

From: Tsai, Shan P SHLOIL-SHS
Sent: Tuesday, October 25, 2005 9:05 PM (GMT)
To: De Jong, Geert G SI-SHS <geert.dejong@shell.com>
Subject: FW: BHRC-TC... 2nd SHS paper in press: Prevalence of MDS Subtypes in Shanghai
Attach: MDSShanghai10_17(Accepted).pdf

Geert,

FYI.

-----Original Message-----

From: Bruce Jarnot [mailto:jarnotb@api.org]
Sent: Tuesday, October 25, 2005 1:24 PM
To: benzconsort-tc@listserve.api.org
Cc: benzconsort-oc@listserve.api.org; benzconsort-cc@listserve.api.org; Howard Feldman
Subject: BHRC-TC... 2nd SHS paper in press: Prevalence of MDS Subtypes in Shanghai
Importance: High

BHRC Technical Committee (TC) –

A second Shanghai Health Study paper by Richard Irons *et al.*, titled "**Prevalence of MDS Subtypes in Shanghai, China: a Comparison of the World Health Organization and French American British Classifications**", has been accepted for publication in *Leukemia Research*. A copy of this manuscript (25-page pdf file) is attached to this e-mail.

Best Regards - Bruce.

From: Bruce Jarnot
Sent: Wednesday, October 12, 2005 5:05 PM
To: benzconsort-tc@listserve.api.org
Cc: benzconsort-oc@listserve.api.org; benzconsort-cc@listserve.api.org; Howard Feldman
Subject: BHRC-TC... In Press: Benzene-Induced Dysplasia
Importance: High

BHRC Technical Committee (TC) –

The first Shanghai Health Study paper by Richard Irons *et al.*, titled "**Chronic exposure to benzene results in a unique form of dysplasia**", has been peer-reviewed and accepted for fast-track publication in *Leukemia Research*. A copy of the 10-page preprint is attached in pdf format.

Dr. Irons' suggested that "fast track" likely means imminent publication for this journal. Rich also noted that 2 additional papers have been submitted, but not yet accepted for publication.

Best Regards - Bruce.

Bruce M. Jarnot, Ph.D., DABT

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Prevalence of MDS Subtypes in Shanghai, China: A Comparison of the World Health Organization and French American British Classifications.

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Abstract

The prevalence of subtypes of the myelodysplastic syndromes (MDS) was determined in a prospective series of 176 patients presenting at 27 Shanghai hospitals. Diagnosis was established in a single laboratory, analyzing morphologic, immunophenotypic, and cytogenetic data, using the World Health Organization (WHO) revised classification and directly compared to the French American British (FAB) criteria. The median age at diagnosis for all cases was 53 y. There was a striking increase in the prevalence of RCMD in younger patients relative to other subtypes (WHO). The overall frequency of clonal cytogenetic abnormalities was 26.5% (WHO) and 31% (FAB). The most frequently encountered lesions were trisomy 8, del(20)q, del(7q) and del(5q). These results are consistent with previously reported age-dependent differences in MDS and a decreased frequency of del(5q) abnormalities between China and the West. These results also indicate that multilineage dysplasia is a prominent feature in MDS developing in younger individuals in Shanghai and suggest distinguishing between RCMD and RA may be important in the design of studies to further understand regional differences in subtype prevalence and to elucidate the pathogenesis of this complex and multifactorial disease.

Keywords

Myelodysplastic syndrome, FAB, WHO, Cytogenetics, RCMD

Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of diseases characterized by ineffective hematopoiesis and abnormal hematopoietic cell morphology, in one or more hematopoietic lineages, and a tendency of progression to bone marrow failure or acute myelogenous leukemia (AML). A central premise in the diagnosis and classification of MDS is the presence of dysplasia in one or more hematopoietic lineages, although other features, such as anemia, cytopenia, bone marrow cellularity, and blast cell count are variously weighted in subtyping the disease. Several reports have suggested an increasing incidence of MDS over the past decade; however, these

observations are tempered by difficulties in assessing and discriminating between regional variations in prevalence, differences in classification, the increasing frequency of diagnosis and differences in the invasiveness or sophistication of diagnostic procedures commonly employed [1]. These problems have been further accentuated by the fact that the distinction of MDS as a clinical and nosologic entity, which began with French-American-British (FAB) Classification in 1982 [2], has been evolving over the past 20 years and has only recently been recognized to be part of the same spectrum of neoplastic diseases as the acute myeloid leukemias [3].

The FAB classification places MDS into a single diagnostic framework, primarily on the basis of blast cell count, presence or absence of ringed sideroblasts (RS) and peripheral blood monocyte count, as refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with excess blasts (RAEB), refractory anemia with excess blasts in transformation (RAEBT) and chronic myelomonocytic leukemia (CMML). The FAB further distinguishes AML, also primarily on the basis of blast cell count, bone marrow and peripheral blood cellularity as well as lineage involvement [2;4]. The recent World Health Organization (WHO) proposal is an attempt to improve the diagnostic and prognostic utility of MDS subclassification. The evolving clinical experience with the WHO classification system, which has been the subject of ongoing debate [5;6], has recently been reviewed [7;8]. The WHO proposal differs in several aspects from the FAB system that include: 1) the cut-off rate for the number of blast cells in the bone marrow necessary to define AML, which traditionally in FAB has been 30% and is 20% in WHO, and 2) the creation of a new subcategory, “refractory cytopenia with multilineage dysplasia (RCMD)”, which distinguishes dysplasia occurring

in a single cell lineage (e.g. erythroid) (i.e. RA) from multilineage dysplasia involving two or more lineages (i.e. RCMD) in the absence of an increase in peripheral blood or bone marrow blasts. The reduction in blast cell threshold for classification of AML has the effect of eliminating the FAB subcategory, RAEBT. The addition of RCMD as a category emphasizes the importance of multilineage dysplasia independent of increased blasts which appears to be of prognostic importance [9].

Chen et al recently reported the results of a retrospective study of MDS which highlighted differences in the age of onset and prevalence of MDS subtypes between Chinese and Western populations diagnosed according to the FAB criteria [10]. We report the results of a concurrent analysis of clinical, morphologic and karyotypic features of an unselected series of 176 cases of de novo MDS diagnosed in patients at Shanghai hospitals. These cases were diagnosed according to the WHO classification and results directly compared with those obtained using FAB criteria.

Patients, materials and methods

Case definitions

All patients, ≥ 18 years of age, presenting at 27 Shanghai hospitals with initial clinical findings consistent with a hematopoietic abnormality between August, 2003 and February, 2005 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, Colorado 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. MDS cases were initially classified according to the WHO as well as the traditional FAB classification systems [2]. Cases of CMML, which are classified as myelodysplastic/myeloproliferative diseases in WHO and as MDS under FAB, were included for comparison purposes. The requisite blast cell percentage for a diagnosis of AML in Shanghai is $\geq 20\%$. For the purpose of comparison in this study, cases of AML originally diagnosed with blast cell percentages between 20-29% were reclassified as RAEBT according to FAB and compared with those classified according to WHO. Patients presenting with concomitant nutritional deficiencies (Vitamin B12, folate or iron), congenital anemias, viral (including HCV or HIV) or bacterial infections, occupational exposure to benzene or receiving cytotoxic therapy with alkylating or anti-metabolic agents were excluded in this analysis.

Peripheral blood

Blood samples were collected by venipuncture and processed for routine complete blood count (CBC) (CellDyne 3700, Abbott, Park, IL), viral screen (HCV and HIV) (Imx, Abbott, Park, IL) and clinical chemistry for liver enzymes (LDH, ALT and AST enzymes) (COBAS, Integra 400 plus, Roche Diagnostics, Shanghai, China). Vitamin B12 and folate were measured by chemical luminescence (Beckman Coulter Dxi800), and total iron binding capacity was measured using a Beckman Coulter LX20. Peripheral blood smears were made from finger sticks.

Bone marrow

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 that included CD4 and CD8. (Beckman Coulter, Immunotech). Morphology and immunophenotype analysis were conducted on both bone marrow aspirate (flow cytometry) and core biopsy (immunohistochemistry) material. Bone marrow aspirate 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 sections stained with Hematoxylin-Eosin, Gomori trichrome, and immunoperoxidase-immunohistochemistry.

Morphologic analysis

Morphologic diagnoses were subjected to a minimum of two and usually three levels of review. Peripheral blood and bone marrow smears and core biopsy slides were evaluated by two of us (J.M, X.D., R.D.I.), and independently reviewed by one or two of us (R.D.I., J.R.). The threshold for lineage involvement was defined as a minimum of 10% of the cells of a given lineage exhibiting dysplastic changes. Dyserythropoiesis was defined by abnormal nuclear morphology, including internuclear bridging, abnormal budding, multiple nucleoli and abnormal mitotic figures or megaloblastoid features.

Myeloid or granulocytic dysplasia was defined by nuclear hypolobulation as observed in pseudo- Pelger Huet cells, hypersegmentation, abnormal mitotic forms, either hypo- or hyper- granulation of the cytoplasm as well as the presence of large irregular granules. Megakaryocyte dysplasia was defined by hypo-lobulated, hyper-lobulated or non-lobulated nuclei, multiple nuclei or prematurely segmented or “shedding” cytoplasm or the presence of micromegakaryocytes in the BM or peripheral blood. Microscopic analysis was performed using an Olympus BX51 bright field microscope (Olympus Optical, Ltd, Tokyo, Japan).

All cases were evaluated for RS and iron utilization by morphologic examination of an iron stain. In practice it is often difficult to distinguish between abnormal iron utilization and iron deficiency on the basis of the bone marrow iron stain alone- a problem that is exacerbated by the relative paucity of RS in the majority of our MDS cases. Similarly, prominent megaloblastic features are often encountered in both MDS as well as folate, iron-folate and Vitamin B12 deficiencies. Therefore, nutritional deficiencies were independently determined by serum clinical chemistry, and patients with demonstrable deficiencies were excluded from this series.

Myelodysplastic syndrome-unclassifiable (MDS-u) is defined by WHO as MDS lacking findings appropriate for classification as RA, RARS, RCMD or RAEB and in which blast cells are not increased. We restricted the use of MDS-u to those cases with features that did not meet either the narrow definition of RA, (i.e. dysplasia involving only the erythroid lineage), or RCMD, (i.e. lacking sufficient evidence of dyserythropoiesis but exhibiting dysplasia in the other two lineages). A total of 9 cases fulfilled these criteria for classification as MDS-u, of which 7 were hypocellular. Using

the FAB criteria 8 of the MDS-u cases would be classified as RA. It is widely appreciated that the differential diagnosis of severely hypoplastic disease as aplastic anemia (AA) or hypoplastic MDS is fraught with difficulty and remains problematic. The use of MDS-u serves to identify cases with evidence of dysplasia but with minimal or inconsistent findings to support further classification.

Cytogenetic and Fluorescence *in situ* Hybridization Analysis

Cytogenetic analyses were performed on unstimulated bone marrow cells following culture. Unstimulated bone marrow or peripheral blood cells were cultured for 24-72 hours, and G-banding analysis performed according to standard techniques. If possible, 20 metaphases were analyzed. Fluorescence *in situ* hybridization (FISH) analysis was performed on short-term cultures of bone marrow or blood cells. Sample preparation and hybridization were performed according to manufacturer's recommendations (Vysis, Downers Grove, IL). Images were viewed using Olympus fluorescence microscopes (Olympus Optical, Tokyo) equipped with appropriate filters and a PowerGene Macprobe image system (Applied Imaging International, Newcastle, UK). Systemic screening for del(5q), del(7q), +8 and 11q23/*MLL* rearrangements was performed on each case. In some cases, additional FISH studies were used to either characterize abnormalities observed in banded studies or confirm the presence of cytogenetic aberrations when suggested by other diagnostic work-up. A minimum of 500 cells and 10 metaphases were scored in interphase and metaphase analysis, respectively. All probes used in the FISH analyses were commercially purchased from Vysis (Downers Grove, IL).

Statistical analysis

The χ^2 , Fisher's exact and Kruskal-Wallis tests were used to examine differences between MDS subtypes employing STATA 8.0 software (StataCorp, College Station, Texas). All *P*-values were two-sided and values less than 0.05 were considered statistically significant.

Results

The total number of cases of MDS diagnosed according to WHO was 176, including 101 males (57%) and 75 females (42.6%). The prevalence of WHO subtypes was: RA: 16 (9%), RARS: 1 (0.5%), RCMD: 122 (69.3 %), RAEB: 28 (16%) and MDS-u: 9 (5 %). The total number of MDS subtypes diagnosed according to FAB (218 cases) was greater than using WHO (176 cases), in part due to the inclusion of CMML (13 cases) as well as 28 cases of RAEBT and 1 RAEB that met a threshold for diagnosis as AML under WHO (Table 1). The majority of RCMD were reclassified as RA or RARS using FAB criteria. Seven cases of RCMD and 1 case of RA (WHO) met a threshold for $\geq 15\%$ RS, all of which were classified as RARS (FAB). Eight of the MDS-u cases were classified as RA under FAB. All cases of AML diagnosed over the same period using WHO criteria that would be classified as RAEBT under FAB criteria were included for purposes of this comparison (Table 2). All had bone marrow blast cell counts less than 30%. Twenty had blast cell counts between 20-29%, one had a blast cell count less than 20% (i.e. acute erythroleukemia) and eight independently met criteria for diagnosis as AML with reoccurring cytogenetic abnormalities according to WHO (i.e. t(8;21),

11q23/MLL rearrangements). Taken together these represent a 20% increase in the number of MDS cases using the FAB compared to WHO criteria. Our findings are consistent with previously reported decreases in the prevalence of CMML and RARS in Asian populations [10-14].

The overall median age for MDS was 53 y (range 19-82) with 25% of the cases $\leq$ 40 y of age (Figure 1). In general, cases of RCMD (WHO) demonstrated the largest prevalence in this series independent of age. However, RCMD showed a significant difference in age-dependent frequency in the early age group (2nd decade) (χ^2 92.21, $P = 0.0001$). With the possible exception of platelets, individual bone marrow and peripheral blood parameters did not vary across MDS subtypes diagnosed according to WHO, and none were statistically significant (Table 3). The diagnosis of RCMD requires scoring a minimum of 10% dysplastic changes in two or more myeloid lineages; however, in the majority of these cases the degree of dysplasia was marked.

G-banded chromosome analysis was completed in 163 of total 176 MDS cases diagnosed according to WHO (92.6%) (Table 4). One case for which conventional cytogenetic analysis was not completed was demonstrated to have clonal abnormalities by FISH. Therefore, informative cytogenetic data was obtained from 164 of 176 patients (93.2%). Among those, 43 cases (26.2%) had clonal abnormalities. The most frequently observed chromosome abnormalities were trisomy 8 (20 cases, 12.2%), del(7q) (8 cases, 4.9%), del(20q) (7 cases, 4.3%), and del(5q) (4 cases, 2.4%) (Table 5). Translocations, each of which was unique, were observed in 7 cases (i.e. 3 RCMD, 3 RAEB and 1 MDS-u) with a median age of 63.3 y. A complex karyotype, defined as more than two independent chromosome aberrations, was detected in 8 cases (4.90%). Using the

definitions outlined by the International Prognostic Scoring System (IPSS) [15], cases with good, intermediate and poor cytogenetic risk features were observed in 76.6%, 12.9% and 10.4% of patients, respectively. No cases of MDS with the del(5q-) as the sole abnormality were observed. The highest frequency of clonal cytogenetic abnormalities was found in cases with RAEB (40.7%), followed by MDS-u (37.5%), RCMD (24.6%) and RA (7.1%). Using case definitions for MDS as defined by FAB, 64 cases (31%) had clonal abnormalities.

Discussion

Characterization of clonal cytogenetic abnormalities has proven to be important in evaluating prognosis in MDS. However, there is only limited retrospective data on the MDS subtype distribution of cytogenetic abnormalities diagnosed according to WHO [10;11]. The frequency of aberrations in *de novo* MDS (FAB) has been previously reported to be 35-40% [10;11;16;17]. In this prospective analysis clonal abnormalities were detected in 26.2% of MDS cases diagnosed according to WHO as compared to 31% diagnosed using FAB criteria. This notwithstanding, informative cytogenetic data was available in more than 93% of our patients, which is significantly higher than 40-70% reported in most MDS (FAB) studies [10;11;18-21]. Differences in the frequency of clonal aberrations between our series and previous reports in large part can be attributed to differences in the WHO and FAB MDS classification criteria. Cases of RAEBT (FAB), which are diagnosed as AML using WHO criteria and therefore not classified as MDS, usually have a high frequency of chromosome abnormalities [10;11;16;17].

Further, cases diagnosed as MDS (FAB) with t(8;21) and 11q23/MLL rearrangements are diagnosed as AML according to WHO [22].

Cytogenetic abnormalities most commonly described in MDS in the West include (in the order of frequency) del(5q), del(7q), +8 and del(20q) [23]. Several studies, including ours, indicate that trisomy 8 is the most common cytogenetic abnormality in Asian MDS. However, inconsistent results are reported for the prevalence of del(5q), del(7q) and del(20q) [10-12;17;24]. In a retrospective study of Chinese MDS patients diagnosed according to FAB, Chen and colleagues reported the frequencies of del(5q) and del(7q) as 4.6%, and 1.6%, respectively [10]. Our findings suggest that Asian patients more frequently present with 7 than 5 abnormalities, and that the frequency of 5 abnormalities is lower than in the West. It is possible that this may be related to the younger median age of MDS cases in Asian versus Western populations. Similar results also have been reported for Japanese patients by Toyama et al [24] and Matsushima et al [17] for overall MDS and by Matsuda et al for RA [25]. According to IPSS, karyotype at diagnosis is an independent prognostic factor to predict survival and leukemic progression in MDS [15]. The frequency of karyotypes in RCMD in our study that are associated with a poor prognosis was low (7.9%) compared to 20% reported by Matsuda in a Japanese population with similar median age [25]. The reason for these differences is not immediately apparent. Using the same criteria, our cases showed frequencies among overall WHO MDS patients that are comparable to other studies (good risk: 76.6%, intermediate risk: 12.9% and poor risk: 10.4%) [11;15]. Follow-up studies are ongoing to determine if the cytogenetic features defined by IPSS also predict risk in overall MDS or subtypes classified according to WHO.

Our results are consistent with studies that have reported a much younger age of onset for MDS, as well as a lower prevalence of RS and CMML in Asian populations relative to those found in the West [1;10-14]. The most striking observations in our patients were the increased prevalence of multilineage dysplasia together with the number of cases presenting at an early age. In contrast to other subtypes of MDS in which there is a tendency toward increased frequency with advancing age, cases of RCMD were often encountered in patients 19-29 years old, and the increased frequency of RCMD in this age group relative to total MDS or the other individual subtypes, such as RAEB or RARS is highly significant ($P < .00001$). The increase in frequency of RCMD relative to RAEB probably accounts for the decreased numbers of RAEB in this series. However, the remarkably low frequency of RS remains unexplained. Cases of childhood MDS, independent of defined congenital hematologic disorders, have been recognized in the West [26]. However, neither the origins of the disease nor its clinical significance in such a young age group are understood. It is interesting that the prevalence of childhood AML in Shanghai is more than twice that reported in the West [27]. Whether there is a biological link between the development of childhood AML and the frequency of multilineage dysplasia in young adults in Shanghai remains unexplored. However, there are a number of factors that could possibly influence the development of MDS in general and multilineage dysplasia specifically. Nutritional factors are well known to play a role in the development of anemias. A total of 44 cases were excluded from our series due to confounding by deficiencies involving iron, folate and Vitamin B12 either singly or in combination. These findings suggest that the frequency of anemia attributable to nutritional deficiency is relatively high in Shanghai and surrounding areas

and is an important complicating factor in the evaluation of regional hematopoietic disease.

It previously has been suggested that regional differences in subtype-specific survival might contribute to artifacts in the prevalence of MDS subtypes and age-distribution. However, it is unlikely that these influences could explain the magnitude of disparity observed in this series, and the trend toward RCMD in a younger age group is more likely to be the result of differences in the etiology and/or pathogenesis of individual MDS subtypes. Chen et al have suggested that differences in the age-dependence of MDS occurring in Asian populations may be explained by exposure to environmental factors including chemicals and infectious agents [10]. However, in our series we excluded 30 cases of MDS with known previous exposure to benzene or alkylating chemotherapy. The features of dysplasia developing in individuals with previous evidence of benzene poisoning appears to have a discrete clinicopathologic signature that is useful in distinguishing benzene-induced persistent dysplasia from previously characterized forms of MDS [28]. Nevertheless, environmental or dietary exposure to chemicals remains an important question with respect to their potential influence on the prevalence and age-dependence of RCMD in our patients. Moreover, the role of infectious agents in the development of MDS is largely unexplored and may be of importance in light of the fact that a great deal of evidence has emerged to implicate a role for altered immune regulation in the pathogenesis of MDS [29-32]. Finally, the role of genetic polymorphisms that may impact on the susceptibility of developing MDS in this population is unknown. Any of these factors, either singly or in combination, might influence the pathogenesis of MDS. However, at present their role in explaining

these results remains undetermined. Our results provide a basis for direct comparison of WHO and FAB criteria in the differential diagnosis of MDS and further identify a marked predisposition toward multilineage dysplasia at a young age in this Asian population. Follow-up of these cases will provide further insight into the role of dysplastic changes in the etiology and pathogenesis of MDS as well as provide a basis for the continuing evaluation of the clinical and biological significance of the WHO diagnostic paradigm.

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Tables

Table 1. Re-evaluation of WHO MDS by FAB

Comparison of WHO and FAB Diagnostic Criteria In MDS Subtypes			
MDS (WHO)	Total Cases	MDS (FAB)	Total Cases
RA	16 (9%)	RA	140 (64.2%)
RARS	1 (0.5%)	RARS	8 (3.6%)
RAEB	28 (16%)	RAEB	29 (13.3%)
RCMD	122 (69.3%)	RAEBT	28 (12.8%)
MDS-u	9 (5%)	CMML	13 (5.9%)
Total	176	Total	218

Table 2. List of abnormal Karyotypes in MDS cases Re-Evaluated by FAB Criteria.

Comparison of WHO and FAB Diagnostic Criteria In Cases of AML			
AML (WHO)	N	Blasts (%)	MDS (FAB)
AML not otherwise categorized	10	20-27	RAEBT
AML with recurrent cytogenetic abnormalities	8	21-29	RAEBT
AML with multilineage dysplasia	10	20-25.5	RAEBT
Acute erythroid leukemia*	1	11	RAEB

*Blasts = 22% of non-erythroid

Table 3. Clinical Observations in MDS subjects diagnosed by WHO criteria

Hematologic and Immune Parameters In Patients Diagnosed with MDS According to WHO Criteria								
MDS (WHO)	Bone Marrow					Peripheral Blood		
	Cellularity			Lymphocytes		WBC x10 ⁹ /L	Hgb g/dL	PLT x10 ⁹ /L
	Hyper	Normo	Hypo	%Lymph	CD4:CD8			
MDS total	88 (50%)	28 (16%)	57 (32%)	21.7 (1.0-82.6)	1.1 (0.1-5.7)	2.62 (0.52-10.6)	7.68 (2.71-15.5)	80 (1.9-468)
RA	10 (62%)	3 (18%)	3 (18%)	20.9 (3.1-54.9)	1.2 (0.1-4.1)	2.89 (1.75-7.82)	7.55 (4.17-12.2)	119 (5.7-393)
RARS	1 (100%)	0	0	2.8	0.8	4.50	7.58	335
RAEB	17 (60%)	3 (10%)	7 (25%)	26.6 (1.9-63.3)	1.0 (0.3-2.2)	2.73 (0.76-10.6)	6.82 (3.71-11.3)	60 (1.9-260)
RCMD	58 (47%)	22 (18%)	40 (32%)	20.9 (1.0-82.6)	1.2 (0.2-5.7)	2.40 (0.52-6.75)	7.84 (2.71-14.3)	78 (4.5-468)
MDS-u	2 (22%)	0	7 (77%)	21.0 (7.7-33.5)	1.1 (0.2-1.7)	2.14 (0.54-5.39)	8.42 (3.93-15.5)	98 (5.7-395)

Bone marrow cellularity was estimated from the trephine core biopsy (Hypo-hypoplastic [$<40\%$ cellularity], Normo-normal [$40\text{--}60\%$ cellularity] and Hyper-hyperplastic [$>60\%$ cellularity]. Lymphocytes in BM aspirates: % lymph- % lymphocytes, CD4:CD8- ratio determined by flow cytometry in BM aspirate cells. Reference ranges in Shanghai: WBC ($4\text{--}10 \times 10^9/\text{L}$); Hgb ($12\text{--}16 \text{ g/dL}$), PLT ($100\text{--}300 \times 10^9/\text{L}$).

Table 4. Analysis in MDS cases by standard cytogenetic criteria

Cytogenetic Analysis of MDS Subtypes			
MDS Subtypes	Total Cases	No. Complete Cytogenetic Analysis	Total % Abnormal Cases
RA	16	14	1 (7.1%)
RARS	1	1	0
RAEB	28	27	11 (40.7%)
RCMD	122	114	28* (24.6%)
MDS-u	9	7	3** (37.5%)
Total	176	163 (92.6%)	43 (26.2%)

* One case with extra Y

** One case with informative FISH without cytogenetic data

Table 5. Frequency of Abnormal Cytogenetics in MDS cases in Shanghai

Clonal Cytogenetic Abnormalities in MDS Subtypes						
Clonal Abnormality (Abnl)	No. Abnl	%Informative (164)	RA (14)	RAEB (27)	RCMD (114)	MDS-u (7)
del(5q)	4	2.4%	0	2(7.4%)	2(1.7%)	0
del(7q)	8	4.9%	0	3(11.1%)	4(3.5%)	1*
Trisomy 8	20	12.2%	1	6(22.2%)	13(11.4%)	0
del(20q)	7	4.3%	0	1(3.7%)	6(5.3%)	0
Monosomy 21	1	0.6%	0	1(3.7%)	0	0
del(9q)	2	1.2%	0	1(3.7%)	1(0.9%)	0
Complex Karyotype ≥ 3 Abnl	8	4.9%	0	3(11.1%)	5(4.4%)	0

* One case with informative FISH without cytogenetic data

Figure Legends

Figure 1. Age distribution of MDS subtypes according to WHO in patients grouped by decades. The age distribution of MDS subtypes is plotted as the percent of total MDS. (*) A higher prevalence of RCMD was observed in all decades relative to other subtypes, but was significant only in the ≤ 29 year age group ($p = 0.0001$).

