available (435) revealed no significant differences between matched unexposed cases and other *de novo* MDS cases (data not shown) [8].

4. Discussion

MDS is a heterogeneous set of disorders for which the pathogenesis remains obscure. Genetic, infectious and environmental influences all have been suggested to play important roles in the development and evolution of MDS, with clonal cytogenetic abnormalities, altered gene regulation, abnormal cytokine production and immune activation variously hypothesized to be responsible for clonal selection and progression in MDS.

MDS developing secondary to therapy with alkylating chemotherapeutic agents is well described, the clinical features of which frequently include multilineage dysplasia, increased ring siderblasts, loss of chromosomes 5 and/or 7, and a rapid progression to AML [1,13]. Recently, distinctive gene expression profiles have been described for several different cytogenetic abnormalities associated with therapy-related MDS, including abnormalities involving chromosome 5 and/or 7. Monosomy 7 is associated with overexpression of several known oncogenes

Table 5
Comparison of hematologic parameters BZ signal cases vs matched unexposed cases.

MDS cases	Bone marrow						Peripheral blood			
	Cellularity			Blasts		MDS-Eo	Neutrophils $\times 10^9$ L	Lymphocytes $\times 10^9$ L	Hg g/dL	Platelets $\times 10^9$ L
	Hypo	Normo	Hyper	0–1.8%	>1.8%					
BZ signal cases	14(61%)	7(30.4%)	2(8.6%)	27(93%)	2 (7%)	21/29** (72.4%)	1.61 (*N* = 29) [0.85]	0.94 (*N* = 29) [0.46]	11.35* (*N* = 29)[2.6]	104.9(*N* = 29)[63]
Matched unexposed cases	26(59%)	10(23%)	8(18%)	46(84%)	9(16%)	1/58** (1.7%)	1.29 (*N* = 53) [1.02]	1.24 (*N* = 53) [0.91]	8.23* (*N* = 55) [2.7]	85.1(*N* = 55)[111]

* $p < .001$ (*t*-test, pooled variance) [Std Dev.].
** $p < .0001$ (hypothesis testing for 2 independent proportions).

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Table 6
Odds ratios for clinical features in benzene signal cases.

Feature	*N*	OR	CI
MDS-Eo	21	∞	
Hg	23	6.4[a]	2.11–19.21
BM Hyper.	2	0.39	0.07–2.05
Clonal Ab. (total)[b]	5	0.46	0.16–1.3
+8	2	0.43	0.081–2.3
−7	1	1.0	0.091–11.02

[a] $p < 0.001$.
[b] For other see text.

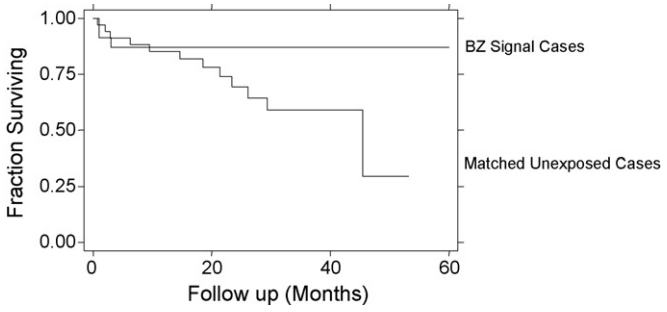

Fig. 6. Kaplan–Meier overall survival analysis for benzene signal cases vs matched unexposed MDS cases.

including HOX9A, BRCA2 and PLAB [14]. In addition, Qian and colleagues have demonstrated a distinct profile for t-MDS/t-AML involving both chromosomes 5 and 7 that is characterized by a higher expression profile of genes involved in cell cycle control and growth [15]. These patterns support a role for clonal cytogenetic or molecular injury early in the clonal evolution of these tumors. In contrast, MDS involving trisomy 8 is associated with higher expression of immune and inflammatory genes, such as TGF-β, IL-10 and VCAM-1 [14], and is consistent with either inflammatory or cytogenetic etiologies playing a dominant role early in the clonal evolution of the disease.

Previous studies, including some from our laboratory, have assumed that clonal cytogenetic injury is a predisposing event in the initiation of BZ-induced BM injury [16–21]. Nevertheless, direct demonstration of clonal CA in the pathogenesis of chronic BZ hematotoxicity has not been established, and several features of MDS associated with BZ exposure reported herein suggest that its pathogenesis is distinct from either therapy-related MDS or *de novo* MDS. These include a decrease in both the frequency and pattern of clonal CA observed in BZ associated MDS, altered prevalence of MDS subtypes, differences in clinical features and a significant variation in the pattern of survival in BZ associated MDS. The predominant morphologic pattern encountered in BM of BZ signal cases, involving MDS-Eo, is essentially identical to that reported by Ruiz et al. in the BM of Brazilian workers heavily exposed to BZ [22].

Whether all forms of MDS have truly clonal origins beginning with a single cytogenetic misadventure has never been established, and it has been suggested that the emergence of cytogenetic clones may be a secondary phenomenon [23]. The evidence implicating immunological targeting of antigens in the hematopoietic environment early in the pathogenesis of MDS is also considerable [24–27]. Frequent findings in MDS include lymphocytopenia, inverted CD4/CD8 T cell ratios, increases in cytotoxic CD8+ T cells (CTL), and evidence of T cell receptor (TCR) gene rearrangements [28–31]. Clonal expansion of T cell subsets is also a prominent finding in the development of aplastic anemia [32–34]. Positive clinical responses to immunosuppressive therapy with anti-thymocyte globulin (ATG) or cyclosporin A is also effective in some patients with MDS [26,30,35]. Independently, there is evidence to suggest that altered patterns of gene regulation observed in tissue-specific autoimmune disease are mediated by collaboration between epigenetic events, such as immune cell activation and leukotriene production [36–39].

We believe that the integration of clinical and histopathologic observations in patients together with molecular findings remains the most promising avenue for hypothesis-based studies on the pathogenesis of BM-induced disease. The predominant pattern of BM pathology in BZ-exposed MDS cases, namely multilineage dysplasia, abnormal eosinophils, hematophagocytosis, together with stromal degeneration, is consistent with an ongoing inflammatory response persisting for many years after cessation of BZ exposure. Immunologic abnormalities are also observed in MDS developing in workers previously exposed to high concentrations of BZ, including increases in circulating large granular lymphocytes (LGLs), altered distribution of CD4 and CD8 T cells and clonal expansion of T cell subpopulations in BM, including prominent clonal proliferations of γδ T cells [11]. Independently, susceptibility to the development of MDS following BZ exposure is associated with an increase in the prevalence of a rare (−238) tumor necrosis factor (TNF-α) polymorphism [40].

Although the mechanisms of persistent BM injury following chronic exposure to BZ remain unknown, a subpopulation of CD34+ BM cells have been implicated as targets, both *in vivo* and *in vitro*, with a variety of studies indicating that BZ metabolites directly alter proliferation and differentiation in BM hematopoietic progenitor cells (HPC). Given the unique ability of BZ among solvents to produce chronic BM damage, it is likely that BZ metabolites play a direct role in establishing the unique pattern of injury that leads to persistent BM pathology long after exposure has ended. Hydroquinone (HQ), a major polyphenolic metabolite of BZ, enhances granulocyte/macrophage-colony stimulating factor (GM-CSF)-dependent clonal proliferation of human CD34+ HPC via activation of extracellular signal-related kinase (ERK) and activation protein-1 (AP-1) [17,41–46]. Hydroquinone also synergizes with TNF-α to produce apoptosis in human CD34+ HPC via a mechanism that involves the inhibition of Nuclear Factor Kappa-B (NFκB) [44]. Eosinophilic dysplasia is a prominent feature in MDS associated with BZ exposure and is also observed in AML with abnormalities involving chromosome 16 (p13; q22) in which production of the CBFβ/MYH11 fusion protein is suggested to result in the expression of antigens which initiate oligoclonal T cell expansion and leukemic blast lysis [37]. It is also possible that epigenetic events may result in comparable alterations in regulation of gene expression. For example, HQ synergizes with LTD4 via the ligand binding domain of the CysLT1 receptor to activate granulocytic/eosinophilic differentiation [47] and γδ T cell subsets that are activated by TNF-α also have been shown to promote hematophagocytosis and modulate eosinophilic inflammation in other tissues. Taken together with the observed human experience in China and Brazil, the evidence strongly suggests that tissue-specific BZ metabolism and altered immune regulation play a predisposing role in the pathogenesis of persistent BM injury and the development of MDS following chronic exposure to BZ.

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