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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