IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION

CHARLES WILSON and LAURA WILSON
Plaintiffs,

VS.

RYCOLINE PRODUCTS, INC.; et al.,
Defendants.

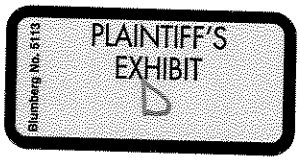
Case No. 2:06:CV-286

****

ORAL DEPOSITION OF
DR. ETHAN NATELSON
SEPTEMBER 17, 2007

****

ORAL DEPOSITION of DR. ETHAN NATELSON, produced as a witness at the instance of the Plaintiffs, and duly sworn, was taken in the above-styled and numbered cause on SEPTEMBER 17, 2007, from 2:16 p.m. to 5:07 p.m., before Denyce M. Sanders, CSR, RPR, in and for the State of Texas, recorded by machine shorthand, at the offices of THOMPSON & KNIGHT, 333 Clay Street, Suite 3300, Houston, Texas, pursuant to the Federal Rules of Civil Procedure and the provisions stated on the record or attached hereto; that the deposition shall be read and signed before any notary public.



APPEARANCES

FOR THE PLAINTIFFS:

SCHMIDT & CLARK
2911 Turtle Creek Boulevard, Suite 1400
Dallas, Texas 75219
Mr. Keith Patton

FOR THE DEFENDANT ASHLAND, INC.:

THOMPSON & KNIGHT
333 Clay Street, Suite 3300
Houston, Texas 77002
Mr. Kevin Parks

EXAMINATION INDEX

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DR. ETHAN NATELSON,
having been first duly sworn, testified as follows:
EXAMINATION
BY MR. PATTON:
Q. Please introduce yourself for the record.
A. My name is Ethan A. Natelson.
Q. You are a doctor, correct?
A. Correct.
Q. Tell us what kind of doctor you are.
MR. PARKS: Before we get into that, we'll agree that the deposition is pursuant to the Federal Rules and the witness will read and sign the transcript?
MR. PATTON: Agreed.
Q. (BY MR. PATTON) Please introduce yourself.
A. I'm -- as I said, my name is Ethan A. Natelson. I'm a hematologist.
Q. And how long have you been a hematologist?
A. I did a fellowship in 1969; and since that time, I've more exclusively seen hematology-type patients.
Q. As we sit here today, do you have a clinical practice where you see hematology patients on a daily and weekly basis?
A. Yes.

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Q. And where is your practice at?
A. At the Methodist Hospital. The address is 6550 Fannin Street.
Q. You are a hired expert in this case, correct?
A. Yes.
Q. Hired by Ashland, correct?
A. Indirectly. Hired by Ricky Raven, but Ashland is the company of record.
Q. Okay. How much are you being paid for your time on the case?
A. I normally charge $250 an hour to review records and $400 an hour for deposition testimony.
Q. And as we sit here today, we marked some exhibits before we started; and Exhibit 50 appears to be your invoicing and billing records, correct?
A. Yes.
Q. How much time did you spend on this case prior to writing your report?
A. I can't tell you that exactly. It's not noted on here, but the total hours are about 15 hours that are listed here; and probably between 10 and 15 hours, I would say, when the report was actually written.
Q. Before September 8, appears you've had ten hours total in the case?
A. Yes.

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Q. And since September 8, you've spent about five hours before coming here for your deposition?
A. Yes.
Q. And that includes four hours to prepare for your deposition?
A. Yes.
Q. Have you been asked to testify at trial in this case?
A. I don't recall that. But that's, essentially, a given. In other words, if I agree to be involved in a case, I typically agree to testify at trial.
Q. Do you know when this case is set for trial?
A. No.
Q. You have served as an expert witness in benzene cases before, correct?
A. Yes.
Q. Have you ever testified in any cases involving MDS or myelodysplastic syndrome?
A. I have to look at the list to be certain about that.
Q. Okay. We can go over that a little bit later. Before we go on to it more later, have you ever testified on behalf of a plaintiff in a chemical exposure case?
A. No.

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Q. Have you ever given the opinion in any litigation that benzene caused a person's given disease, whatever that disease may be?
A. No.
Q. Is it your opinion in this case that Charles Wilson's MDS is not related to any benzene exposure?
A. He has a form -- he has an illness that can be classified as a form of MDS. I don't believe that particular illness can be caused by benzene.
Q. Just so I have your opinions clear. It's your opinion in this case, that Charles Wilson's MDS/RARS is not related to any exposure to benzene?
A. Yes. That's correct.
Q. Stated another way, you will testify at trial in this case that benzene exposure played absolutely zero causative factor in Charles Wilson's MDS or RARS, correct?
A. There's no "or." He has one illness, which is RARS; and I don't believe that benzene is related to that illness.
Q. Okay. My question is a little more specific. Do you believe that benzene in any way caused or contribute or played any causative factor in Charles Wilson's RARS?
A. No.

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do with sideroblastic anemia; and then within that chapter, RARS is a subheading.
Q. And in the Wintrobe text, is benzene identified as a potential cause of RARS?
A. No.
Q. The article that you've written that is planned for publication, when -- when do you expect that will be published?
A. Well, it's listed as November -- in the November edition.
Q. And was that article peer reviewed?
A. Yes.
Q. What was your purpose in writing that article?
A. Well, my purpose was this: I don't think this is a question that has been discussed much in the hematologic literature. In other words, there are a number of articles out there that deal with the subject; but I don't think anybody, since the time of Dr. Damashek, has written a general discussion about this situation.
Q. And by this general subject, do you mean whether benzene can cause RARS?
A. Yes.
Q. And what is the conclusion in your article?
A. Well, my conclusion is that there's no

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epidemiologic evidence or case report data to suggest that benzene can cause RARS and considerable data that would suggest that it can't.
Q. Mr. Patton also asked you questions, for instance, as to whether benzene can cause AML?
A. Yes.
Q. And you agreed that it could?
A. Yes.
Q. And then he asked you questions about the fact that RARS can develop into AML as the natural -- in the natural history -- in the course of the disease, if you will?
A. Yes.
Q. But if you have a patient who has been diagnosed with RARS who then develops AML, wouldn't you rule out benzene as a potential cause because you know that benzene does not cause RARS?
A. Both situations. We know that AML is the natural progression of a number of cases of RARS, and we also know that there is no relationship between benzene and RARS. So if a person with RARS develops AML, I would think that's just simply the natural progression of the illness.
Q. So even if Mr. Wilson does proceed on to develop AML, your opinion would remain that benzene did

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not cause that?
A. That's correct.
MR. PARKS: Thank you for your time. I'll pass the witness.
FURTHER EXAMINATION
BY MR. PATTON:
Q. Dr. Natelson, what is the multiple hit theory as it relates to bone marrow diseases?
A. Well, the notion is that, for example, someone gets, let's say, damage to a chromosome. It may be minor damage or major damage. And that changes the milieu, for example. It produces a growth factor or a growth factor that has a certain advantage and starts proliferating cells in the bone marrow; and then a second genetic event occurs which takes those proliferating cells and makes them leukemic.
And so it takes multiple hits to drive a normal bone marrow to a leukemic bone marrow because of the fact that the bone marrow has tremendous reparative processes. And it's very difficult to -- to sustain damage in the bone marrow because of the fact that even chromosome defects can be repaired.
Q. Would you agree with me that some individuals are more susceptible or more likely to take those hits to the bone marrow, so to speak, a little harder than

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others?
A. That's theoretically possible, yes.
Q. Is it theoretically likely, as far as the concept of susceptibility goes, for people who have different forms of cancer. Some people are more likely to get cancer than others for one reason or another, right?
A. That is so. We don't know the reasons for that. But that is true, that in some instances there's genetic predisposition, but -- but those -- the research in that is really in its infancy.
Q. But you agree with me that some people have a genetic predisposition to be more susceptible to contracting, say, leukemia or a bone marrow disease from exposure to benzene than other people would be; fair statement?
A. Well, certainly. I don't know the answer to that. I would say it's quite -- it's possible. I don't know that for a fact.
MR. PATTON: All right. Thank you for your time.
MR. PARKS: Reserve the rest of our questions until the time of trial.
MR. PATTON: And for the record, can we have the agreement, Kevin, that you will, for cost

sake, take the exhibits, copy them, return the original to Dr. Natelson, send me a copy within, say, five days; is that fair?

MR. PARKS: That's fair.

WITNESS CORRECTIONS AND SIGNATURE

Please indicate changes on this sheet of paper, giving the change, page number, line number and reason for the change. Please sign each page of changes.

PAGE/LINE CORRECTION REASON FOR CHANGE

_____

_____

DR. ETHAN NATELSON

SIGNATURE OF WITNESS

I, DR. ETHAN NATELSON, solemnly swear or affirm under the pains and penalties of perjury that the foregoing pages contain a true and correct transcript of the testimony given by me at the time and place stated with the corrections, if any, and the reasons therefor noted on the foregoing correction page(s), and that I am signing this before a Notary Public.

_____

DR. ETHAN NATELSON

STATE OF T E X A S *
COUNTY OF _____ *

SUBSCRIBED AND SWORN TO BEFORE ME BY _____ on this, the _____ day of _____, 2007.

_____

Notary Public, State of Texas

My Commission Expires: _____

Job 2-64038

THE STATE OF TEXAS :
COUNTY OF HARRIS :

I, DENYCE SANDERS, a Certified Shorthand Reporter and Notary Public in and for the State of Texas, do hereby certify that the facts as stated by me in the caption hereto are true; that the above and foregoing answers of the witness, DR. ETHAN NATELSON, to the interrogatories as indicated were made before me by the said witness after being first duly sworn to testify the truth, and same were reduced to typewriting under my direction; that the above and foregoing deposition as set forth in typewriting is a full, true, and correct transcript of the proceedings had at the time of taking of said deposition.

I further certify that I am not, in any capacity, a regular employee of the party in whose behalf this deposition is taken, nor in the regular employ of this attorney, and I certify that I am not interested in the cause, nor of kin or counsel to either of the parties.

That the amount of time used by each party at the deposition is as follows:
Mr. Keith Patton - 02:28:54
Mr. Kevin Parks - 00:05:48

GIVEN UNDER MY HAND AND SEAL OF OFFICE, on this, the 25th day of September, 2007.

_____

DENYCE SANDERS, CSR, RPR
Notary Public in and for
Harris County, T E X A S

My Commission Expires: 4-6-09
Certification No.: 4038
Expiration Date: 12-31-07
U.S. LEGAL Support, Inc.
363 N. Sam Houston Parkway, 9th Floor, Houston, Texas 77060; 713.653.7100
Firm No. 122

UNITED STATES DISTRICT COURT

for the

District of South Carolina

Joyce Barrois, et al
Plaintiff
v.
Fireman's Fund Insurance Company, et al
Defendant

Civil Action No. 09-380

(If the action is pending in another district, state where: Eastern District of Louisiana)

SUBPOENA TO PRODUCE DOCUMENTS, INFORMATION, OR OBJECTS OR TO PERMIT INSPECTION OF PREMISES IN A CIVIL ACTION

To: David W. Ploth, Editor, The American Journal of the Medical Sciences, 2491 River Bluff Ln, Mount Pleasant, SC 29466 or 1088 Johnnie Dodds Blvd., Mount Pleasant, SC 29464

☑ *Production:* **YOU ARE COMMANDED** to produce at the time, date, and place set forth below the following documents, electronically stored information, or objects, and permit their inspection, copying, testing, or sampling of the material: A certified, true,correct copy of the cover page, title page and manuscript of "Benzene Exposure and Refractory Sideroblastic Erythropoiesis:Is There An Association? by Ethan A. Natelson, MD, The Am J of the Med Sci., 2007, 334(5):356-360 attached as Exhibit A. Please include all supporting documentation, rough drafts, funding sources, notes, possible conflict of interest disclosures and peer review comments.

Place:	Date and Time:
Williams Law Office, LLC 3000 W. Esplanade Ave., Ste.200 Metairie, Louisiana 70002	02/01/2010 5:00 pm

☐ *Inspection of Premises:* **YOU ARE COMMANDED** to permit entry onto the designated premises, land, or other property possessed or controlled by you at the time, date, and location set forth below, so that the requesting party may inspect, measure, survey, photograph, test, or sample the property or any designated object or operation on it.

Place:	Date and Time:

The provisions of Fed. R. Civ. P. 45(c), relating to your protection as a person subject to a subpoena, and Rule 45 (d) and (e), relating to your duty to respond to this subpoena and the potential consequences of not doing so, are attached.

Date: 1/19/10

CLERK OF COURT

OR

Signature of Clerk or Deputy Clerk

Attorney's signature

The name, address, e-mail, and telephone number of the attorney representing *(name of party)* Joyce Barrois, et al, who issues or requests this subpoena, are:

L.Eric Williams, Jr., Williams Law Office, LLC, 3000 W. Esplanade Ave., Ste. 200, Metairie, LA 70002, Tel:(504) 832-9898, eric@amlbenzene.net

PLAINTIFF'S EXHIBIT E

Blumberg No. 5113

Civil Action No. 09-380

PROOF OF SERVICE

(This section should not be filed with the court unless required by Fed. R. Civ. P. 45.)

This subpoena for *(name of individual and title, if any)* _____

was received by me on *(date)* _____ .

❒ I served the subpoena by delivering a copy to the named person as follows: _____

_____

_____ on *(date)* _____ ; or

❒ I returned the subpoena unexecuted because: _____

_____ .

Unless the subpoena was issued on behalf of the United States, or one of its officers or agents, I have also tendered to the witness fees for one day's attendance, and the mileage allowed by law, in the amount of

$ _____ .

My fees are $ _____ for travel and $ _____ for services, for a total of $ 0.00 .

I declare under penalty of perjury that this information is true.

Date: _____

_____
Server's signature

_____
Printed name and title

_____
Server's address

Additional information regarding attempted service, etc:

Federal Rule of Civil Procedure 45 (c), (d), and (e) (Effective 12/1/07)

(c) Protecting a Person Subject to a Subpoena.

(1) ***Avoiding Undue Burden or Expense; Sanctions.*** A party or attorney responsible for issuing and serving a subpoena must take reasonable steps to avoid imposing undue burden or expense on a person subject to the subpoena. The issuing court must enforce this duty and impose an appropriate sanction — which may include lost earnings and reasonable attorney's fees — on a party or attorney who fails to comply.

(2) ***Command to Produce Materials or Permit Inspection.***

(A) *Appearance Not Required.* A person commanded to produce documents, electronically stored information, or tangible things, or to permit the inspection of premises, need not appear in person at the place of production or inspection unless also commanded to appear for a deposition, hearing, or trial.

(B) *Objections.* A person commanded to produce documents or tangible things or to permit inspection may serve on the party or attorney designated in the subpoena a written objection to inspecting, copying, testing or sampling any or all of the materials or to inspecting the premises — or to producing electronically stored information in the form or forms requested. The objection must be served before the earlier of the time specified for compliance or 14 days after the subpoena is served. If an objection is made, the following rules apply:

(i) At any time, on notice to the commanded person, the serving party may move the issuing court for an order compelling production or inspection.

(ii) These acts may be required only as directed in the order, and the order must protect a person who is neither a party nor a party's officer from significant expense resulting from compliance.

(3) ***Quashing or Modifying a Subpoena.***

(A) *When Required.* On timely motion, the issuing court must quash or modify a subpoena that:

(i) fails to allow a reasonable time to comply;

(ii) requires a person who is neither a party nor a party's officer to travel more than 100 miles from where that person resides, is employed, or regularly transacts business in person — except that, subject to Rule 45(c)(3)(B)(iii), the person may be commanded to attend a trial by traveling from any such place within the state where the trial is held;

(iii) requires disclosure of privileged or other protected matter, if no exception or waiver applies; or

(iv) subjects a person to undue burden.

(B) *When Permitted.* To protect a person subject to or affected by a subpoena, the issuing court may, on motion, quash or modify the subpoena if it requires:

(i) disclosing a trade secret or other confidential research, development, or commercial information;

(ii) disclosing an unretained expert's opinion or information that does not describe specific occurrences in dispute and results from the expert's study that was not requested by a party; or

(iii) a person who is neither a party nor a party's officer to incur substantial expense to travel more than 100 miles to attend trial.

(C) *Specifying Conditions as an Alternative.* In the circumstances described in Rule 45(c)(3)(B), the court may, instead of quashing or modifying a subpoena, order appearance or production under specified conditions if the serving party:

(i) shows a substantial need for the testimony or material that cannot be otherwise met without undue hardship; and

(ii) ensures that the subpoenaed person will be reasonably compensated.

(d) Duties in Responding to a Subpoena.

(1) ***Producing Documents or Electronically Stored Information.*** These procedures apply to producing documents or electronically stored information:

(A) *Documents.* A person responding to a subpoena to produce documents must produce them as they are kept in the ordinary course of business or must organize and label them to correspond to the categories in the demand.

(B) *Form for Producing Electronically Stored Information Not Specified.* If a subpoena does not specify a form for producing electronically stored information, the person responding must produce it in a form or forms in which it is ordinarily maintained or in a reasonably usable form or forms.

(C) *Electronically Stored Information Produced in Only One Form.* The person responding need not produce the same electronically stored information in more than one form.

(D) *Inaccessible Electronically Stored Information.* The person responding need not provide discovery of electronically stored information from sources that the person identifies as not reasonably accessible because of undue burden or cost. On motion to compel discovery or for a protective order, the person responding must show that the information is not reasonably accessible because of undue burden or cost. If that showing is made, the court may nonetheless order discovery from such sources if the requesting party shows good cause, considering the limitations of Rule 26(b)(2)(C). The court may specify conditions for the discovery.

(2) ***Claiming Privilege or Protection.***

(A) *Information Withheld.* A person withholding subpoenaed information under a claim that it is privileged or subject to protection as trial-preparation material must:

(i) expressly make the claim; and

(ii) describe the nature of the withheld documents, communications, or tangible things in a manner that, without revealing information itself privileged or protected, will enable the parties to assess the claim.

(B) *Information Produced.* If information produced in response to a subpoena is subject to a claim of privilege or of protection as trial-preparation material, the person making the claim may notify any party that received the information of the claim and the basis for it. After being notified, a party must promptly return, sequester, or destroy the specified information and any copies it has; must not use or disclose the information until the claim is resolved; must take reasonable steps to retrieve the information if the party disclosed it before being notified; and may promptly present the information to the court under seal for a determination of the claim. The person who produced the information must preserve the information until the claim is resolved.

(e) Contempt. The issuing court may hold in contempt a person who, having been served, fails without adequate excuse to obey the subpoena. A nonparty's failure to obey must be excused if the subpoena purports to require the nonparty to attend or produce at a place outside the limits of Rule 45(c)(3)(A)(ii).

Benzene Exposure and Refractory Sideroblastic Erythropoiesis: Is There an Association?

ETHAN A. NATELSON, MD

ABSTRACT: The myelodysplastic syndromes (MDS) consist of a group of diverse hematological disorders that carry an increased risk of transforming into acute myeloid leukemia. They may appear de novo and without obvious cause (primary or de novo MDS) or be induced by certain mutagenic environmental or therapeutic toxins (secondary MDS). Excessive exposures to benzene are generally considered to be a potential environmental risk factor for both MDS and acute myeloid leukemia. However, such risk is unproven for each disease component within the MDS classification. A critical review of the refractory sideroblastic disorders strongly suggests that benzene exposure is not a potential cause of this distinct and still-evolving subset of MDS. The widely disparate nature of MDS suggests that epidemiologic studies can only provide meaningful data on associations and potential causation of its component syndromes by a disease-specific analysis, as is currently advocated for other hematological malignancies. **KEY INDEXING TERMS:** Benzene; Myelodysplasia; MDS; Refractory Sideroblastic Anemia. **[Am J Med Sci 2007; 334(5):356–360.]**

The terms myelodysplasia and the myelodysplastic syndromes (MDS) refer to a classification of heterogenous and distinct disease forms linked by the presence of oligoclonal hematopoietic stem cell abnormalities, ineffective hematopoiesis characterized by abnormal differentiation and maturation of myeloid cells, and a variable but increased, risk of progression toward acute myeloid leukemia.[1–6] A common etiology among each of the various myelodysplastic disorders that is also shared with acute myeloid leukemia (AML) has not been proposed, and little is known about the major causes of MDS.[6,7] Unfortunately, epidemiological surveys seeking statistical information on hematological malignancies in cohorts potentially exposed to environmental chemicals or toxins seldom provide specific data on MDS, despite its higher incidence in the general population than AML.[4] Moreover, in rare instances when information on MDS is collected and presented, it is usually treated as a single, distinct disease entity, and its numbers may be combined with those of AML or even other unrelated hematological malignancies in an attempt to achieve statistical significance in the analysis.[8] Such approach obscures identification of potential etiologic risk factors that might be specific to the individual disease components within MDS and to the various AML syndromes. Thus, under certain circumstances, benzene exposure is an accepted, although uncommon, potential risk factor for both MDS and AML.[1,6,7,9] However, when carefully studied (vide infra), such risk does not necessarily apply to all components of the MDS. This report reviews the clinical observations that provide specific information concerning the question of causal associations between benzene as an environmental toxin and the acquired refractory sideroblastic disorders, a readily identifiable subset of the MDS classification system.[10,11]

Discussion

Refractory anemia with ringed sideroblasts (RARS) was a well-described and stand-alone hematopoietic disorder until 1982, when it was included among other hematological syndromes grouped under a then newly defined heading of MDS.[12–15] Typically discussed in hematologic text chapters dealing with congenital and acquired sideroblastic anemias of diverse cause, RARS has been described under a variety of titles including acquired idiopathic refractory sideroblastic anemia and sideroachrestic anemia. Its unifying morphological feature is the presence of ringed sideroblasts, as identified by iron stains, in at least 15% of the bone marrow erythroid precursors throughout multiple areas of the bone marrow films or biopsy.[1,4] Such histologic stains have been a part of the routine evaluation of bone marrow specimens for nearly 60 years.[16] The variable but clearly preleukemic nature of RARS has

From the Transitional Residency Program, The Methodist Hospital, and Weill Medical College of Cornell University at The Methodist Hospital, Houston, Texas.

Submitted January 16, 2007; accepted in revised form May 2, 2007.

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Table 1. Myelodysplastic Syndromes

Original FAB Divisions	Current WHO Divisions
Refractory anemia	Refractory anemia
RA-Ringed sideroblasts	RC-Multilineage dysplasia
RA-Excess (5–20%) blasts	RA-Ringed sideroblasts
RA-Excess (21–30%) blasts in transition	RC-RS–Multilineage dysplasia
	RA-Excess (5–9%) blasts type I
Chronic myelomonocytic leukemia	RA-Excess (9–19%) blasts type II
	MDS isolated deletion 5 q
	MDS unclassifiable

FAB, French-American-British format; WHO, World Health Organization; RA, refractory anemia; RC, refractory cytopenia; RS, ringed sideroblasts.

also been known since its earliest descriptions.[13,15] In the original MDS classification scheme, the subset of individuals with RARS comprised about 10% to 20% of the total cases in most series, with the relative frequency, perhaps, age of onset-related.[17] They exhibit the lowest incidence of classical chromosomal aberrations of all MDS subsets and the least frequency of progression to AML—approximately 15%.[18–21] Certain chromosomal aberrations have been associated with RARS including long arm deletions of chromosomes 5, 11, and 20, as well as trisomy 8.[21] None are specific, however, and particularly deletions of 20q and trisomy 8 are identified in a number of diverse hematological disorders.[21–23]

Modern revised classification systems of MDS attempt to separate RARS into 2 general subsets, 1 currently referred to as refractory cytopenia with multilineage dysplasia-ringed sideroblasts (RCMD-RS), and a second entity with little evident morphologic cytologic dysplasia outside the erythroid cell line, and a lesser risk of AML (Table 1). It remains under the designation of RARS.[1,11,19,20,24] Recently, a further clinically-useful separation of the refractory sideroblastic disorders has been suggested, segregating all patients with ringed sideroblasts and markedly elevated platelet counts into yet another subset designated RARS-T or RST to emphasize the presence of thrombocytosis.[24–27] Many of the individuals in this third grouping may also possess the newly described JAK-2 V617F chromosomal abnormality, commonly identified in polycythemia vera and other myeloproliferative disorders.[26–28] RARS-T could then also be listed under the newly suggested descriptive designation of myelodysplastic/myeloproliferative syndromes.[25] The recognition that some individuals with RARS and marked thrombocytosis might exhibit a clinical course more in keeping with a myeloproliferative disorder, with megakaryocytic hyperplasia and bone marrow fibrosis, similar to the natural history of polycythemia vera, primary thrombocythemia and agnogenic myeloid metaplasia, is not a new observation.[29] The presence of the JAK-2 V617F mutation among the RARS-T group provides a unifying molecular explanation. Importantly, myeloproliferative disorders have not been epidemiologically linked with exposures to either benzene or to modern mutagenic chemotherapy agents.

The frequency distribution of these 3 refractory sideroblastic syndromes, particularly between RARS and RCMD-RS, is clearly observer-dependent and also varies with the alternate classifications systems for MDS of the World Health Organization and the French-American-British (FAB) format.[10,30] An estimate would place about 10% of the total as RARS-T. However, if the bone marrow blast cell count increases above 5%, and even if large numbers of ringed sideroblasts are retained, under these classification schemes individuals are now moved from an RARS syndrome into another MDS category, refractory anemia with excess blasts (RAEB). In effect, this movement arbitrarily diminishes the pool of cases with refractory sideroblastic hematopoiesis available for epidemiological or clinical analysis, and is a weakness of all MDS classifications.[18] Importantly, occasional patients with RARS, even those with apparent multilineage cytologic dysplasia, may favorably respond to immunosuppressive therapy, and in rare instances the hematologic syndrome is actually consequent to unusual deficiency states.[31–34] Thus, progression to AML may not be a part of the natural history in certain forms of acquired sideroblastic anemia, emphasizing that all such disorders do not necessarily represent hematologic malignancies.

From the earliest descriptions, authors reporting on RARS pondered potential causes of this uncommon disorder. Wintrobe's experience in a single institution along with review of many additional cases led him and his associates to conclude that no specific drugs, environmental chemicals, alcohol, or cytostatic agents, could be confirmed as potential risk factors, and that the cause of RARS was unknown.[12] Benzene had previously been used as an oral chemotherapeutic agent in forms of chronic leukemia, and it was well known to cause bone marrow damage and potentially eventuate in AML. Exposure to this agent was specifically sought in an analysis of 84 patients with RARS, but only 1 of these subjects could recall any previous benzene exposure. Furthermore, that patient also had received radiation therapy.[19] The collective experience now involving thousands of reported patients having received chemotherapy and then developing secondary hematologic syndromes including MDS, has only supplied rare case reports describing a chronic acquired sideroblastic process. This phenomenon has usually occurred in the presence of an underlying hematological malignancy, such as multiple myeloma or Hodgkin's disease, and is then noted after treatment including both alkylating agents and radiation.[35] Here, the acquired sideroblastic process, itself, is often transient and not evident in follow-up bone marrow examinations.[36,37] Thus, neither exposure to

benzene nor to modern chemotherapeutic mutagenic agents has been shown to be a risk factor for RARS.

There are 3 major epidemiological studies often cited as offering detailed observations, including bone marrow analysis, which routinely includes iron stains, concerning the hematological effects of chronic, high-level benzene exposure. In the Hayes[8] report of about 74,000 factory workers, primarily immersion painters in China, 28 individuals with either AML or MDS were cited. Analysis of the individual slide material from these MDS cases, and which specifically described bone marrow iron examination, showed no examples of RARS.[38] In the Aksoy[39] report of 29,000 Turkish shoe workers heavily exposed to benzene-containing glues, 42 individuals were described as developing forms of AML, among other hematological disorders. Yet, in his description of the specific hematologic abnormalities in this cohort, there is no observation consistent with RARS.[40] Finally, in the frequently-analyzed United States Pliofilm factory cohort of about 1,800 individuals exposed to large concentrations of pure benzene for many years, there are no reported details of bone marrow examinations, but no instance of a listed diagnosis consistent with RARS.[41] A recent report from The MD Anderson Cancer Center also could not confirm solvent exposure as a significant predisposing event in their patients with RARS, or with chronic myelomonocytic leukemia, an original, and now former, member within the MDS classification system.[7] In that study, such exposures were apparent risk factors for other forms of MDS such as refractory anemia with increased blasts (RAEB). In reality, there does not appear to be a single epidemiological study or even an isolated case report in the world's medical literature that links RARS with benzene exposure.

Recently, Irons et al[42] carefully analyzed blood counts and serial bone marrow examinations on a small cohort of 23 Chinese workers heavily exposed to benzene. They reaffirmed earlier sporadic observations of marrow hypoplasia and eosinophilia in benzene-exposed workers but failed to identify the presence of ringed sideroblasts. Similarly, an absence of ringed sideroblasts was noted in careful bone marrow studies of 33 additional individuals with chronic benzene poisoning.[43] These marrow studies also demonstrated eosinophilia and dyspoietic erythroblasts. In general, the type of MDS bone marrow histology usually described in both heavily benzene-exposed individuals and therapy-related MDS is a hypocellular bone marrow with cellular dyspoiesis, and distinct from the usual findings in RARS which typically reflect a normocellular to hypercellular bone marrow pattern.[38,44]

Cigarette smoke is considered a risk factor for both AML and MDS, but the specific element or mechanism involved is unknown.[9,45–53] Cigarette smoke does contain some benzene, but the cumulative dose achieved by smokers is less than thought necessary to increase the risk of AML, and the chromosomal anomalies identified in smokers with AML are somewhat different from those typically reported for benzene-induced and other secondary forms of AML.[9,48–53] The chromosomal aberrations seen in the vast majority of benzene-induced AML and modern day chemotherapy-induced AML, or t-AML, are similar, with a predominance of deletions in chromosomes 5 and 7.[9] This is not surprising, since postulated mechanisms of leukemogenesis in benzene-induced and t-AML, are also thought to be similar, if not identical.[54,55] Some reports have suggested cigarette smoking is linked with RARS, although the mechanism is not known.[7,45]

One feature of MDS that further confounds epidemiological analysis of its individual disease components is the fact that unlike AML, MDS is not a final illness designation, and individuals frequently move within the MDS classification over time. This is particularly true with respect to RARS syndromes. Thus, it would be expected that as the often slow progression to AML from the 3 refractory sideroblastic variants described here occurs, the percentage of blast cells in the bone marrow may increase gradually from its initially normal values. As previously mentioned, ringed sideroblasts are often still present when the bone marrow blast percentage reaches 5% to 10%, but the MDS diagnosis now moves to RAEB.[17,56] When the blast percentage eventually reaches 20%, by convention, the diagnosis of AML is now allowed. Even if ringed sideroblasts remain evident in excess along with marked erythroid dyspoiesis, the specific diagnosis would be an FAB-M6 AML or erythroleukemia. Obviously, for such individual, this transition reflects a single disease process, and not 3 separate illnesses. This logic was first explained by Dameshek[15] in 1965. For epidemiological purposes, should such a patient remain an example of an RARS syndrome or be considered to have the same illness as one with a sudden appearance of FAB-M6 AML without ringed sideroblasts or a prior diagnosis of MDS?

As our understanding of the molecular events leading to MDS and AML increases, epidemiologists and clinicians should work together to carefully analyze representative cohorts so that truly like illnesses are compared, in order to better identify potential risk factors. Simply abstracting a diagnosis from an often misleading and incomplete death certificate should become obsolete. A disease-specific approach is currently the rule in clinical studies that analyze the natural history and responses to therapy among the various disorders that make up the MDS classification.[57] Similar coordinated analyses by epidemiologists, pathologists and clinicians have been advocated and implemented for epidemiologic studies of the lymphoproliferative disorders, which also appear to have a number of distinct and disease

pattern-specific potential causes.[58,59] Despite these caveats, based upon the considerable evidence presented here, benzene exposure cannot be considered a potential etiology for RARS, although it remains a risk factor for certain forms of MDS such as RAEB and unclassified and/or hypocellular MDS syndromes, and for AML.

References

1. **Catenacci DVT, Schiller GJ.** Myelodysplasic (sic) syndromes: a comprehensive review. Blood Rev 2005;19:301–19.
2. **Kantarjian H.** Myelodysplastic syndromes (MDS): what is new? Leukemia Insights 2006;11:1–7.
3. **Aul C, Bowen DT, Yoshida Y.** Pathogenesis, etiology and epidemiology of myelodysplastic syndromes. Haematol 1998; 83:71–86.
4. **Cazzola M, Malcovati I.** Myelodysplastic syndromes: coping with ineffective hematopoiesis. N Engl J Med 2005;352: 536–8.
5. **Heaney M, Golde DW.** Myelodysplasia. N Engl J Med 1999; 340:1649–60.
6. **Breccia M, Mengarelli A, Mancini M, et al.** Myelodysplastic syndromes in patients under 50 years old: a single institution experience. Leuk Res 2005;29:749–54.
7. **Strom SS, Gu Y, Gruschkus SK, et al.** EH Risk factors of myelodysplastic syndrome: a case control study. Leukemia 2005;19:1912–8.
8. **Hayes RB, Yin S-N, Dosemici M, et al.** Benzene and the dose-related incidence of hematologic neoplasms in China. J Natl Cancer Inst 1997;89:1065–71.
9. **Estey E, Döhner H.** Acute myeloid leukaemia. Lancet 2006; 368:1894–907.
10. **Malcovati L, Della Porta MG, Pascutto C, et al.** Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 2005;23:7594–603.
11. **Müller-Berndorff H, Haas PS, Kunzmann R, et al.** Comparison of five prognostic scoring systems, the French-American-British (FAB) and World Health Organization (WHO) classifications in patients with myelodysplastic syndromes: results of a single-center analysis. Ann Hematol 2006;85: 502–13.
12. **Kushner JP, Lee GR, Wintrobe MM, et al.** Idiopathic refractory sideroblastic anemia: clinical and laboratory investigation of 17 patients and review of the literature. Medicine 1971;50:139–59.
13. **Cheng DS, Kushner JP, Wintrobe MM.** Idiopathic refractory sideroblastic anemia: incidence and risk factors for leukemic transformation. Cancer 1979;44:724–51.
14. **Cazzola M, Barosi G, Gobbi PG, et al.** Natural history of idiopathic refractory sideroblastic anemia. Blood 1988;71: 305–12.
15. **Dameshek W.** Sideroblastic anaemia: is this a malignancy? Br J Haematol 1965;11:52–8.
16. **Beutler E, Robson MJ, Buttenwieser B.** A comparison of the plasma iron, iron-binding capacity, sternal marrow iron and other methods in the clinical evaluation of iron stores. Ann Intern Med 1958;48:60–82.
17. **Kuendgen A, Strupp C, Aivado M, et al.** Myelodysplastic syndromes in patients younger than age 50. J Clin Oncol 2006;24:5358–65.
18. **Vandermolen L, Rice L, Rose MA, et al.** Ringed sideroblasts in primary myelodysplasia: leukemic propensity and prognostic factors. Ann Intern Med 1988;148:653–6.
19. **Garand R, Gardais J, Bizet M, et al.** Heterogeneity of acquired idiopathic sideroblastic anaemia (AISA). Leukemia Res 1992;16:463–8.
20. **Germing U, Gattermann N, Arvedo M, et al.** Two types of acquired idiopathic sideroblastic anaemia (AlSA): a time tested distinction. Br J Haematol 2000;108:724–8.
21. **Schwartz S, Jiji R, Meekins J, et al.** Chromosome abnormalities in acquired idiopathic sideroblastic anemia with subsequent leukemic transformation. Can Gen Cytogenet 1986;19:291–9.
22. **Olney HJ, Le Beau MM.** Evaluation of recurring cytogenetic abnormalities in the treatment of myelodysplastic syndromes. Leuk Res 2007;31:427–34.
23. **Kurtin PJ, Dewald GW, Shields DJ, et al.** Hematologic disorders associated with deletions of chromosome 20q: a clinicopathologic study of 107 patients. Am J Clin Pathol 1996;106:680–8.
24. **Cabello AI, Collado R, Ruiz MÁ, et al.** A retrospective analysis of myelodysplastic syndromes with thrombocytosis: reclassification of the cases by WHO proposals. Leuk Res 2005;29:365–70.
25. **Shaw GR.** Ringed sideroblasts with thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol 2005;31:180–4.
26. **Szpurka R, Tiu G, Murugesan S, et al.** Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood 2006;2173–81.
27. **Boissinot M, Garand R, Hamidou M, et al.** The JAK2-V61F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood 2006;108:1781–2.
28. **Villeval J-L, James C, Pisani DF, et al.** New insights into the pathogenesis of JAK2 V617F-positive myeloproliferative disorders and consequences for the management of patients. Semin Thromb Haemost 2006;32:341–351.
29. **Scully RE, Mark EJ, McNeely WF, et al.** BU Weekly Clinicopathologial Exercises (case 17-1992). N Engl J Med 1992;326:1137–46.
30. **Malcovati L, Germing U, Kuendgen A, et al.** Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–10.
31. **Zervas J, Geary CG, Oleesky S.** Sideroblastic anemia treated with immunosuppressive therapy. Blood 1974;44: 117–23.
32. **Lin J-T, Wang W-S, Yen C-C, et al.** Myelodysplastic syndrome complicated by autoimmune hemolytic anemia: remission of refractory anemia following mycophenolate mofetil. Ann Hematol 2002;81:723–6.
33. **Natelson E.** Pregnancy-induced pancytopenia: distinctive hematological features. Am J Med Sci 2006;332:205–7.
34. **Huff JD, Keung Y, Thakuri M, et al.** Copper deficiency causes reversible myelodysplasia. Am J Hematol 2007;82 advance e-publication.
35. **Kitahara M, Cosgriff TM, Eyre HJ.** Sideroblastic Anemia as a preleukemic event in patients treated for Hodgkin's disease. Ann Intern Med 1980;92:625–7.
36. **Magalhães SMM, Durate FB, Ribeiro SCC, et al.** Sideroblastic anemia following treatment of chronic myeloid leukemia with busulfan. Leukemia 2002;14:214–5.
37. **Fernandez LA, Zayed E.** Busulfan-induced sideroblastic anemia. Am J Hematol 1998;28:199–200.
38. **Linet MS, Yin S-N, Travis LB, et al.** Clinical features of hematopoietic malignancies and related disorders among

benzene exposed workers in china. Environ Health Perspect 1996;104:1353–64.
39. **Aksoy M.** Different types of malignancies due to occupational exposure to benzene: a review of recent observations in Turkey. Environ Res 1980;23:181–90.
40. **Aksoy M, Dinçol K, Akgün T, et al.** Haematological effects of chronic benzene poisoning in 217 workers. Br J Indust Med 1971;28:296–302.
41. **Rinsky RA, Hornung RW, Silver SR, et al.** Benzene exposure and hematopoietic mortality: a long-term epidemiologic risk assessment. Am J Indust Med 2002;42:474–80.
42. **Irons RD, Lv L, Gross SA, et al.** Chronic exposure to benzene results in a unique form of dysplasia. Leukemia Res 2005;29:1371–80.
43. **Ruiz MA, Augusto LGS, Vassallo J, et al.** Bone marrow morphology in patients with neutropenia due to chronic exposure to organic solvents (benzene): early lesions. Path Res Pract 1994;190:151–4.
44. **Orazi A.** Histopathology in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes and myelodysplastic/myeloproliferative diseases. Pathobid 2007;74: 97–114.
45. **Björk J, Albin M, Mauritzson N, et al.** Smoking and myelodysplastic syndromes. Epidemiol 2000;11:285–91.
46. **Pasqualetti P, Festucia V, Acitelli P, et al.** Tobacco smoking and risk of haematological malignancies in adults: a case-control study. Br J Haematol 1997;97:659–62.
47. **Carmona R.** The Surgeon General's 2004 report, The Health Consequences of Smoking. US Dept Health and Human Services, Chap 2, p 254.
48. **Chelghoum Y, Danaïla C, Belhabri A, et al.** Influence of cigarette smoking on the presentation and course of acute myeloid leukemia. Ann Oncol 2002;13:1621–7.
49. **Lichtman MA.** Cigarette smoking, cytogenetic abnormalities, and acute myelogenous leukemia. Leuk 2007;21: 1137–40.
50. **Thomas X, Chelghoum Y.** Cigarette smoking and acute leukemia. Leuk Lymphoma 2004;45:1103–9.
51. **Kane EV, Roman E, Cartwright R, et al.** Tobacco and the risk of acute leukaemia in adults. Br J Cancer 1999;81:1228–33.
52. **Davico L, Sacerdote C, Ciccone G, et al.** Chromosome 8, occupational exposures, smoking, and acute nonlymphocytic leukemias: a population-based study. Can Epidemiol Biomarkers Prev 1998;7:1123–5.
53. **Moorman AV, Roman E, Cartwright RA, et al.** Smoking and the risk of acute myeloid leukaemia in cytogenetic subgroups. Br J Cancer 2002;86:60–2.
54. **Smith MA, McCaffrey RP, Karp JE.** The secondary leukemias: challenges and research directions. J Natl Cancer Inst 1996;88:407–18.
55. **Morgan GJ, Alvares CL.** Benzene and the hematopoietic stem cell. Chemico-Biol Interact 2005;153–154:217–222.
56. **Yoshida Y, Oguma S, Tohyama K, et al.** Diagnostic and biological significance of sideroblastic erythropoiesis in the myelodysplastic syndromes. Int J Hematol 1998;67:137–44.
57. **List A, Kurtin S, Roe DJ, et al.** Efficacy of Lenalidomide in myelodysplastic syndromes. N Engl J Med 2005;352: 549–57.
58. **Alexander DD, Mink PJ, Adami HO, et al.** The non-Hodgkin lymphomas: A review of the epidemiologic literature. Int J Can 2007;120:1–39.
59. **Wang SS, Slager SL, Brennan P, et al.** Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10211 cases and 11905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007;109:3479–88.

Eric Williams

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Dear Mr. Williams:
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Thank you,
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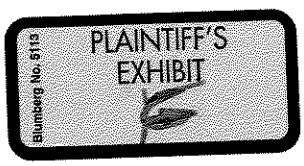

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Subject: A manuscript number has been assigned (AJMS 07-031)

Feb 13, 2007

Associate Professor Natelson,

Thank you for submitting your manuscript to The The American Journal of the Medical Sciences. Your submission entitled "Benzene Exposure and Refractory Sideroblastic Erythropoiesis - Is There an Association?" has been assigned the following manuscript number: AJMS 07-031.

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Subject: AJMS 07-031 Decision

April 25, 2007

RE: AJMS 07-031, entitled "Benzene Exposure and Refractory Sideroblastic Erythropoiesis - Is There an Association?"

Good Morning Dr. Natelson,

I am pleased to inform you that your paper has been found acceptable for publication pending minor revision. Below you will find the reviewers comments for your review. I anticipate that you will easily be able to answer the criticisms of the reviewers in a satisfactory manner and will verify those changes upon receipt of the revised manuscript.

Please include with your revised (highlighted) submission an itemized, point-by-point response to the comments of each reviewer. Thank you, I look forward to receiving your completed revisions by May 09, 2007.

To submit a revision, go to http://maj.edmgr.com/ and log in as an Author. You will see a menu item called "Submission Needing Revision." Please click on this item to obtain your submission record and begin the revision process.

Your username is: ******
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With kind regards,
Ms. Kathleen Kelly
Managing Editor
The American Journal of the Medical Sciences

Reviewer Comments:

Reviewer #1: I found this to be a very interesting, if somewhat abbreviated review of this subject. This topic is also quite timely as benzene related issues (mostly toxic tort litigation) are on the rise and MDS is frequently at issue. Most epidemiologists and toxicologists consider MDS to be a single disease entity, particularly from an etiological point of view. Therefore, this review makes a useful contribution to the scientific literature by providing a needed, informed opposing viewpoint. Additionally, physicians are becoming increasingly involved in this discussion (both formally and informally), and in many cases they come in with a very poor background or understanding of benzene toxicology or pathology. In both of these areas, this review provides something of value and would likely be widely read and appreciated.

General Comments:

The abstract is perhaps overly concise and would benefit from a bit more information.

I think this manuscript would also benefit from a more robust discussion of MDS and the FAB method of classification (the 'FAB' 5). A more detailed discussion regarding the accepted and suspected risk factors for the various subtypes of MDS would also be very germane to this review. How might this change within the WHO classification system?

There were several areas where some light editing is needed. Additionally, the draft tends to have a lot of technical terms with (in some cases) insufficient introduction to that topic. I have provided a few examples below.

There was a great deal of effort on the part of the author to identify the 3 subtypes of RARS, but that didn't seem to influence the overall conclusions of the manuscript, namely that MDS is not a single disease and that benzene exposure is not associated with one type, RARS. Perhaps a rationale for why the 3 different forms of RARS are important epidemiologically might be included for clarity.

Specific Comments:

P3: Is myelodysplasia the same as myelodysplastic syndrome? I think the author's answer would be yes, but this needs to made clear at the outset of the introduction.

P3, L4: Suggested edit...delete "That there is"...and begin the sentence with"a".

P3, L15: The sentence needs some additional justification. The approach as described only 'blurs' things if, in fact, the various diseases under the rubric of MDS actually have different etiologies. Later, some evidence is offered that supports this sentence, but at this point in the progression of the discussion, no data has been discussed.

P5, L12: The sentence that starts with 'RARS' is somewhat confusing.

P6, L9: Please define the T in RARS-T...it is identified later in the section.

P7, L11: What does 'obvious multi-lineage dysplasia' have to do with IS therapy. This section jumped around a bit too much for a non-hematologist to follow. Also, does it indicate the different forms of RARS have diverse causes or really just evidence of a diverse response to treatment?

P8: Is the point of this section that neither benzene nor chemotherapy exposure is associated with an increased risk of RARS?

P9: Were the pathological descriptions published in Aksoy's work sufficiently detailed to determine if any RARS cases were present? Did they use the appropriate assays and stains? Also, the 'pliofilm' cohort was based on death certificates. Is it possible that some had RARS and then progressed and died from AML without RARS being identified (or identifiable)? If so, does this change the authors position?

P10: If topoisomerase inhibition is a potential mechanism for benzene and AML, why is there no involvement of chromosomes 11 in any benzene exposure worker or occupational AML case? Involvement with (11q23) is extremely common in topoisomerase inhibition induced t-AML, but not in benzene cohorts? This reviewer has serious doubts about this mechanism playing a role in benzene induced AML.

P12: What are the recommendations of the author regarding MDS epidemiology? How should the hypothetical patient discussed on page 11-12 be handled in epidemiology studies? How would that ever be identified by the investigators...particularly in a retrospective mortality study? I totally agree that this is a significant issue and would

therefore be interested in reading any recommendations regarding this short-coming in available or forthcoming studies.

P12: Last line: I would suggest the following editorial change...although it remains a risk factor for other certain forms of AML and for AML. This line also begs the question...which forms of MDS might be positively associated with benzene? Perhaps a table could be included with the various diseases and the relative strength of the epidemiology evidence.

Complete Revision Instructions:

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David Pyatt, MD (Reviewer 1)

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AJMS 07-031
"Benzene Exposure and Refractory Sideroblastic Erythropoiesis - Is There an Association?"
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Additional Comments to Editorial Office:

Comments to Author: I found this to be a very interesting, if somewhat abbreviated review of this subject. This topic is also quite timely as benzene related issues (mostly toxic tort litigation) are on the rise and MDS is frequently at issue. Most epidemiologists and toxicologists consider MDS to be a single disease entity, particularly from an etiological point of view. Therefore, this review makes a useful contribution to the scientific literature by providing a needed, informed opposing viewpoint. Additionally, physicians are becoming increasingly involved in this discussion (both formally and informally), and in many cases they come in with a very poor background or understanding of benzene toxicology or pathology. In both of these areas, this review provides something of value and would likely be widely read and appreciated.

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The abstract is perhaps overly concise and would benefit from a bit more information.

I think this manuscript would also benefit from a more robust discussion of MDS and the FAB method of classification (the 'FAB' 5). A more detailed discussion regarding the accepted and suspected risk factors for the various subtypes of MDS would also be very germane to this review. How might this change within the WHO classification system?

There were several areas where some light editing is needed. Additionally, the draft tends to have a lot of technical terms with (in some cases) insufficient introduction to that topic. I have provided a few examples below.

There was a great deal of effort on the part of the author to identify the 3 subtypes of RARS, but that didn't seem to influence the overall conclusions of the manuscript, namely that MDS is not a single disease and that benzene exposure is not associated with one type, RARS. Perhaps a rationale for why the 3 different forms of RARS are important epidemiologically might be included for clarity.

Specific Comments:

P3: Is myelodysplasia the same as myelodysplastic syndrome? I think the author's answer would be yes, but this needs to made clear at the outset of the introduction.

P3, L4: Suggested edit...delete "That there is"...and begin the sentence with"a".

P3, L15: The sentence needs some additional justification. The approach as described only 'blurs' things if, in fact, the various diseases under the rubric of MDS actually have different etiologies. Later, some evidence is offered that supports this sentence, but at this point in the progression of the discussion, no data has been discussed.

P5, L12: The sentence that starts with 'RARS' is somewhat confusing.

P6, L9: Please define the T in RARS-T...it is identified later in the section.

P7, L11: What does 'obvious multi-lineage dysplasia' have to do with IS therapy. This section jumped around a bit too much for a non-hematologist to follow. Also, does it indicate the different forms of RARS have diverse causes or really just evidence of a diverse response to treatment?

P8: Is the point of this section that neither benzene nor chemotherapy exposure is associated with an increased risk of RARS?

P9: Were the pathological descriptions published in Aksoy's work sufficiently detailed to determine if any RARS cases were present? Did they use the appropriate assays and stains? Also, the 'pliofilm' cohort was based on death certificates. Is it possible that some had RARS and then progressed and died from AML

without RARS being identified (or identifiable)? If so, does this change the authors position?

P10: If topoisomerase inhibition is a potential mechanism for benzene and AML, why is there no involvement of chromosomes 11 in any benzene exposure worker or occupational AML case? Involvement with (11q23) is extremely common in topoisomerase inhibition induced t-AML, but not in benzene cohorts? This reviewer has serious doubts about this mechanism playing a role in benzene induced AML.

P12: What are the recommendations of the author regarding MDS epidemiology? How should the hypothetical patient discussed on page 11-12 be handled in epidemiology studies? How would that ever be identified by the investigators... particularly in a retrospective mortality study? I totally agree that this is a significant issue and would therefore be interested in reading any recommendations regarding this short-coming in available or forthcoming studies.

P12: Last line: I would suggest the following editorial change... although it remains a risk factor for other certain forms of AML and for AML. This line also begs the question...which forms of MDS might be positively associated with benzene? Perhaps a table could be included with the various diseases and the relative strength of the epidemiology evidence.

More Reviewer Details

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Benzene Exposure and Refractory Sideroblastic Erythropoiesis: Is There an Association?

ETHAN A. NATELSON, MD

Editor in Chief

ABSTRACT: The myelodysplastic syndromes (MDS) consist of a group of diverse hematological disorders that carry an increased risk of transforming into acute myeloid leukemia. They may appear de novo and without obvious cause (primary or de novo MDS) or be induced by certain mutagenic environmental or therapeutic toxins (secondary MDS). Excessive exposures to benzene are generally considered to be a potential environmental risk factor for both MDS and acute myeloid leukemia. However, such risk is unproven for each disease component within the MDS classification. A critical review of the refractory sideroblastic disorders strongly suggests that benzene exposure is not a potential cause of this distinct and still-evolving subset of MDS. The widely disparate nature of MDS suggests that epidemiologic studies can only provide meaningful data on associations and potential causation of its component syndromes by a disease-specific analysis, as is currently advocated for other hematological malignancies. **KEY INDEXING TERMS:** Benzene; Myelodysplasia; MDS; Refractory Sideroblastic Anemia. **[Am J Med Sci 2007; 334(5):356–360.]**

The terms myelodysplasia and the myelodysplastic syndromes (MDS) refer to a classification of heterogenous and distinct disease forms linked by the presence of oligoclonal hematopoietic stem cell abnormalities, ineffective hematopoiesis characterized by abnormal differentiation and maturation of myeloid cells, and a variable but increased, risk of progression toward acute myeloid leukemia.[1–6] A common etiology among each of the various myelodysplastic disorders that is also shared with acute myeloid leukemia (AML) has not been proposed, and little is known about the major causes of MDS.[6,7] Unfortunately, epidemiological surveys seeking statistical information on hematological malignancies in cohorts potentially exposed to environmental chemicals or toxins seldom provide specific data on MDS, despite its higher incidence in the general population than AML.[4] Moreover, in rare instances when information on MDS is collected and presented, it is usually treated as a single, distinct disease entity, and its numbers may be combined with those of AML or even other unrelated hematological malignancies in an attempt to achieve statistical significance in the analysis.[8] Such approach obscures identification of potential etiologic risk factors that might be specific to the individual disease components within MDS and to the various AML syndromes. Thus, under certain circumstances, benzene exposure is an accepted, although uncommon, potential risk factor for both MDS and AML.[1,6,7,9] However, when carefully studied (vide infra), such risk does not necessarily apply to all components of the MDS. This report reviews the clinical observations that provide specific information concerning the question of causal associations between benzene as an environmental toxin and the acquired refractory sideroblastic disorders, a readily identifiable subset of the MDS classification system.[10,11]

Discussion

Refractory anemia with ringed sideroblasts (RARS) was a well-described and stand-alone hematopoietic disorder until 1982, when it was included among other hematological syndromes grouped under a then newly defined heading of MDS.[12–15] Typically discussed in hematologic text chapters dealing with congenital and acquired sideroblastic anemias of diverse cause, RARS has been described under a variety of titles including acquired idiopathic refractory sideroblastic anemia and sideroachrestic anemia. Its unifying morphological feature is the presence of ringed sideroblasts, as identified by iron stains, in at least 15% of the bone marrow erythroid precursors throughout multiple areas of the bone marrow films or biopsy.[1,4] Such histologic stains have been a part of the routine evaluation of bone marrow specimens for nearly 60 years.[16] The variable but clearly preleukemic nature of RARS has

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Table 1. Myelodysplastic Syndromes

Original FAB Divisions	Current WHO Divisions
Refractory anemia	Refractory anemia
RA-Ringed sideroblasts	RC-Multilineage dysplasia
RA-Excess (5–20%) blasts	RA-Ringed sideroblasts
RA-Excess (21–30%) blasts in transition	RC-RS–Multilineage dysplasia
	RA-Excess (5–9%) blasts type I
Chronic myelomonocytic leukemia	RA-Excess (9–19%) blasts type II
	MDS isolated deletion 5 q
	MDS unclassifiable

FAB, French-American-British format; WHO, World Health Organization; RA, refractory anemia; RC, refractory cytopenia; RS, ringed sideroblasts.

also been known since its earliest descriptions.[13,15] In the original MDS classification scheme, the subset of individuals with RARS comprised about 10% to 20% of the total cases in most series, with the relative frequency, perhaps, age of onset-related.[17] They exhibit the lowest incidence of classical chromosomal aberrations of all MDS subsets and the least frequency of progression to AML—approximately 15%.[18–21] Certain chromosomal aberrations have been associated with RARS including long arm deletions of chromosomes 5, 11, and 20, as well as trisomy 8.[21] None are specific, however, and particularly deletions of 20q and trisomy 8 are identified in a number of diverse hematological disorders.[21–23]

Modern revised classification systems of MDS attempt to separate RARS into 2 general subsets, 1 currently referred to as refractory cytopenia with multilineage dysplasia-ringed sideroblasts (RCMD-RS), and a second entity with little evident morphologic cytologic dysplasia outside the erythroid cell line, and a lesser risk of AML (Table 1). It remains under the designation of RARS.[1,11,19,20,24] Recently, a further clinically-useful separation of the refractory sideroblastic disorders has been suggested, segregating all patients with ringed sideroblasts and markedly elevated platelet counts into yet another subset designated RARS-T or RST to emphasize the presence of thrombocytosis.[24–27] Many of the individuals in this third grouping may also possess the newly described JAK-2 V617F chromosomal abnormality, commonly identified in polycythemia vera and other myeloproliferative disorders.[26–28] RARS-T could then also be listed under the newly suggested descriptive designation of myelodysplastic/myeloproliferative syndromes.[25] The recognition that some individuals with RARS and marked thrombocytosis might exhibit a clinical course more in keeping with a myeloproliferative disorder, with megakaryocytic hyperplasia and bone marrow fibrosis, similar to the natural history of polycythemia vera, primary thrombocythemia and agnogenic myeloid metaplasia, is not a new observation.[29] The presence of the JAK-2 V617F mutation among the RARS-T group provides a unifying molecular explanation. Importantly, myeloproliferative disorders have not been epidemiologically linked with exposures to either benzene or to modern mutagenic chemotherapy agents.

The frequency distribution of these 3 refractory sideroblastic syndromes, particularly between RARS and RCMD-RS, is clearly observer-dependent and also varies with the alternate classifications systems for MDS of the World Health Organization and the French-American-British (FAB) format.[10,30] An estimate would place about 10% of the total as RARS-T. However, if the bone marrow blast cell count increases above 5%, and even if large numbers of ringed sideroblasts are retained, under these classification schemes individuals are now moved from an RARS syndrome into another MDS category, refractory anemia with excess blasts (RAEB). In effect, this movement arbitrarily diminishes the pool of cases with refractory sideroblastic hematopoiesis available for epidemiological or clinical analysis, and is a weakness of all MDS classifications.[18] Importantly, occasional patients with RARS, even those with apparent multilineage cytologic dysplasia, may favorably respond to immunosuppressive therapy, and in rare instances the hematologic syndrome is actually consequent to unusual deficiency states.[31–34] Thus, progression to AML may not be a part of the natural history in certain forms of acquired sideroblastic anemia, emphasizing that all such disorders do not necessarily represent hematologic malignancies.

From the earliest descriptions, authors reporting on RARS pondered potential causes of this uncommon disorder. Wintrobe's experience in a single institution along with review of many additional cases led him and his associates to conclude that no specific drugs, environmental chemicals, alcohol, or cytostatic agents, could be confirmed as potential risk factors, and that the cause of RARS was unknown.[12] Benzene had previously been used as an oral chemotherapeutic agent in forms of chronic leukemia, and it was well known to cause bone marrow damage and potentially eventuate in AML. Exposure to this agent was specifically sought in an analysis of 84 patients with RARS, but only 1 of these subjects could recall any previous benzene exposure. Furthermore, that patient also had received radiation therapy.[19] The collective experience now involving thousands of reported patients having received chemotherapy and then developing secondary hematologic syndromes including MDS, has only supplied rare case reports describing a chronic acquired sideroblastic process. This phenomenon has usually occurred in the presence of an underlying hematological malignancy, such as multiple myeloma or Hodgkin's disease, and is then noted after treatment including both alkylating agents and radiation.[35] Here, the acquired sideroblastic process, itself, is often transient and not evident in follow-up bone marrow examinations.[36,37] Thus, neither exposure to

benzene nor to modern chemotherapeutic mutagenic agents has been shown to be a risk factor for RARS.

There are 3 major epidemiological studies often cited as offering detailed observations, including bone marrow analysis, which routinely includes iron stains, concerning the hematological effects of chronic, high-level benzene exposure. In the Hayes[8] report of about 74,000 factory workers, primarily immersion painters in China, 28 individuals with either AML or MDS were cited. Analysis of the individual slide material from these MDS cases, and which specifically described bone marrow iron examination, showed no examples of RARS.[38] In the Aksoy[39] report of 29,000 Turkish shoe workers heavily exposed to benzene-containing glues, 42 individuals were described as developing forms of AML, among other hematological disorders. Yet, in his description of the specific hematologic abnormalities in this cohort, there is no observation consistent with RARS.[40] Finally, in the frequently-analyzed United States Pliofilm factory cohort of about 1,800 individuals exposed to large concentrations of pure benzene for many years, there are no reported details of bone marrow examinations, but no instance of a listed diagnosis consistent with RARS.[41] A recent report from The MD Anderson Cancer Center also could not confirm solvent exposure as a significant predisposing event in their patients with RARS, or with chronic myelomonocytic leukemia, an original, and now former, member within the MDS classification system.[7] In that study, such exposures were apparent risk factors for other forms of MDS such as refractory anemia with increased blasts (RAEB). In reality, there does not appear to be a single epidemiological study or even an isolated case report in the world's medical literature that links RARS with benzene exposure.

Recently, Irons et al[42] carefully analyzed blood counts and serial bone marrow examinations on a small cohort of 23 Chinese workers heavily exposed to benzene. They reaffirmed earlier sporadic observations of marrow hypoplasia and eosinophilia in benzene-exposed workers but failed to identify the presence of ringed sideroblasts. Similarly, an absence of ringed sideroblasts was noted in careful bone marrow studies of 33 additional individuals with chronic benzene poisoning.[43] These marrow studies also demonstrated eosinophilia and dyspoietic erythroblasts. In general, the type of MDS bone marrow histology usually described in both heavily benzene-exposed individuals and therapy-related MDS is a hypocellular bone marrow with cellular dyspoiesis, and distinct from the usual findings in RARS which typically reflect a normocellular to hypercellular bone marrow pattern.[38,44]

Cigarette smoke is considered a risk factor for both AML and MDS, but the specific element or mechanism involved is unknown.[9,45–53] Cigarette smoke does contain some benzene, but the cumulative dose achieved by smokers is less than thought necessary to increase the risk of AML, and the chromosomal anomalies identified in smokers with AML are somewhat different from those typically reported for benzene-induced and other secondary forms of AML.[9,48–53] The chromosomal aberrations seen in the vast majority of benzene-induced AML and modern day chemotherapy-induced AML, or t-AML, are similar, with a predominance of deletions in chromosomes 5 and 7.[9] This is not surprising, since postulated mechanisms of leukemogenesis in benzene-induced and t-AML, are also thought to be similar, if not identical.[54,55] Some reports have suggested cigarette smoking is linked with RARS, although the mechanism is not known.[7,45]

One feature of MDS that further confounds epidemiological analysis of its individual disease components is the fact that unlike AML, MDS is not a final illness designation, and individuals frequently move within the MDS classification over time. This is particularly true with respect to RARS syndromes. Thus, it would be expected that as the often slow progression to AML from the 3 refractory sideroblastic variants described here occurs, the percentage of blast cells in the bone marrow may increase gradually from its initially normal values. As previously mentioned, ringed sideroblasts are often still present when the bone marrow blast percentage reaches 5% to 10%, but the MDS diagnosis now moves to RAEB.[17,56] When the blast percentage eventually reaches 20%, by convention, the diagnosis of AML is now allowed. Even if ringed sideroblasts remain evident in excess along with marked erythroid dyspoiesis, the specific diagnosis would be an FAB-M6 AML or erythroleukemia. Obviously, for such individual, this transition reflects a single disease process, and not 3 separate illnesses. This logic was first explained by Dameshek[15] in 1965. For epidemiological purposes, should such a patient remain an example of an RARS syndrome or be considered to have the same illness as one with a sudden appearance of FAB-M6 AML without ringed sideroblasts or a prior diagnosis of MDS?

As our understanding of the molecular events leading to MDS and AML increases, epidemiologists and clinicians should work together to carefully analyze representative cohorts so that truly like illnesses are compared, in order to better identify potential risk factors. Simply abstracting a diagnosis from an often misleading and incomplete death certificate should become obsolete. A disease-specific approach is currently the rule in clinical studies that analyze the natural history and responses to therapy among the various disorders that make up the MDS classification.[57] Similar coordinated analyses by epidemiologists, pathologists and clinicians have been advocated and implemented for epidemiologic studies of the lymphoproliferative disorders, which also appear to have a number of distinct and disease

pattern-specific potential causes.[58,59] Despite these caveats, based upon the considerable evidence presented here, benzene exposure cannot be considered a potential etiology for RARS, although it remains a risk factor for certain forms of MDS such as RAEB and unclassified and/or hypocellular MDS syndromes, and for AML.

References

1. **Catenacci DVT, Schiller GJ.** Myelodysplasic (sic) syndromes: a comprehensive review. Blood Rev 2005;19:301–19.
2. **Kantarjian H.** Myelodysplastic syndromes (MDS): what is new? Leukemia Insights 2006;11:1–7.
3. **Aul C, Bowen DT, Yoshida Y.** Pathogenesis, etiology and epidemiology of myelodysplastic syndromes. Haematol 1998; 83:71–86.
4. **Cazzola M, Malcovati I.** Myelodysplastic syndromes: coping with ineffective hematopoiesis. N Engl J Med 2005;352: 536–8.
5. **Heaney M, Golde DW.** Myelodysplasia. N Engl J Med 1999; 340:1649–60.
6. **Breccia M, Mengarelli A, Mancini M, et al.** Myelodysplastic syndromes in patients under 50 years old: a single institution experience. Leuk Res 2005;29:749–54.
7. **Strom SS, Gu Y, Gruschkus SK, et al.** EH Risk factors of myelodysplastic syndrome: a case control study. Leukemia 2005;19:1912–8.
8. **Hayes RB, Yin S-N, Dosemici M, et al.** Benzene and the dose-related incidence of hematologic neoplasms in China. J Natl Cancer Inst 1997;89:1065–71.
9. **Estey E, Döhner H.** Acute myeloid leukaemia. Lancet 2006; 368:1894–907.
10. **Malcovati L, Della Porta MG, Pascutto C, et al.** Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol 2005;23:7594–603.
11. **Müller-Berndorff H, Haas PS, Kunzmann R, et al.** Comparison of five prognostic scoring systems, the French-American-British (FAB) and World Health Organization (WHO) classifications in patients with myelodysplastic syndromes: results of a single-center analysis. Ann Hematol 2006;85: 502–13.
12. **Kushner JP, Lee GR, Wintrobe MM, et al.** Idiopathic refractory sideroblastic anemia: clinical and laboratory investigation of 17 patients and review of the literature. Medicine 1971;50:139–59.
13. **Cheng DS, Kushner JP, Wintrobe MM.** Idiopathic refractory sideroblastic anemia: incidence and risk factors for leukemic transformation. Cancer 1979;44:724–51.
14. **Cazzola M, Barosi G, Gobbi PG, et al.** Natural history of idiopathic refractory sideroblastic anemia. Blood 1988;71: 305–12.
15. **Dameshek W.** Sideroblastic anaemia: is this a malignancy? Br J Haematol 1965;11:52–8.
16. **Beutler E, Robson MJ, Buttenwieser B.** A comparison of the plasma iron, iron-binding capacity, sternal marrow iron and other methods in the clinical evaluation of iron stores. Ann Intern Med 1958;48:60–82.
17. **Kuendgen A, Strupp C, Aivado M, et al.** Myelodysplastic syndromes in patients younger than age 50. J Clin Oncol 2006;24:5358–65.
18. **Vandermolen L, Rice L, Rose MA, et al.** Ringed sideroblasts in primary myelodysplasia: leukemic propensity and prognostic factors. Ann Intern Med 1988;148:653–6.
19. **Garand R, Gardais J, Bizet M, et al.** Heterogeneity of acquired idiopathic sideroblastic anaemia (AISA). Leukemia Res 1992;16:463–8.
20. **Germing U, Gattermann N, Arvedo M, et al.** Two types of acquired idiopathic sideroblastic anaemia (AISA): a time tested distinction. Br J Haematol 2000;108:724–8.
21. **Schwartz S, Jiji R, Meekins J, et al.** Chromosome abnormalities in acquired idiopathic sideroblastic anemia with subsequent leukemic transformation. Can Gen Cytogenet 1986;19:291–9.
22. **Olney HJ, Le Beau MM.** Evaluation of recurring cytogenetic abnormalities in the treatment of myelodysplastic syndromes. Leuk Res 2007;31:427–34.
23. **Kurtin PJ, Dewald GW, Shields DJ, et al.** Hematologic disorders associated with deletions of chromosome 20q: a clinicopathologic study of 107 patients. Am J Clin Pathol 1996;106:680–8.
24. **Cabello AI, Collado R, Ruiz MÁ, et al.** A retrospective analysis of myelodysplastic syndromes with thrombocytosis: reclassification of the cases by WHO proposals. Leuk Res 2005;29:365–70.
25. **Shaw GR.** Ringed sideroblasts with thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol 2005;31:180–4.
26. **Szpurka R, Tiu G, Murugesan S, et al.** Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood 2006;2173–81.
27. **Boissinot M, Garand R, Hamidou M, et al.** The JAK2-V61F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood 2006;108:1781–2.
28. **Villeval J-L, James C, Pisani DF, et al.** New insights into the pathogenesis of JAK2 V617F-positive myeloproliferative disorders and consequences for the management of patients. Semin Thromb Haemost 2006;32:341–351.
29. **Scully RE, Mark EJ, McNeely WF, et al.** BU Weekly Clinicopathologial Exercises (case 17-1992). N Engl J Med 1992;326:1137–46.
30. **Malcovati L, Germing U, Kuendgen A, et al.** Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes. J Clin Oncol 2007;25:3503–10.
31. **Zervas J, Geary CG, Oleesky S.** Sideroblastic anemia treated with immunosuppressive therapy. Blood 1974;44: 117–23.
32. **Lin J-T, Wang W-S, Yen C-C, et al.** Myelodysplastic syndrome complicated by autoimmune hemolytic anemia: remission of refractory anemia following mycophenolate mofetil. Ann Hematol 2002;81:723–6.
33. **Natelson E.** Pregnancy-induced pancytopenia: distinctive hematological features. Am J Med Sci 2006;332:205–7.
34. **Huff JD, Keung Y, Thakuri M, et al.** Copper deficiency causes reversible myelodysplasia. Am J Hematol 2007;82 advance e-publication.
35. **Kitahara M, Cosgriff TM, Eyre HJ.** Sideroblastic Anemia as a preleukemic event in patients treated for Hodgkin's disease. Ann Intern Med 1980;92:625–7.
36. **Magalhães SMM, Durate FB, Ribeiro SCC, et al.** Sideroblastic anemia following treatment of chronic myeloid leukemia with busulfan. Leukemia 2002;14:214–5.
37. **Fernandez LA, Zayed E.** Busulfan-induced sideroblastic anemia. Am J Hematol 1998;28:199–200.
38. **Linet MS, Yin S-N, Travis LB, et al.** Clinical features of hematopoietic malignancies and related disorders among

benzene exposed workers in china. Environ Health Perspect 1996;104:1353–64.
39. **Aksoy M.** Different types of malignancies due to occupational exposure to benzene: a review of recent observations in Turkey. Environ Res 1980;23:181–90.
40. **Aksoy M, Dinçol K, Akgün T, et al.** Haematological effects of chronic benzene poisoning in 217 workers. Br J Indust Med 1971;28:296–302.
41. **Rinsky RA, Hornung RW, Silver SR, et al.** Benzene exposure and hematopoietic mortality: a long-term epidemiologic risk assessment. Am J Indust Med 2002;42:474–80.
42. **Irons RD, Lv L, Gross SA, et al.** Chronic exposure to benzene results in a unique form of dysplasia. Leukemia Res 2005;29:1371–80.
43. **Ruiz MA, Augusto LGS, Vassallo J, et al.** Bone marrow morphology in patients with neutropenia due to chronic exposure to organic solvents (benzene): early lesions. Path Res Pract 1994;190:151–4.
44. **Orazi A.** Histopathology in the diagnosis and classification of acute myeloid leukemia, myelodysplastic syndromes and myelodysplastic/myeloproliferative diseases. Pathobid 2007;74: 97–114.
45. **Björk J, Albin M, Mauritzson N, et al.** Smoking and myelodysplastic syndromes. Epidemiol 2000;11:285–91.
46. **Pasqualetti P, Festucia V, Acitelli P, et al.** Tobacco smoking and risk of haematological malignancies in adults: a case-control study. Br J Haematol 1997;97:659–62.
47. **Carmona R.** The Surgeon General's 2004 report, The Health Consequences of Smoking. US Dept Health and Human Services, Chap 2, p 254.
48. **Chelghoum Y, Danaïla C, Belhabri A, et al.** Influence of cigarette smoking on the presentation and course of acute myeloid leukemia. Ann Oncol 2002;13:1621–7.
49. **Lichtman MA.** Cigarette smoking, cytogenetic abnormalities, and acute myelogenous leukemia. Leuk 2007;21: 1137–40.
50. **Thomas X, Chelghoum Y.** Cigarette smoking and acute leukemia. Leuk Lymphoma 2004;45:1103–9.
51. **Kane EV, Roman E, Cartwright R, et al.** Tobacco and the risk of acute leukaemia in adults. Br J Cancer 1999;81:1228–33.
52. **Davico L, Sacerdote C, Ciccone G, et al.** Chromosome 8, occupational exposures, smoking, and acute nonlymphocytic leukemias: a population-based study. Can Epidemiol Biomarkers Prev 1998;7:1123–5.
53. **Moorman AV, Roman E, Cartwright RA, et al.** Smoking and the risk of acute myeloid leukaemia in cytogenetic subgroups. Br J Cancer 2002;86:60–2.
54. **Smith MA, McCaffrey RP, Karp JE.** The secondary leukemias: challenges and research directions. J Natl Cancer Inst 1996;88:407–18.
55. **Morgan GJ, Alvares CL.** Benzene and the hematopoietic stem cell. Chemico-Biol Interact 2005;153–154:217–222.
56. **Yoshida Y, Oguma S, Tohyama K, et al.** Diagnostic and biological significance of sideroblastic erythropoiesis in the myelodysplastic syndromes. Int J Hematol 1998;67:137–44.
57. **List A, Kurtin S, Roe DJ, et al.** Efficacy of Lenalidomide in myelodysplastic syndromes. N Engl J Med 2005;352: 549–57.
58. **Alexander DD, Mink PJ, Adami HO, et al.** The non-Hodgkin lymphomas: A review of the epidemiologic literature. Int J Can 2007;120:1–39.
59. **Wang SS, Slager SL, Brennan P, et al.** Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10211 cases and 11905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007;109:3479–88.

* Response to Reviewers

4-27-07

Ms. Kathleen Kelly
Managing Editor
The American Journal of the Medical Sciences

RE: Manuscript AJMS 07-031, Response to Reviewers

Dear Ms. Kelly,

Thank you for the opportunity to respond to the reviewer's comments and suggestions. I must say that I appreciate the detailed analysis and the time spent in this review. I hope you will agree that the revised manuscript flows more easily as it is read and more clearly highlights the points I am trying to make. This improves its usefulness to both general physicians and epidemiologists who are not always familiar with the descriptive and often confusing language used in discussing MDS. My point by point responses to the specific comments are as follows:

P3: Yes, myelodysplasia, myelodysplastic syndrome, myelodysplasic disorders and myelodysplastic syndromes all refer to the same process and classification, and are terms used interchangeably throughout the medical literature. I have clarified this in the abstract and the opening sentence of the introduction.

P3, L4: I have modified this sentence as suggested.

P3, L15: I have clarified this language and added the term, *vide infra*, to emphasize that while no data has yet been presented, it is to follow.

P5, L12: This confusing sentence has been re-worked.

P6, L9: The T, as used in RARS-T, has been defined in the sentence.

P7, L11: This sentence has been re-worked and added to in order to make the point clear that even a very dyspoietic and sick appearing RARS marrow, with obvious multilineage dysplasia, does not mean that the patient has a neoplastic condition destined to evolve into leukemia. Thus, the RARS syndromes are very heterogenous in causation. Truthfully, in my and other hematologist's opinions, they should exit MDS as has chronic myelomonocytic leukemia, but this is too big a leap to propose in a focused article like this one.

P8: Yes, that is the point. I have added a summation sentence to the paragraph to emphasize this conclusion.

P9: Here the reviewer reveals that he/she is not a clinician. The Aksoy and Pliofilm studies were done in the 1970's and 1980's. As a medical student in 1963-4 I was doing

iron stains on bone marrows, as they were already a routine procedure at that time. I did add a reference from 1958 (now 16), by Dr. Ernst Beutler, who, almost 50 years later, remains an eminent hematologist, clinician and lecturer, and who reviewed the subject of bone marrow iron stains at that time. I also discussed the use of iron stains and death certificates in the text.

P10: The reviewer is alluding to a topic that, "has not yet been introduced in evidence", as the lawyers often say. Nowhere in the manuscript did I use the term Topoisomerase II inhibitor. Actually, I agree with the reviewer on this concept. Thus, I removed the 2 references (original 48-49) that specifically deal with Topoisomerase II inhibition as a possible mechanism of benzene leukemogenic action, and used alternate references (now 54-55) that state that the leukemogenic effects of benzene and modern chemotherapy are thought to be identical, without specifying the proposed Topoisomerase II related mechanism that the reviewer finds controversial.

P12: This is an important topic and has been more and more emphasized in the medical literature, but, until recently, been given more lip service than application. I added a few sentences and a very new reference (now 59) that shows, in concert with the preceding reference (now 58) how this was suggested and then actually has been implemented in the field of lymphoma epidemiology and causation.

P12, Last Line: I did add a comment as to which types of MDS might be benzene-induced. I did add a table to show how the classification of MDS began, and what it consists of today. There is simply no data to define the strength of the evidence for each subtype of MDS – this can only be addressed as I have done to exclude RARS and CMML (an entity now removed from the original classification system) and plea for specific data in all of the categories.

Going back to the general comments, I have expanded the abstract, adding information, and in the table show how the MDS classification has been modified from the original "FAB 5" the reviewer mentions.

Please let me know if these revisions and additions have satisfactorily addressed the reviewer's comments.

Cordially yours,

Ethan A. Natelson, M.D.

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Date: 05/02/2007
To: "Ethan Allen Natelson" enatelson@tmh.tmc.edu
From: "The American Journal of the Medical Sciences" ajms@musc.edu
Subject: AJMS 07-031R1 Decision

May 02, 2007

RE: AJMS 07-031R1, entitled "Benzene Exposure and Refractory Sideroblastic Erythropoiesis - Is There an Association?"

Good Morning Dr. Natelson,

I am pleased to inform you that your work has now been accepted for publication in The American Journal of the Medical Sciences. Congratulations to you and your co-authors! While there are no additional changes to be made at this time, there may be some minor changes which may need to be addressed once you receive the proofs back from the publisher. When you receive the page proofs, please check both the text and bibliography carefully as we must rely on the authors for the accuracy of the references.

Your manuscript materials will now be forwarded to production for placement in an upcoming issue.

Thank you for submitting your interesting and important work to The Journal. It has been a pleasure working with you during the review process, and I do hope we will have a future opportunity of working with you again.

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With kind regards,

Ms. Kathleen Kelly
Managing Editor
The American Journal of the Medical Sciences

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The American Journal of the Medical Sciences

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from any of the following organizations: National Institutes of Health (NIH), Wellcome Trust, Howard Hughes Medical Institute (HHMI), or other(s); (5) disclaimers, if any, including notices of the manuscript's prior appearance as a preliminary report or abstract; and (6) a short title of no more than 40 characters and spaces.

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Parkin DM, Clayton D, Black RJ, et al. Childhood leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, et al, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

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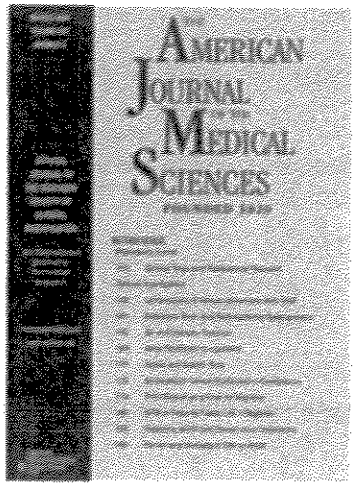

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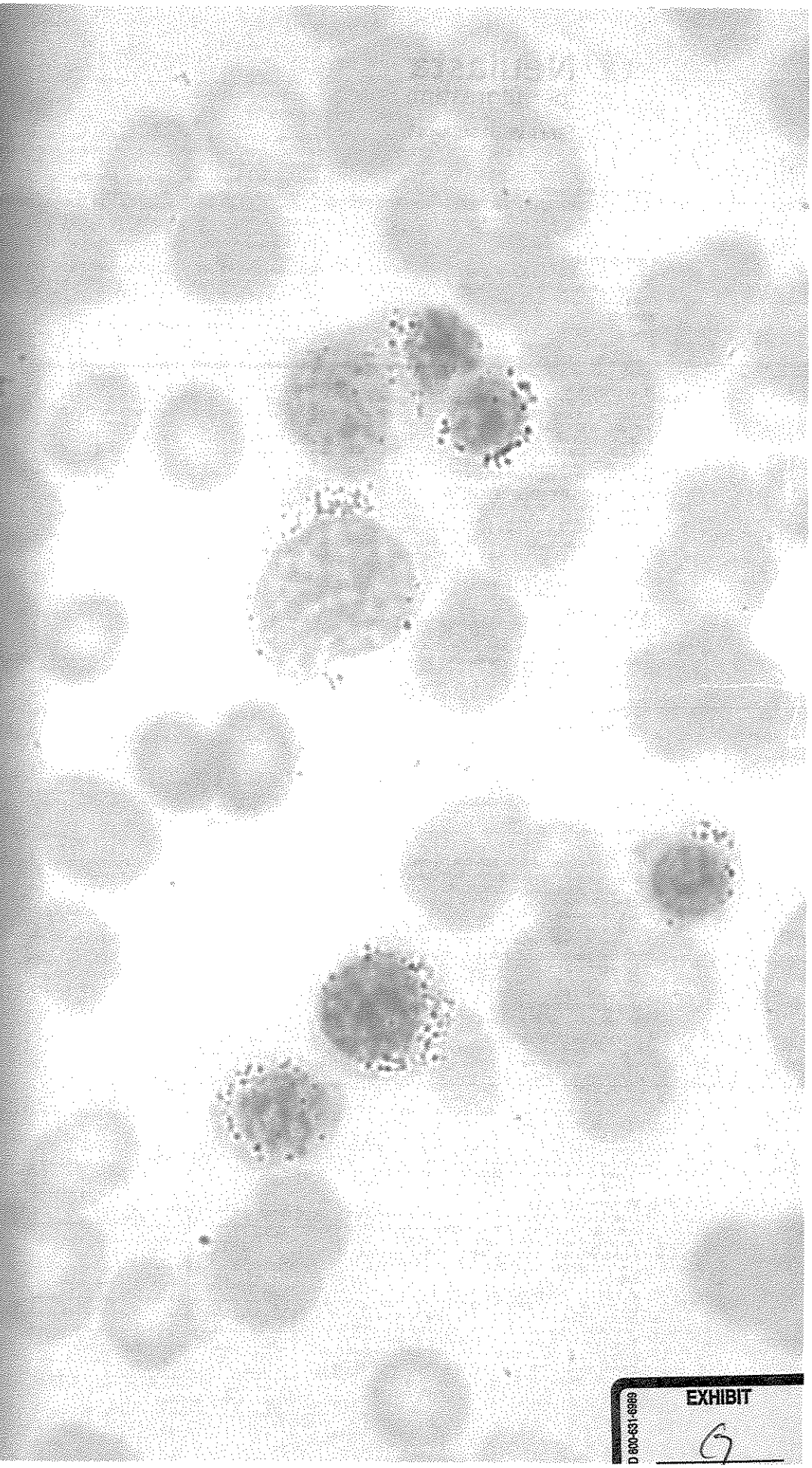

Neulasta®
(pegfilgrastim)
Start with support

BRIEF SUMMARY OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

Neulasta® is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with nonmyeloid malignancies receiving myelosuppressive anticancer drugs associated with a clinically significant incidence of febrile neutropenia.

CONTRAINDICATIONS

Neulasta® is contraindicated in patients with known hypersensitivity to *E coli*-derived proteins, pegfilgrastim, Filgrastim, or any other component of the product.

WARNINGS

General

The safety and efficacy of Neulasta® for peripheral blood progenitor cell (PBPC) mobilization has not been evaluated in adequate and well-controlled studies. Neulasta® should not be used for PBPC mobilization.

Splenic Rupture

RARE CASES OF SPLENIC RUPTURE HAVE BEEN REPORTED FOLLOWING THE ADMINISTRATION OF NEULASTA®. SPLENIC RUPTURE, IN SOME CASES RESULTING IN DEATH, HAS ALSO BEEN ASSOCIATED WITH FILGRASTIM, THE PARENT COMPOUND OF NEULASTA®. PATIENTS RECEIVING NEULASTA® WHO REPORT LEFT UPPER ABDOMINAL AND/OR SHOULDER TIP PAIN SHOULD BE EVALUATED FOR AN ENLARGED SPLEEN OR SPLENIC RUPTURE.

Adult Respiratory Distress Syndrome (ARDS)

Adult respiratory distress syndrome (ARDS) has been reported in neutropenic patients with sepsis receiving Filgrastim, the parent compound of Neulasta®, and is postulated to be secondary to an influx of neutrophils to sites of inflammation in the lungs. Neutropenic patients receiving Neulasta® who develop fever, lung infiltrates, or respiratory distress should be evaluated for the possibility of ARDS. In the event that ARDS occurs, Neulasta® should be discontinued and/or withheld until resolution of ARDS and patients should receive appropriate medical management for this condition.

Allergic Reactions

Allergic reactions to Neulasta®, including anaphylaxis, skin rash, and urticaria, have been reported in post marketing experience. The majority of reported events occurred upon initial exposure. In some cases, symptoms recurred with rechallenge, suggesting a causal relationship. In rare cases, allergic reactions including anaphylaxis, recurred within days after initial anti-allergic treatment was discontinued. If a serious allergic reaction occurs, appropriate therapy should be administered, with close patient follow-up over several days. Neulasta® should be permanently discontinued in patients with serious allergic reactions.

Sickle Cell Disease

Severe sickle cell crises have been associated with the use of Neulasta® in patients with sickle cell disease. Severe sickle cell crises, in some cases resulting in death, have also been associated with Filgrastim, the parent compound of pegfilgrastim. Only physicians qualified by specialized training or experience in the treatment of patients with sickle cell disease should prescribe Neulasta® for such patients, and only after careful consideration of the potential risks and benefits.

PRECAUTIONS

General

Use With Chemotherapy and/or Radiation Therapy

Neulasta® should not be administered in the period between 14 days before and 24 hours after administration of cytotoxic chemotherapy (see **DOSAGE AND ADMINISTRATION**) because of the potential for an increase in sensitivity of rapidly dividing myeloid cells to cytotoxic chemotherapy.

The use of Neulasta® has not been studied in patients receiving chemotherapy associated with delayed myelosuppression (eg, nitrosoureas, mitomycin C).

The administration of Neulasta® concomitantly with 5-fluorouracil or other antimetabolites has not been evaluated in patients. Administration of pegfilgrastim at 0, 1, and 3 days before 5-fluorouracil resulted in increased mortality in mice; administration of pegfilgrastim 24 hours after 5-fluorouracil did not adversely affect survival.

The use of Neulasta® has not been studied in patients receiving radiation therapy.

Potential Effect on Malignant Cells

Pegfilgrastim is a growth factor that primarily stimulates neutrophils and neutrophil precursors; however, the G-CSF receptor through which pegfilgrastim and Filgrastim act has been found on tumor cell lines, including some myeloid, T-lymphoid, lung, head and neck, and bladder tumor cell lines. The possibility that pegfilgrastim can act as a growth factor for any tumor type cannot be excluded. Use of Neulasta® in myeloid malignancies and myelodysplasia (MDS) has not been studied. In a randomized study comparing the effects of the parent compound of Neulasta®, Filgrastim, to placebo in patients undergoing remission induction and consolidation chemotherapy for acute myeloid leukemia, important differences in remission rate between the two arms were excluded. Disease-free survival and overall survival were comparable; however, the study was not designed to detect important differences in these endpoints.[1]

Information for Patients

Patients should be informed of the possible side effects of Neulasta®, and be instructed to report them to the prescribing physician. Patients should be informed of the signs and symptoms of allergic drug reactions and be advised of appropriate actions. Patients should be counseled on the importance of compliance with their Neulasta® treatment, including regular monitoring of blood counts.

If it is determined that a patient or caregiver can safely and effectively administer Neulasta® (pegfilgrastim) at home, appropriate instruction on the proper use of Neulasta® (pegfilgrastim) should be provided for patients and their caregivers, including careful review of the "Information for Patients and Caregivers" insert. Patients and caregivers should be cautioned against the reuse of needles, syringes, or drug product, and be thoroughly instructed in their proper disposal. A puncture-resistant container for the disposal of used syringes and needles should be available.

Laboratory Monitoring

To assess a patient's hematologic status and ability to tolerate myelosuppressive chemotherapy, a complete blood count and platelet count should be obtained before chemotherapy is administered. Regular monitoring of hematocrit value and platelet count is recommended.

Drug Interaction

No formal drug interaction studies between Neulasta® and other drugs have been performed. Drugs such as lithium may potentiate the release of neutrophils; patients receiving lithium and Neulasta® should have more frequent monitoring of neutrophil counts.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No mutagenesis studies were conducted with pegfilgrastim. The carcinogenic potential of pegfilgrastim has not been evaluated in long-term animal studies. In a toxicity study of 6 months duration in rats given once weekly subcutaneous injections of up to 1000 mcg/kg of pegfilgrastim (approximately 23-fold higher than the recommended human dose), no precancerous or cancerous lesions were noted.

When administered once weekly via subcutaneous injections to male and female rats at doses up to 1000 mcg/kg prior to, and during mating, reproductive performance, fertility, and sperm assessment parameters were not affected.

Pregnancy Category C

Pegfilgrastim has been shown to have adverse effects in pregnant rabbits when administered subcutaneously every other day during gestation at doses as low as 50 mcg/kg/dose (approximately 4-fold higher than the recommended human dose). Decreased maternal food consumption, accompanied by a decreased maternal body weight gain and decreased fetal body weights were observed at 50 to 1000 mcg/kg/dose. Pegfilgrastim doses of 200 and 250 mcg/kg/dose resulted in an increased incidence of abortions. Increased post-implantation loss due to early resorptions was observed at doses of 200 to 1000 mcg/kg/dose, and decreased numbers of live rabbit fetuses were observed at pegfilgrastim doses of 200 to 1000 mcg/kg/dose, given every other day.

Subcutaneous injections of pegfilgrastim of up to 1000 mcg/kg/dose every other day during the period of organogenesis in rats were not associated with an embryotoxic or fetotoxic outcome. However, an increased incidence (compared to historical controls) of wavy ribs was observed in rat fetuses at 1000 mcg/kg/dose every other day. Very low levels (< 0.5%) of pegfilgrastim crossed the placenta when administered subcutaneously to pregnant rats every other day during gestation.

Once weekly subcutaneous injections of pegfilgrastim to female rats from day 6 of gestation through day 18 of lactation at doses up to 1000 mcg/kg/dose did not result in any adverse maternal effects. There were no deleterious effects on the growth and development of the offspring and no adverse effects were found upon assessment of fertility indices.

There are no adequate and well-controlled studies in pregnant women. Neulasta® should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether pegfilgrastim is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Neulasta® is administered to a nursing woman.

Pediatric Use

The safety and effectiveness of Neulasta® in pediatric patients have not been established. The 6 mg fixed dose single-use syringe formulation should not be used in infants, children, and smaller adolescents weighing less than 45 kg.

Geriatric Use

Of the 932 patients with cancer who received Neulasta® in clinical studies, 139 (15%) were age 65 and over, and 18 (2%) were age 75 and over. No overall differences in safety or effectiveness were observed between patients age 65 and older and younger patients.

ADVERSE REACTIONS

See WARNINGS sections regarding Splenic Rupture, ARDS, Allergic Reactions, and Sickle Cell Disease.

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of Neulasta® cannot be directly compared to rates in the clinical trials of other drugs and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to Neulasta® use and for approximating rates.

The data described below reflect exposure to Neulasta® in 932 patients. Neulasta® was studied in placebo- and active-controlled trials (n = 467, and n = 465, respectively). The population encompassed an age range of 21 to 88 years. Ninety-two percent of patients were female. The ethnicity of the patients was as follows: 75% Caucasian, 18% Hispanic, 5% Black, and 1% Asian. Patients with solid tumors (breast [n = 823], lung and thoracic tumors [n = 53]) or lymphoma (n = 56) received Neulasta® after nonmyeloablative cytotoxic chemotherapy. Most patients received a single 100 mcg/kg (n = 259) or a single 6 mg (n = 546) dose per chemotherapy cycle over 4 cycles.

In the placebo-controlled trial, bone pain occurred at a higher incidence in Neulasta®-treated patients as compared to placebo-treated patients. The incidence of other commonly reported adverse events were similar in the Neulasta®- and placebo-treated patients, and were consistent with the underlying cancer diagnosis and its treatment with chemotherapy. The data in Table 1 reflect those adverse events occurring in at least 10% of patients treated with Neulasta® in the placebo-controlled study.

TABLE 1. Adverse Events Occurring in ≥ 10%* of Patients in The Placebo-Controlled Study.

Event	Neulasta® (n = 467)	Placebo (n = 461)
Alopecia	48%	47%
Bone Pain†	31%	26%
Diarrhea	29%	28%
Pyrexia (not including febrile neutropenia)	23%	22%
Myalgia	21%	18%
Headache	16%	14%
Arthralgia	16%	13%
Vomiting	13%	11%
Asthenia	13%	11%
Edema peripheral	12%	10%
Constipation	10%	6%

*Events occurring in ≥ 10% of Neulasta®-treated patients and at a higher incidence as compared to placebo-treated patients.

†Bone pain is limited to the specified adverse event term, "bone pain."

In the active-controlled studies, common adverse events occurred at similar rates and severities in both treatment arms (Neulasta®, n = 465; Filgrastim, n = 331). These adverse experiences occurred at rates between 72% and 15% and included: nausea, fatigue, alopecia, diarrhea, vomiting, constipation, fever, anorexia, skeletal pain, headache, taste perversion, dyspepsia, myalgia, insomnia, abdominal pain, arthralgia, generalized weakness, peripheral edema, dizziness, granulocytopenia, stomatitis, mucositis, and neutropenic fever.

Bone Pain

The analysis of bone pain described below is based on a composite analysis using multiple, related, adverse event terms.

In the placebo-controlled study, the incidence of bone pain was 57% in Neulasta®-treated patients compared to 50% in placebo-treated patients. Bone pain was generally reported to be of mild-to-moderate severity.

Among patients experiencing bone pain, approximately 37% of Neulasta®- and 31% of placebo-treated patients utilized non-narcotic analgesics and 10% of Neulasta®- and 9% of placebo-treated patients utilized narcotic analgesics.

In the active-controlled studies, the use of non-narcotic and narcotic analgesics in association with bone pain was similar between Neulasta®- and Filgrastim-treated patients. No patient withdrew from study due to bone pain.

Laboratory Abnormalities

In clinical studies, leukocytosis (WBC counts > 100 x 10^9/L) was observed in less than 1% of 932 patients with nonmyeloid malignancies receiving Neulasta®. Leukocytosis was not associated with any adverse effects.

In the placebo-controlled study, reversible elevations in LDH, alkaline phosphatase, and uric acid that did not require treatment occurred at similar rates in Neulasta®- and placebo-treated patients.

Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients receiving Neulasta® has not been adequately determined. While available data suggest that a small proportion of patients developed binding antibodies to Filgrastim or pegfilgrastim, the nature and specificity of these antibodies has not been adequately studied. No neutralizing antibodies have been detected using a cell-based bioassay in 45 patients who apparently developed binding antibodies. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay, and the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, concomitant medications, and underlying disease. Therefore, comparison of the incidence of antibodies to Neulasta® with the incidence of antibodies to other products may be misleading.

Cytopenias resulting from an antibody response to exogenous growth factors have been reported on rare occasions in patients treated with other recombinant growth factors. There is a theoretical possibility that an antibody directed against pegfilgrastim may cross-react with endogenous G-CSF, resulting in immune-mediated neutropenia, but this has not been observed in clinical studies.

OVERDOSAGE

The maximum amount of Neulasta® that can be safely administered in single or multiple doses has not been determined. Single subcutaneous doses of 300 mcg/kg have been administered to 8 healthy volunteers and 3 patients with non-small cell lung cancer without serious adverse effects. These patients experienced a mean maximum ANC of 55 x 10^9/L, with a corresponding mean maximum WBC of 67 x 10^9/L. The absolute maximum ANC observed was 96 x 10^9/L with a corresponding absolute maximum WBC observed of 120 x 10^9/L. The duration of leukocytosis ranged from 6 to 13 days. Leukapheresis should be considered in the management of symptomatic individuals.

DOSAGE AND ADMINISTRATION

The recommended dosage of Neulasta® is a single subcutaneous injection of 6 mg administered once per chemotherapy cycle. Neulasta® should not be administered in the period between 14 days before and 24 hours after administration of cytotoxic chemotherapy (see **PRECAUTIONS**).

The 6 mg fixed-dose formulation should not be used in infants, children, and smaller adolescents weighing less than 45 kg.

No dosing adjustment is necessary for renal dysfunction (see **CLINICAL PHARMACOLOGY, Special Populations**).

Rx Only

This product, its production, and/or its use may be covered by one or more US Patents, including US Patent Nos. 5,824,784; 4,810,643; 4,999,291; 5,582,823; 5,580,755 as well as other patents or patents pending.

Reference

*1Heil G, Hoelzer D, Sanz MA, et al. A randomized, double-blind, placebo-controlled, phase III study of Filgrastim in remission induction and consolidation therapy for adults with de novo Acute Myeloid Leukemia. *Blood.* 1997;90:4710-4718.

Issue Date: 09/15/2005

References: 1. Neulasta® (pegfilgrastim) Prescribing Information. Thousand Oaks, Calif: Amgen. **2.** Vogel CL, Wojtukiewicz MZ, Carroll RR, et al. First and subsequent cycle use of pegfilgrastim prevents febrile neutropenia in patients with breast cancer: a multicenter, double-blind, placebo-controlled phase III study. *J Clin Oncol.* 2005;23:1178-1184.

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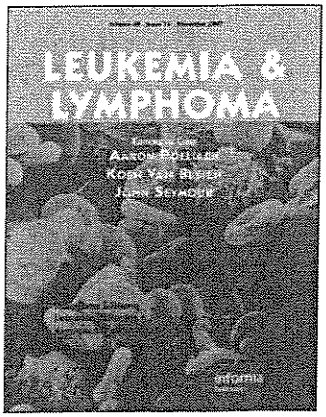

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Hematopoietic Malignancies and Related Disorders Among Benzene-Exposed Workers in China

Lois B. Travis [a]; Chin-Yang Li [b]; Zhi-Nan Zhang [c]; De-Gao Li [c]; Song-Nian Yin [d]; Wong-Ho Chow [a]; Gui-Lan Li [d]; Mustafa Dosemeci [a]; William Blot [a]; Joseph F. Fraumeni Jr. [a]; Richard B. Hayes [a]; Martha S. Linet [a]
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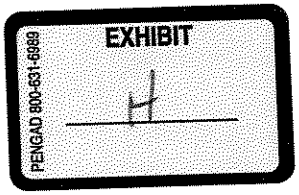

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Hematopoietic Malignancies and Related Disorders Among Benzene-Exposed Workers in China

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Although the relationship between benzene and acute nonlymphocytic leukemia (ANLL) is well established, most of the analytic cohort investigations examining the relationship between benzene and hematologic neoplasms have evaluated only death certificates to validate diagnoses. In a follow-up study of 74,828 benzene-exposed and 35,805 non-exposed workers in China, pathology reports, medical records, and/or histopathologic material were reviewed for all patients with hematopoietic malignancies to ensure correct classification and to provide clinicopathologic descriptions. Eighty-two patients with hematopoietic neoplasms and related disorders were identified among benzene-exposed workers, including 32 cases of acute leukemia, 7—myelodysplastic syndrome (MDS), 9—chronic granulocytic leukemia (CGL), 20—malignant lymphoma or related disorder (ML), 9—aplastic anemia, and 5 others. Among the comparison group, 13 hematologic malignancies were observed, including 6 patients with acute leukemia, 2—CGL, 3—ML, and 2 others. The hematopathologic characteristics of the benzene-exposed ANLL cases resembled those following chemotherapy or radiotherapy. ANLL in workers exposed to benzene may represent a distinct clinicopathologic entity, with characteristics similar to treatment-related ANLL, including a preceding preleukemic phase in some patients. Results in our series, one of the largest to date, also indicate that a greater diversity of hematologic neoplasms is evident among benzene-exposed workers than previously described.

KEY WORDS: Benzene leukemia dysplasia

INTRODUCTION

The International Agency for Research on Cancer (IARC) concluded in the early 1980s that there was sufficient evidence that benzene could cause acute myelogenous leukemia (AML).[1] At that time, the major studies implicating benzene in the occurrence of AML were two US cohort mortality investigations,[2,3] a clinical series drawn from Turkish shoe and leather manufacturing workers,[4,5] and case-control studies in France, Sweden and the US.[6–9] Worldwide sources of exposure to benzene include its widespread use in industry and its presence in cigarette smoke, gasoline, and automobile exhaust,[1] thus potentially exposing a large population to its carcinogenic risks. Since the IARC report, investigators have also raised concerns about possible associations of benzene with non-

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Hodgkin's lymphoma,[10] multiple myeloma,[11] and other hematopoietic malignancies.[12] Clarification of the role of benzene exposure in the etiology of various hematopoietic malignancies requires accurate classification. However, most of the investigations examining the relationship between benzene and hematologic neoplasms have evaluated only death certificates, with very few studies reviewing medical record or histologic data to provide detailed clinicopathologic descriptions and ensure correct categorization.[4,6,13,14] Thus, a major objective of a recent epidemiologic study among a large number of benzene-exposed (and non-exposed) workers in China was to obtain medical record data and histopathologic specimens for patients with hematopoietic malignancies to enable classification according to recently published criteria and to identify unusual clinical and pathologic features.

SUBJECTS AND METHODS

A retrospective cohort study of incident cases of hematopoietic neoplasms and related disorders was conducted among 74,828 benzene-exposed workers employed between January 1, 1972 and December 31, 1987 in 672 factories in 12 Chinese cities (Shanghai, Tianjin, Chengdu, Chongqing, Harbin, Shenyang, Jinzhou, Zhengzhou, Luoyang, Guangzhou, Nanchang and Kaifeng).[15] A concurrent comparison group consisted of 35,805 workers not occupationally exposed to benzene who were employed during the same period in 109 factories in the 12 cities. Careful review of factory records identified eligible exposed and unexposed persons. Data abstracted from these files included name, job title and information describing the types of work units and dates employed.

Since the factories usually provide all health care for current and retired workers, follow-up of benzene-exposed and unexposed subjects through December 31, 1987 was carried out utilizing factory occupational and medical records to ascertain hematopoietic malignancies and related disorders, vital status, and, for those deceased, cause of death. Additional means of follow-up were utilized for a small number of subjects who had left the factories prior to retirement. For workers reported to have developed hematologic neoplasms and related disorders, pertinent histopathologic material, pathology reports and medical records were requested for confirmation. Clinical, laboratory and pathology data for all patients were abstracted onto standardized forms by physician investigators who were not aware of the exposure status of the subjects, nor of the numbers of exposed and non-exposed cases. All slides were reviewed systematically using structured, detailed abstract forms to objectively characterize hematopoiesis. Evaluation of the peripheral blood smear included review of polymorphonuclear (PMN) leukocytes for hypersegmentation, hyposegmentation (the pseudo-Pelger-Huet anomaly), hypogranularity, abnormal granules and the presence of vacuolization. Platelet size and granularity and red blood cell shape, size and general morphology were also noted. Eosinophilia was defined as an absolute count of more than 450 cells/uL; basophilia was defined as an absolute count of more than 200 cells/uL.[16]

Hematologic parameters recorded for bone marrow aspirates included the following: the estimated erythrocyte:granulocyte ratio, per cent blasts and morphologic changes. Evidence of dyserythropoiesis included the presence of ringed sideroblasts, multinuclearity, karyorrhexis, nuclear fragmentation, abnormal nuclear shape, impaired hemoglobinization, megaloblastoid changes or other nuclear or cytoplasmic abnormalities. Dysgranulopoiesis was characterized as for the peripheral blood smear. Dysmegakaryopoietic features included the presence of micromegakaryotes, large mononuclear forms, multiple small nuclei, or giant or abnormal granules. The upper limit of the normal range for marrow basophils was defined as 0.2 per cent of total nucleated cells (TNC), and for eosinophils, 6.2 per cent of TNC.[17]

Diagnoses were assigned after evaluation of all available clinical, laboratory and pathologic data without knowledge of the patient's benzene exposure status. Published criteria were utilized to classify myelodysplastic syndrome (MDS),[18] acute nonlymphocytic leukemia (ANLL),[19-21] acute lymphoblastic leukemia (ALL),[19] and non-Hodgkin's lymphoma (NHL).[22] Since study objectives did not include quality control of the previously assigned Chinese diagnoses, which derived from a variety of sources, a systematic comparison of these diagnoses with the results of the current review was not undertaken.

RESULTS

Eighty-two hematopoietic malignancies and related disorders were documented among benzene-exposed workers, with 13 such cases identified among the non-exposed comparison group. Frequencies of the major diagnostic categories of hematologic neoplasms in

benzene-exposed and non-exposed subjects are summarized in Table 1. Diagnoses confirmed by review of pathology reports and medical records for 51 benzene-exposed subjects included acute leukemia (17 patients [pts]), MDS (2 pts), chronic granulocytic leukemia (CGL) (5 pts), malignant lymphoma or related malignancy (ML) (16 pts), aplastic anemia (8 pts) and 3 other disorders. For an additional 31 benzene-exposed subjects, diagnoses based on evaluation of histopathologic material included 9 cases of ANLL; 5 acute leukemias which could not be classified further; 5 MDS; 4 CGL; 4 ML; 1 ALL; and 1 aplastic anemia (Tables 2–5). Findings in an additional patient were considered compatible with malignant histiocytosis and those in another subject were thought consistent with some type of toxic bone marrow effect.

Diagnoses confirmed by review of histopathologic material for the non-exposed comparison group included ANLL-M2 (1 pt), multiple myeloma (1 pt) and lymphoproliferative disorder (1 pt). Evaluation of medical records and pathology reports for 10 additional patients indicated diagnoses of acute leukemia (5 pts), CGL (2 pt), NHL (2 pt) and 1 leukemia, not otherwise specified. Diagnoses observed among only benzene-exposed cases included aplastic anemia and MDS (Table 1).

Preliminary analyses indicate that the age- and sex-adjusted relative risk of all confirmed lymphohematopoietic disorders in exposed versus unexposed workers is 3.4 (95% confidence interval: 1.9–6.1). The results of future analyses (which will evaluate risk by industry, occupational category, level of benzene exposure and type of hematologic malignancy) will be reported separately. Available clinicopathologic data for 31 of the benzene-exposed cases are presented below by diagnostic subgroup; data for patients 32–34, who were not exposed to benzene, are summarized in Tables 2–4.

Table 1 Frequency of major diagnostic groups of all hematopoietic malignancies and related disorders among benzene-exposed and non-exposed workers

Diagnosis	*Number of cases (%)**	
	Exposed workers	*Non-exposed workers*
Aplastic anemia	9 (11%)	0
Acute leukemia	32 (39%)	6 (46%)
Myelodysplastic syndrome	7 (9%)	0
Chronic granulocytic leukemia	9 (11%)	2 (15%)
Malignant lymphoma and related disorders	20 (24%)	3 (23%)
Other	5† (6%)	2‡ (15%)
Total	82	13

*Numbers may not sum to 100 due to rounding error.
†Includes diagnoses of agranulocytosis (2), multiple myeloma (1), leukemia, not otherwise specified (NOS) (1), and toxic effect (1).
‡Includes diagnoses of multiple myeloma (1) and leukemia, NOS (1).

ANLL Histopathologic data were evaluated for 5 men and 4 women with ANLL (Table 2); patients typically presented with anemia and thrombocytopenia, with white blood cell counts ranging from 1,200 to 16,800 per uL (Table 3). Dyspoiesis was common (Tables 4 and 5). Three cases of ANLL-M2 and four of ANLL-M3 were included in the histopathologic review. In two patients (numbers 4 and 9), subtyping of ANLL was not possible. The leukemic bone marrow of Patient 4 was thought consistent with either ANLL-M2 or ANLL-M4. Review of bone marrow for Patient 9 revealed peroxidase-positive blasts, which demonstrated prominent nucleoli and a moderate amount of cytoplasm, some with granules. This patient was thought to have either a myelomonocytic or monocytic leukemia. An unusual increase in small, atypical basophils or mast cells was also noted in this patient, who had a prior diagnosis of chronic benzene poisoning.

Acute leukemia, not otherwise specified (AL, NOS) In five persons with acute leukemia, the cell type could not be established after review of histopathology, without the availability of additional cytochemical stains. Data available for two of these cases suggested blastic transformations of antecedent myeloproliferative disorders: Patient 10 presented with hepatosplenomegaly; basophilia and large platelets were among the features noted on the peripheral blood smear. Bone marrow examination revealed approximately 35 per cent blasts and right-shifted granulopoiesis with numerous PMNs, many of which were hypogranular. The peripheral blood smear for Patient 11 showed dacrocytes, basophilia and large platelets, many in aggregates, features consistent with the prior diagnosis of CGL noted in the medical record. Dysplasia was evident in peripheral blood and bone marrow, with undifferentiated blasts comprising approximately 30–35% of marrow cellularity. Other characteristics supporting the underlying diagnosis of CGL included bone marrow basophilia and available laboratory values (Table 3) which included a low leukocyte alkaline phosphatase (LAP) score.

More than 90% of marrow cellularity in Patient 12 consisted of blasts, which did not stain with peroxidase according to an accompanying report. The sig-

Table 2 Clinical presentation of 34 patients with hematopoietic malignancies and related disorders*

Patient no.	*Age/Sex (yr)*	*Symptoms/Signs*	*Physical examination*		*LN*	*Diagnosis*
			HM	*SM*		
1	40/M	lower limb ulceration, ecchymoses, petechiae	−	−	+	ANLL-M2
2	45/F	vertigo, weakness, palpitations	−	−	−	ANLL-M2
3	24/M	fever	−	−	−	ANLL-M2
4	25/M	fever, malaise	−	−	−	ANLL-M2 or M4
5	43/M	sternal tenderness, gingival infiltration	−	−	+	ANLL-M3, microgranular
6	47/F	petechiae, sternal tenderness, gingival bleeding, ecchymoses	−	−	−	ANLL-M3
7	23/M	N/A	N/A	N/A	N/A	ANLL-M3
8	43/F	petechiae, ecchymoses	−	−	−	ANLL-M3
9†	53/F	fever, malaise, vertigo, pallor, dyspnea, mucosal ulceration	−	−	−	ANLL-M4 or M5
10	70/M	bronchitis	+	+	−	AL, NOS
11	64/M	N/A	N/A	N/A	N/A	AL, NOS
12	42/M	sternal tenderness, ecchymoses	+	−	+	AL, NOS
13	42/M	N/A	−	−	−	AL, NOS
14	42/M	fever	+	−	+	AL, NOS
15	53/M	N/A	−	−	−	RA
16†	48/F	lower limb ulceration	−	N/A	+	CMML
17	30/F	N/A	−	−	−	RAEBT
18	41/F	N/A	N/A	N/A	N/A	MDS, NOS
19†	40/M	fever, joint pain	+	−	−	MDS, NOS
20	28/F	petechiae	+	+	+	CGL
21	62/M	abdominal mass, malaise, weight loss	+	+	−	CGL
22	30/M	N/A	−	−	−	CGL, atypical
23	N/A	N/A	N/A	N/A	N/A	CGL
24	20/M	fever, malaise vertigo	+	+	−	NHL (possible)
25	41/F	malnutrition, pallor	−	−	+	NHL
26	40/M	fever	−	−	+	NHL
27	34/M	fever	+	+	+	NHL
28	23/M	sternal tenderness, gingival pain	+	+	+	ALL
29	45/M	gingival bleeding, petechiae	+	N/A	N/A	Aplastic Anemia
30	58/M	fever, malaise	−	−	+	Malignant Histiocytosis (possible)
31‡	33/M	weakness, vertigo	+	+	−	Toxic effect
32	49/M	epistaxis, malaise, weight loss	−	−	+	ANLL-M2
33	52/M	chest wall mass	−	−	−	Multiple Myeloma
34	54/M	N/A	N/A	N/A	N/A	LPD

Abbreviations: AL: acute leukemia; ALL: acute lymphoblastic leukemia; ANLL: acute non-lymphocytic leukemia; CGL: chronic granulocytic leukemia; CMML: chronic myelomonocytic leukemia; HM: hepatomegaly; LN: lymphadenopathy; LPD: lymphoproliferative disorder; MDS: myelodysplastic syndrome; N/A: not ascertained; NHL: non-Hodgkin's lymphoma; NOS: not otherwise specified; RA: refractory anemia; RAEBT: refractory anemia with excess blasts in transformation; SM: splenomegaly; +: present; −: absent.

*This table is limited to those patients for whom histopathologic materials were reviewed (Tables 3–5). Patients 1–31 were occupationally exposed to benzene.

†Patient had been previously diagnosed with chronic benzene poisoning.

‡Available data preceded the reviewed bone marrow aspirate by 10 years.

nificant variation in blast size together with the presence of cytoplasmic granules and budding seemed suggestive of a megakaryocytic lineage. However, in the absence of immunohistochemical stains to evaluate a possible diagnosis of acute megakaryocytic leukemia (ANLL-M7),[21] this patient was also considered to have AL, NOS.

An unusual feature of increased lymphocytes with large cytoplasmic granules was present in both the peripheral blood smear and marrow aspirate of Patient 13. Otherwise, findings in this patient were consistent with AL, NOS (Table 4).

MDS Myelodysplastic syndromes were evident in five subjects based upon review of histopathologic material. Findings in Patient 15 included bone marrow hypocellularity, with markedly dyserythropoietic features, including multinuclearity, megaloblastoid changes, and abnormal nuclear shape; the general

Table 3 Laboratory data and peripheral blood morphologic findings in patients* with hematopoietic malignancies and related disorders†

Patient no.	*Hemoglobin (g/dl)*	*WBC ($\times 10^3$/uL)*	*% blasts*	*Platelets ($\times 10^3$/uL)*	*Peripheral blood morphologic findings*	*Diagnosis*
1	8.6	16.8	20	N/A	pseudo-Pelger-Huet PMNS, Auer rods, thrombocytopenia	ANLL-M2
2	4.4	4.4	0	105	N/A	ANLL-M2
4	3.0	13.7	0	N/A	anisocytosis, poikilocytosis, pseudo Pelger-Huet PMNs, pancytopenia, type II blasts	ANLL-M2 or M4
5	7.0	1.6	20	15	thrombocytopenia, hypogranular promyelocytes or monocytic cells	ANLL-M3, microgranular
6	8.8	1.2	10	66	N/A	ANLL-M3
8	5.2	3.1	0	40	N/A	ANLL-M3
9‡	4.6	3.4	38	10	N/A	ANLL-M4 or M5
10	8.0	15.2	23	56	polychromasia, NRBCs, hypersegmented, hyposegmented and hypogranular PMNs, large platelets, basophilia	AL, NOS
11§	N/A	84.6¶	4	350	dacrocytes, left-shifted granulopoiesis, large platelets, basophilia	AL, NOS
12	5.9	2.9	50	10	N/A	AL, NOS
13	14.7	2.3	0	86	oval macrocytes, neutropenia, thrombocytopenia, immature lymphoid cells with granules	AL, NOS
14	8.0	17.5	0	0.7	N/A	AL, NOS
15	4.0	1.8	0	16	N/A	RA
16‡	6.5	36.2	2	17	oval macrocytes, target cells, megaloblastic and megaloblastoid NRBCs, hypogranular PMNs, giant bands, basophilia, thrombocytopenia	CMML
17	8.1	4.6	N/A	36	N/A	RAEBT
19‡	10.0	4.7	0	50	N/A	MDS, NOS
20‖	7.0	198.0**	2	134	hypogranular PMNs and myelocytes, rodent nuclei, basophilia, eosinophilia	CGL
21	10.0	121.6	3	242	oval macrocytes, target cells, NRBCs, basophilia	CGL
22	7.0	32.8††	0	300	left-shifted granulopoiesis, no basophilia nor eosinophilia	CGL, atypical
23	N/A	N/A	N/A	N/A	left-shifted granulopoiesis with mild basophilia	CGL
24	8.4	2.6	0	37	N/A	NHL (possible)
25	8.5	16.8	0	N/A	N/A	NHL
26	11.0	8.3	0	210	N/A	NHL
27‡‡	7.0	5.5	0	N/A	N/A	NHL
28	9.5	23.0	96	51	oval macrocytes, thrombocytopenia	ALL
29	4.0	2.2	0	26	N/A	Aplastic Anemia
30	11.5	4.4	0	100	pancytopenia, large atypical monocytoid cells present	Malignant Histiocytosis (possible)
31§§	8.0	220.0¶¶	0	406	N/A	Toxic effect
32	6.0	4.9	64	N/A	anisocytosis, poikilocytosis, numerous Auer rods	ANLL-M2
33	5.5	2.9	0	76	rouleaux	Multiple Myeloma

Abbreviations: AL: acute leukemia; ALL: acute lymphoblastic leukemia; ANLL: acute non-lymphocytic leukemia; CGL: chronic granulocytic leukemia; CMML: chronic myelomonocytic leukemia; MDS: myelodysplastic syndrome; N/A: not available; NHL: non-Hodgkin's lymphoma; NOS: not otherwise specified; NRBC: nucleated red blood cells; PMN: polymorphonuclear leukocyte; RA: refractory anemia; RAEBT: refractory anemia with excess blasts in transformation; WBC: white blood cell.

*Neither laboratory data nor peripheral blood smears were available for patient nos. 3, 7, 18, and 34.

†Patients 1–31 were occupationally exposed to benzene.

‡Patient had been previously diagnosed with chronic benzene poisoning.

§Laboratory values preceded the reviewed peripheral blood and bone marrow specimens by 3 years.

¶WBC differential: 22% PMN, 33% bands, 8% metamyelocytes, 6% myelocytes, 5% promyelocytes, 4% blasts, 7% basophils, 4% eosinophils, 11% lymphocytes.

‖Patient was Philadelphia-chromosome positive.

**WBC differential: 27% PMN, 18% bands, 13% metamyelocytes, 25% myelocytes, 5% promyelocytes, 2% blasts, 4% eosinophils, 4% basophils, 2% lymphocytes.

††WBC differential: 49% PMN, 11% bands, 7.5% metamyelocytes, 20.5% myelocytes, 5% promyelocytes, 5% lymphocytes, 2% other.

‡‡Laboratory values were obtained approximately 1 month after lymph node biopsy.

§§Laboratory values preceded the reviewed bone marrow aspirate by 10 years.

¶¶WBC differential: 40% PMNs, 20% bands, 15% metamyelocytes, 25% promyelocytes.

Table 4 Bone marrow features in patients* with hematopoietic malignancies and related disorders††

Patient no.	*E:G*	*Cellularity/Dyspoiesis*			*% blasts*	*Comments*	*Diagnosis*
		Erythrocytes	*Granulocytes*	*Megakaryocytes*			
1	1:50	Decr/+	Incr/−	Decr/−	60	peroxidase stain: positive	ANLL-M2
2	1:30	Decr/+	Incr/−	Decr/†	30–35	—	ANLL-M2
3	1:10	Decr/−	Incr/−	Decr/†	40	—	ANLL-M2
4	1:100	Decr/†	Incr/−	Decr/†	40	numerous immature cells, either monocytes or granulocytes	ANLL-M2 or M4
5	1:20	NI/−	Incr/−	Decr/†	60	—	ANLL-M3, microgranular
6	1:20	Decr/+	Incr/−	Decr/†	—	hypergranular promyelocytes: 90%	ANLL-M3
7	1:15	Decr/+	Incr/−	Decr/†	—	hypergranular promyelocytes: 90%	ANLL-M3
8	50:1	Decr/+	Incr/+	Decr/−	—	hypergranular and hypogranular promyelocytes: 90%	ANLL-M3
9‡	1:100	Decr/+	Incr/+	Decr/†	80	increase in basophils or mast cells (5%), which are dysplastic; increase in monocytes; peroxidase stain: positive	ANLL-M4 or M5
10	1:8	Decr/+	Incr/+	Decr/†	35	preceding MPD	AL, NOS
11	1:100	Decr/+	Incr/−	Decr/+	30–35	undifferentiated blasts; preceding CGL	AL, NOS
12	§	§	Decr/−	Decr/−	90	blasts demonstrate cytoplasmic budding and granules	AL, NOS
13	1:1	N/A	N/A	N/A	see comment	immature lymphoid-like cells (some with granules): 90%	AL, NOS
14	1:50	Decr/−	Incr/−	Decr/†	90	—	AL, NOS
15	3:1	Incr/+	Decr/−	Decr/†	<5	significant dyserythropoiesis; marked granulocytic hypoplasia	Refractory Anemia
16‡	1:6	NI/+	Incr/+	Decr/+	5–10	incr. basophils: 1–2%. incr. monocytes: 20–25%	CMML
17	1:3	Incr‖/+	Incr/+	Decr/†	5–10	Auer rods noted	RAEBT
18	5:2	Incr¶/+	Decr/+	Decr/†	<5	—	MDS, NOS
19‡	N/A	N/A	N/A	N/A	N/A	pseudo-Pelger-Huet PMNs	MDS, NOS
21	1:15	NI/+	Incr/+	NI/−	<5	low LAP score, slight increase in basophils	CGL
22	1:10	NI/+	Incr/+	NI/−	<5	no basophilia nor eosinophilia, low LAP score	CGL, atypical
23	1:5	NI/+	Incr/−	NI/−	<5	eosinophilia: 5% basophilia: <1%	CGL
24	1:1	Incr¶/+	Decr/+	NI/+	<5	increase in granular lymphocytes; hemophagocytosis	NHL (possible)
28	5:1	NI/−	Decr/†	Decr/†	90%	no Auer rods	ALL
29	10:1	Incr**/+	Decr/†	Decr/†	<5	megaloblastoid RBCs	Aplastic Anemia
30	1:2	NI/+	Decr/−	Decr/†	<5	—	Malignant Histiocytosis (possible)
31	1:18	NI/+	Incr/−	NI/−	<5	eosinophils: 5–8%; extensive dyserythropoiesis	Toxic effect
32	1:100	Decr/−	Incr/−	Decr/†	70	numerous Auer rods	ANLL-M2
33	1:3	Decr/−	Decr/−	NI/−	<5	myeloma cells: 50%	Multiple Myeloma
34	1:10	Decr/−	Decr/−	Decr/†	<5	lymphocytes: 50–60%	LPD

Abbreviations: AL: acute leukemia; ALL: acute lymphoblastic leukemia; ANLL: acute non-lymphocytic leukemia; CGL: chronic granulocytic leukemia; CMML: chronic myelomonocytic leukemia; Decr: decreased; E:G: erythrocyte: granulocyte ratio; Incr: increased; LAP: leukocyte alkaline phosphatase; LPD: lymphoproliferative disorder; MDS: myclodysplastic syndrome; MPD: myeloproliferative disorder; N/A: not ascertained; NHL: non-Hodgkin's lymphoma; NOS: not otherwise specified; PMN: polymorphonuclear leukocyte; RAEBT: refractory anemia with excess blasts in transformation; RBC: red blood cell; +: present; −: absent.

*Bone-marrow was not available for review in Pt. 22. Lymph node biopsies (Pts. 25, 26, and 27) are not included in the table.

††Patients 1–31 were occupationally exposed to benzene.

†Dyspoiesis could not be assessed since so few cells were available.

‡Patient had been previously diagnosed with chronic benzene poisoning.

§No erythroblasts were identified on the slide.

‖Erythroblasts: 30%

¶Erythroblasts: 60%

**Erythroblasts: 80%

Table 5 Summary of dysplastic morphologic findings in the peripheral blood or bone marrow of benzene-exposed workers with hematopoietic malignancies and related disorders*

Morphologic finding	*Diagnostic category (no. of patients)*								
	ANLL (9)	*AL. NOS (4)*	*MDS (5)*	*CGL (4)*	*NHL (possible) (1)*	*ALL (1)*	*AA (1)*	*MH (possible) (1)*	*Toxic effect (1)*
Dyserythropoiesis (all types)	6	3	5	3	1	0	1	1	1
Multinuclearity	0	0	2	1	1	0	0	0	1
Impaired hemoglobinization	5	2	3	1	1	0	1	1	1
Abnormal nuclear shape	0	0	3	0	0	0	0	0	1
Megaloblastoid changes	3	0	4	2	1	0	1	0	1
Dysgranulopoiesis (all types)	5	1	4	3	1	1	0	0	0
Hypersegmented PMNs	0	1	0	0	0	0	0	0	0
Hypogranulation	1	1	2	1	0	0	0	0	0
Pseudo-Pelger-Huet anomaly	2	0	3	0	1	0	0	0	0
Dysmegakaryocytopoiesis (Micromegakaryocytes)	0	1	0	0	1	0	0	0	0

Abbreviations: AA: aplastic anemia; ALL: acute lymphoblastic leukemia; AL. NOS: acute leukemia, not otherwise specified; ANLL: acute non-lymphocytic leukemia; CGL: chronic granulocytic leukemia; MDS: myelodysplastic syndrome; MH: malignant histiocytosis; NHL: non-Hodgkin's lymphoma.

*This table is limited to benzene-exposed subjects (patient numbers 1–31). Specimens from Patient 13 were not evaluable for dysplasia. The 3 subjects for whom lymph node biopsies were reviewed are not included in this table.

morphology of the red cells was dimorphic. Features of this case were consistent with refractory anemia (RA).

The findings in Patient 16, who had a prior diagnosis of chronic benzene poisoning, were most consistent with chronic myelomonocytic leukemia (CMML). Peripheral blood smear features included oval macrocytes, megaloblastoid nucleated red blood cells (NRBCs), hypogranular PMNs and monocytosis. Bone marrow demonstrated megaloblastoid dyserythropoiesis and giant bands and metamyelocytes, as well as an increase in monocytes (20–25%). Excess numbers of both peripheral blood and marrow basophils were also apparent. Information about the size of the spleen on physical examination was not available, and cytogenetic studies and a LAP score had not been performed to exclude a diagnosis of CGL. However, the extensive dyspoiesis evident on both the peripheral smear and the bone marrow, together with the increase in marrow monocytes, seemed to favor a diagnosis of CMML.

Although a marrow smear from Patient 17 contained only 5–10% blasts, the presence of Auer rods, together with other findings, met criteria for a diagnosis of refractory anemia with excess blasts in transformation (RAEBT). Dyserythropoiesis in this subject consisted of abnormal nuclear shape, megaloblastoid changes, and impaired hemoglobinization. Hypogranular PMNs and the pseudo-Pelger-Huet anomaly were also evident.

For 2 of the cases, the MDS could not be subclassified further. Dyspoietic features in Patient 18 included megaloblastoid, multinucleated red cells, impaired hemoglobinization, and hypogranular and pseudo-Pelger-Huet granulocytes. Patient 19, who had a prior history of chronic benzene poisoning, was considered to have a possible MDS, based on the peripheral blood counts, presence of pseudo-Pelget-Huet cells on the reviewed slide, and a bone marrow report which noted 4 per cent blasts. Unfortunately, the available marrow aspirate was of poor quality.

CGL Four cases of CGL are summarized in Tables 2–5. Although a bone marrow aspirate was not available for examination in Patient 20, clinical findings and laboratory studies (which included the presence of the Philadelphia chromosome) were consistent with the diagnosis of CGL indicated in the medical record. Patient 21 presented with hepatosplenomegaly, a high white blood cell (WBC) count with basophilia and a LAP score of 8. Peripheral blood and bone marrow findings, which were consistent with CGL, also included dyspoiesis. Patient 22 had a medical record diagnosis of CGL. Given his peripheral blood counts, myelocyte bulge, LAP score of 6, and striking granulocytic hyperplasia on the marrow aspirate, we maintained CGL as the study diagnosis. However, in

the absence of basophilia, we considered this to be an atypical variant.

Malignant lymphoma Patient 24 presented with constitutional symptoms, hepatosplenomegaly and pancytopenia. Marrow review revealed hemophagocytosis, multinucleated and megaloblastoid dyserythropoiesis, slight impairment of hemoglobinization, pseudo-Pelger-Huet PMNs, hyposegmented eosinophils, eosinophils with pseudopodia and micromegakaryocytes. Several very large cells with cytoplasmic granules, possibly suggestive of malignant T-lymphocytes, were also present. Although this patient may have had an unusual peripheral T-cell lymphoma,[23] in the absence of additional immunohistochemical stains, only a diagnosis of possible non-Hodgkin's lymphoma (NHL) could be assigned.

Evaluation of lymph node biopsies disclosed three cases of NHL. Two patients (25 and 26) were both considered to have malignant lymphoma, diffuse mixed small and large cell, based on samples of tonsillar tissue and a cervical lymph node, respectively. Patient 27 was thought to have diffuse large cell lymphoma based on review of a supraclavicular lymph node biopsy.

Other diagnoses Bone marrow from Patient 28 generally demonstrated characteristic findings of ALL. The hypoplastic bone marrow of Patient 29, which was consistent with aplastic anemia, demonstrated impaired hemoglobinization and megaloblastoid dyserythropoiesis; the peripheral blood WBC differential was remarkable for a mild relative lymphocytosis (47%).

Patient 30, who presented with fever, malaise, lymphadenopathy and pancytopenia, had a medical record diagnosis of malignant histiocytosis. Although special stains to confirm this cell lineage were not available for our review, both the peripheral blood and marrow smears showed large, erythrophagocytic, monocytoid cells suggestive of malignant histiocytes. Therefore, since the patient's clinical and pathologic features were consistent with the medical record diagnosis, we maintained it for study classification. Dyspoietic changes observed for this case included slightly impaired hemoglobinization.

Patient 31, a 33 year old man, had been exposed to benzene for approximately 11 years at the time the specimen described in Table 3 was obtained. This marrow aspirate demonstrated marked dyserythropoiesis, including multinuclearity, megaloblastoid changes, abnormal nuclear shape and cytoplasmic vacuolization. These changes were thought most consistent with some type of toxic bone marrow effect, with further hematologic classification not possible.

DISCUSSION

The present report represents one of the largest series of benzene-related hematopoietic malignancies, identified from a cohort study, in which diagnoses were confirmed by review of pathology reports, medical records, and/or histopathologic material and in which individual clinicopathologic descriptions are provided.[4,6,13,14] Important findings include the observation that the hematopathologic features of ANLL associated with benzene exposure resemble those following chemotherapy or radiotherapy, and documentation of diagnoses of MDS. Taken together, these observations suggest that benzene-related ANLL may represent a distinct clinicopathologic entity and perhaps display a preleukemic phase in some patients, as does therapy-related ANLL.

Another new finding in our study is the greater diversity of hematologic malignancies than usually reported with benzene exposure, perhaps due in part to the sizable number of subjects in a variety of occupational settings. In previous investigations of cancer risk among various groups of benzene-exposed workers, excesses of several forms of hematopoietic malignancies have been described, but only AML and aplastic anemia appear to be consistently increased.[1] Results have, otherwise, varied considerably between studies. For example, preleukemia and acute erythroleukemia were especially common among Turkish workers with chronic benzene poisoning,[24] and erythroleukemia was also noted among Italian workers exposed to benzene.[25] In the Paris region, CGL and CLL accounted for 26 and 16%, respectively, of all leukemias associated with benzene exposure.[6] In addition, elevated risks for multiple myeloma, although based on only four cases, were reported among benzene-exposed workers in the US.[26] Reasons for the variation in the distribution of hematopoietic malignancies between studies may include the presence of concomitant exposures in the workplace, the duration and/or level of benzene exposure, or dissimilarities in case ascertainment and reporting. Furthermore, differences in genetic or other susceptibility factors in the at-risk populations may also affect the distribution of malignancies which are observed. For instance, although CLL was not seen among benzene-exposed

workers in our cohort and only one case of multiple myeloma was evident, population-based incidence data for China indicate that rates for these malignancies are very low.[27,28] Similarly, acute erythroleukemia, also rare in our series, comprises only 5% of ANLL in China.[27] Otherwise, the spectrum of hematopoietic disorders in our series includes many of the conditions reported in prior studies of benzene-exposed workers. Clinicopathologic features of the benzene-exposed cases are discussed below by diagnostic subgroup and compared with observations in prior reports.

ANLL Although the presenting signs and symptoms of the benzene-exposed patients with ANLL typical of acute leukemia occurring de novo,[29] many of the hematologic features resembled those reported in leukemias secondary to chemotherapy or radiotherapy.[30] Characteristics typical of t-ANLL which were observed in our patients included the presence of dysmyelopoietic changes,[30–33] the common occurrence of anemia and thrombocytopenia,[30–32] and increased bone marrow cellularity.[30,31,33] Basophilia, which has been reported in t-ANLL,[30,34] was evident in 2 of 3 patients with a prior history of chronic benzene poisoning, although other clinicopathologic series of benzene-induced leukemias have not noted increased numbers of basophils.[4,13,14] Also, basophilia does not seem to be a typical feature of chronic benzene toxicity.[35]

Treatment-related leukemias, which may demonstrate a preleukemic phase,[30–33] are generally considered distinct clinicopathologic entities with characteristic non-random cytogenetic changes.[30,36,37] Morphologic and hematologic similarities between our benzene-exposed cases and t-ANLL suggest an overlap in pathogenesis, perhaps including damage to marrow stem cells and to extra-medullary mediators of carcinogenesis. One of the metabolic products of benzene postulated to be leukemogenic includes trans-trans-muconaldehyde, which shares functional similarities with alkylating agents.[38] Non-random abnormalities of chromosomes 5 and 7 have been reported among patients with ANLL and past occupational exposure to either solvents or petroleum products[39,40] and subjects with t-ANLL following alkylating agent therapy.[36] More recently, Faglioli *et al.*[41] noted that the clinicopathologic features of ANLL following exposure to organic solvents also resembled those seen in t-ANLL. Another metabolite of benzene, hydroquinone, may inhibit the production of interleukin-1, a cytokine necessary for hematopoiesis.[42] These and other potential mechanisms of benzene hematotoxicity and carcinogenicity were recently reviewed by Cox.[43]

Acute promyelocytic leukemia has been reported infrequently in benzene-exposed groups[4] as well as in t-ANLL.[30–33] Although ANLL-M3 occurred in at least 4 patients in this series, its general representation among the subtypes of ANLL was similar to its distribution in *de novo* ANLL in China.[27]

MDS The occasional reports of preleukemia in association with benzene exposure[4,14] have only rarely included descriptions of dysplasia, such as megaloblastoid dyserythropoiesis.[4] To our knowledge, diagnoses of MDS *per se* have not been previously reported in benzene-exposed workers, perhaps due in part to the relatively recent recognition of some of these disorders.[18] Among benzene-exposed workers in general, reports of dyspoiesis have usually been limited to the rare occurrence of the pseudo-Pelger-Huet anomaly.[35,44] To date, dysplastic findings have not been observed in pancytopenic patients with chronic exposure to benzene.[45]

It is likely that, with increasing appreciation of various types of hematologic dyspoiesis, MDS may be recognized more often among benzene-exposed subjects, enabling further characterization of the dysplastic features associated with benzene toxicity. Correlations between specific cytologic and cytogenetic aberrations might aid in understanding the genetic basis of morphologic dysplasia, as suggested by the possible association reported between pseudo-Pelger-Huet anomaly and monosomy 17.[46] A study of the natural history of benzene-related MDS, including its possible progression to acute leukemia as reported in t-ANLL,[30–33] should help elucidate mechanisms of leukemogenesis.

CGL Although secondary CGL has been reported following chemotherapy,[47] it is mainly considered to be radiogenic.[48] Other clinicopathologic series of benzene-exposed workers have found variable proportions of CGL among reported hematopoietic malignancies.[6,14] Among all benzene-exposed workers in our cohort, CGL accounted for approximately 11% of hematologic malignancies. The four patients with CGL included in our histopathologic review generally showed features characteristic of that diagnosis,[49] as reported in other benzene-exposed workers with this disorder.[6,14] However, dysplastic changes were noted in all cases, and two subjects (numbers 20 and 22) were relatively young at diagnosis, given that CGL typically does not show a sharp increase in incidence

until after age 50.[27] The absence of basophilia in the latter patient was unusual, but has been noted in atypical CGL.[50]

Malignant lymphoma Although lymphoma and related disorders (ML) accounted for approximately 24% of the hematopoietic malignancies among benzene-exposed patients in our series, to date there have been only scattered clinicopathologic reports of ML in benzene-exposed workers, as summarized recently by Aksoy.[12] Most of these descriptions have utilized older classification systems, thus limiting comparisons with our cases. It is noteworthy that two of the four cases of NHL among benzene-exposed patients in this series for whom slides were reviewed represent one subtype of lymphoma as classified by the Working Formulation, although this could represent a chance observation, given the small numbers. Further investigations including immunohistochemical studies as well as histopathologic review should help to clarify the relationship of NHL subtypes to benzene exposure.

Other diagnoses There have been occasional clinicopathologic descriptions of ALL in benzene-exposed workers.[4,6,14,51] Leukocyte counts (median: 4,200/uL; range 1,700–39,000/uL) cited in these reports tend to be somewhat lower than in de novo ALL,[52] but the cases seem otherwise typical. Clinicopathologic features of our patient were also similar to those of de novo ALL.[52]

Although the association between benzene exposure and pancytopenia or aplastic anemia (AA) is well established,[1] there is considerable variability in bone marrow cellularity among pancytopenic patients, even when clinical and other hematologic features are similar.[45] Although benzene-related AA is frequently accompanied by an absolute lymphopenia,[45] a mild relative lymphocytosis was noted in the patient for whom histopathologic material was reviewed. Dyserythropoiesis was minimal in this subject, in accordance with previous reports of benzene-related AA.[45]

The marked degree of toxic changes observed in erythropoietic cells in Patient 31 seems to have not been previously reported among benzene-exposed workers. Cytoplasmic vacuolization of erythroid (n = 1) or myeloid cells (n = 2) was noted on bone marrow examination in 3 apparently asymptomatic benzene-exposed workers with peripheral blood cytopenias, all of whom also demonstrated maturation arrests in the myeloid series.[35] Similarly, cytoplasmic vacuolization has been reported in the bone marrow of benzene-intoxicated rats.[53]

Marrow eosinophilia was also evident in Patient 31. Proportions of eosinophilic leukocytes greater than 8 per cent were noted in the peripheral blood of 6 of 217 benzene-exposed workers in one series,[35] and in 2 of 30 women with chronic benzene poisoning reported by Smith.[54] Relative or absolute numbers of eosinophils were not increased in the peripheral blood or bone marrow of the three patients in our series with prior diagnoses of chronic benzene poisoning.

Rapidly expanding knowledge of the importance of oncogenes in hematologic malignancies,[55] the application of molecular genetic techniques to hematology,[56] and characterization of the importance of the bone marrow stromal environment hold promise for identifying potential similarities, as well as differences, in the mechanisms of action of known leukemogens, such as benzene, radiation, and chemotherapy. It is hoped that the results of this clinicopathologic evaluation of hematologic neoplasms among benzene-exposed workers will encourage further incidence studies to clarify risks by subtype in the general population and in occupational cohorts with excessive hematopoietic malignancies. In addition, cytogenetic and molecular studies of benzene-related leukemias, along with corresponding studies of t-ANLL, should deepen our insights into the underlying mechanisms of leukemogenesis.

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REFERENCES

1. International Agency for Research on Cancer (IARC) and International Association of Cancer Registries. (1982) Benzene. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: some industrial chemicals and dyestuffs*, vol. 29, pp. 93–148. Lyon, France: IARC.
2. Ott, M. G., Townsend, J. C., Rishbeck, W. A. *et al.* (1978) Mortality among individuals occupationally exposed to benzene. *Arch Environ. Health*, **33**, 3–10.
3. Rinsky, R. A., Young, R. J. and Smith, A. B. (1981) Leukemia in benzene workers. *Am. J. Ind. Med.*, **2**, 217–245.

4. Aksoy, M., Erdem, S. and Dincol, G. (1974) Leukemia in shoe-workers exposed chronically to benzene. *Blood*, **44**, 837–841.
5. Aksoy, M. and Erdem, S. (1978) Follow-up study on the mortality and the development of leukemia in 44 pancytopenic patients with chronic benzene exposure. *Blood*, **44**, 285–292.
6. Goguel, P. A., Cavigneaux, A. and Bernard, J. (1967) Les Leucémies Benzéniques De La Région Parisienne Entre 1950 et 1965 (Etude de 50 observations). *Nouv. Rev. Fr. D'Hémat.*, **7**, 465–480.
7. Girard, P. R. and Revol, L. (1970) La frequence d'une exposition benzenique au cours des hemopathies graves. *Nouv. Rev. Fr. Hematol.*, **10**, 477–484.
8. Brandt, L., Nilsson, P. G. and Mitelman, F. (1978) Occupational exposure to petroleum products in men with acute non-lymphocytic leukemia. *Br. Med. J.*, **1**, 553–554.
9. Linos, A., Kyle, R. A., O'Fallon, W. M. *et al.* (1980) A case-control study of occupational exposures and leukemia. *Int. J. Epidemiol.*, **9**, 131–135.
10. Young, N. (1989) Benzene and lymphoma. *Am. J. Ind. Med.*, **15**, 495–498.
11. Goldstein, B. D. (1990) Is exposure to benzene a cause of human multiple myeloma? *Ann. NY Acad. Sci.*, **609**, 225–234.
12. Aksoy, M. (1988) Benzene Carcinogenicity. In *Benzene Carcinogenicity*, edited by M. Aksoy, pp. 113–151. Boca Raton, Florida: CRC Press.
13. Aksoy, M., Dincol, K. and Erdem, S. (1972) Acute leukemia due to chronic exposure to benzene. *Am. J. Med.*, **52**, 160–166.
14. Aksoy, M., Erdem, S. and Dincol, G. (1976) Types of leukemia in chronic benzene poisoning. A study in thirty-four patients. *Acta Haematol.*, **55**, 65–72.
15. Yin, S.-N., Linet, M. S., Hayes, R. B. *et al.* A cohort study of benzene-exposed workers in China. I. General Methods and Resources. *Am. J. Indust. Med.*, (in press).
16. Lehmann, H. P. and Henry J. B. (1991) SI Units. In *Clinical Diagnosis and Management by Laboratory Methods*, edited by J. B. Henry, D. A. Nelson, R. H. Tomar, J. A. Washington and G. A. Threatte, pp. 1366–1382. Philadelphia: Saunders.
17. Jandl, J. H. (1987) Blood and blood forming tissues. In *Blood: Textbook of Hematology*, edited by J. H. Jandl, pp. 1–48. Boston: Little, Brown, and Co.
18. Bennett, J. M., Catovsky, D., Daniel, M. T. *et al.* (1982) Proposals for the classification of the myelodysplastic syndromes. *Br. J. Haematol.*, **51**, 189–199.
19. Bennett, J. M., Catovsky, D., Daniel, M. T. *et al.* (1976) Proposals for the classification of the acute leukaemias. *Br. J. Haematol.*, **33**, 451–458.
20. Bennett, J. M. (1980) Correspondence: A variant form of hypergranular promyelocytic leukaemia (M3). *Br. J. Haematol.*, **44**, 169–170.
21. Bennett, J. M., Catovsky, D., Daniel, M. T. *et al.* (1985) Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7). *Ann. Intern. Med.*, **103**, 460–462.
22. National Cancer Institute Sponsored Study of Classifications of Non-Hodgkin's Lymphomas: (1982) Summary and Description of a Working Formulation for Clinical Usage. *Cancer*, **49**, 2112–2135.
23. Diez-Martin, J. L., Lust, J. A., Witzig, T. E., Banks, P. M. and Li, C. Y. (1991) Unusual presentation of extranodal peripheral T-cell lymphomas with multiple paraneoplastic features. *Cancer*, **68**, 834–841.
24. Aksoy, M. (1985) Malignancies due to occupational exposure to benzene. *Am. J. Indust. Med.*, **7**, 395–402.
25. Vigliani, E. C. (1978) Leukemia associated with benzene exposure. *Ann. NY Acad. Sci.*, **271**, 143–145.
26. Rinsky, R. A., Smith, A. B., Hornung, R. *et al.* (1987) Benzene and leukemia: An epidemiologic risk assessment. *N. Engl. J. Med.*, **316**, 1044–1050.
27. Yang, C. and Zhang, X. (1991) Incidence survey of leukemia in China. *Chin. Med. Sci. J.*, **6**, 65–70.
28. International Agency for Research on Cancer (IARC) and International Association of Cancer Registries. (1987) Age-specific and standardized incidence rates—China. In *Cancer Incidence in Five Continents*, edited by C. Muir, J. Waterhouse, T. Mack, J. Powell and S. Whelan, vol. V, pp. 394–401. Lyon, France: IARC.
29. Burns, C. P., Armitage, J. O., Frey, A. L. *et al.* (1981) Analysis of the presenting features of adult acute leukemia: The French-American-British Classification. *Cancer*, **47**, 2460–2469.
30. Michels, S. D., McKenna, R. W., Arthur, D. C. and Brunning, R. D. (1985) Therapy-related acute myeloid leukemia and myelodysplastic syndrome: A clinical and morphologic study of 65 cases. *Blood*, **65**, 1364–1372.
31. Pedersen-Bjergaard, J., Philip, P., Pedersen, N. T. *et al.* (1984) Acute nonlymphocytic leukemia, preleukemia, and acute myeloproliferative syndrome secondary to treatment of other malignant diseases. II. Bone marrow cytology, cytogenetics, results of HLA typing, response to antileukemic chemotherapy, and survival in a total series of 55 patients. *Cancer*, **54**, 452–462.
32. Brusamolino, E., Papa, G., Valagussa, P. *et al.* (1987) Treatment-related leukemia in Hodgkin's disease: A multi-institution study on 75 cases. *Hematol. Oncol.*, **5**, 83–98.
33. Pedersen-Bjergaard, J., Philip, P., Mortensen, B. T. *et al.* (1981) Acute nonlymphocytic leukemia, preleukemia, and acute myeloproliferative syndrome secondary to treatment of other malignant diseases. Clinical and cytogenetic characteristics and results of in vitro culture of bone marrow and HLA typing. *Blood*, **57**, 712–723.
34. Vardiman, J. W., Golomb, H. M., Rowley, J. D. and Variakojis, D. (1978) Acute nonlymphocytic leukemia in malignant lymphoma. A morphologic study. *Cancer*, **42**, 229–242.
35. Aksoy, M., Dincol, K., Akgün, T. *et al.* (1971) Haematological effects of chronic benzene poisoning in 217 workers. *Br. J. Ind. Med.*, **28**, 296–305.
36. Le Beau, M. M., Albain, K. S., Larson, R. A. *et al.* (1986) Clinical and cytogenetic correlations in 63 patients with therapy-related myelodysplastic syndromes and acute nonlymphocytic leukemia: Further evidence for characteristic abnormalities of chromosomes no. 5 and 7. *J. Clin. Oncol.*, **4**, 325–345.
37. Whitlock, J. A., Greer, J. P. and Lukens, J. N. (1991) Epipodophyllotoxin-related leukemia. Identification of a new subset of secondary leukemia. *Cancer*, **68**, 600–604.
38. Witz, G., Latriano, L. and Goldstein, B. D. (1989) Metabolism and toxicity of trans, trans-muconaldehyde, and open-ring microsomal metabolite of benzene. *Environ. Health Perspect.*, **82**, 19–22.
39. Mitelman, F., Nilsson, P. G., Brandt, L. *et al.* (1981) Chromosome pattern, occupation, and clinical features in patients with acute nonlymphocytic leukemia. *Cancer Genet. Cytogenet.*, **4**, 197–214.
40. Golomb, H. M., Alimena, G., Rowley, J. D. *et al.* (1982) Correlation of occupation and karyotype in adults with acute nonlymphocytic leukemia. *Blood*, **60**, 404–411.
41. Fagioli, F., Cuneo, A., Piva, N. *et al.* (1992) Distinct cytogenetic and clinicopathologic features in acute myeloid leukemia after occupational exposure to pesticides and organic solvents. *Cancer*, **70**, 77–85.
42. Renz, J. F. and Kalf, G. F. (1991) Role for interleukin-1 (IL-1) in benzene-induced hematotoxicity: Inhibition of conversion of pre-IL-1α to mature cytokine in murine macrophages by hydroquinone and prevention of benzene-induced hematotoxicity in mice by IL-1α. *Blood*, **78**, 938–944.

43. Cox, L. A., Jr. (1991) Biological basis of chemical carcinogenesis: Insights from benzene. *Risk Analysis*, **11**, 453–464.
44. Paterni, L. and Russo, G. (1960) Il Primo caso di anomalia Pelgheriana nella emopatia benzenica; associazione con nanismo cellulare. *Haematologica*, **45**, 213–220.
45. Aksoy, M., Dincol, K., Erdem, S. *et al.* (1972) Details of blood changes in 32 patients with pancytopenia associated with long-term exposure to benzene. *Br. J. Ind. Med.*, **29**, 56–64.
46. Lai, J. L., Zandecki, M., Fenaux, P. *et al.* (1990) Translocations (5;17) and (7;17) in patients with de novo or therapy-related myelodysplastic syndromes or acute nonlymphocytic leukemia: a possible association with acquired pseudo-Pelger-Huet anomaly and small vacuolated granulocytes. *Cancer Genet. Cytogenet.*, **46**, 173–183.
47. Whang-Peng, J., Young, R. C., Lee, E. C. *et al.* (1988) Cytogenetic studies in patients with secondary leukemia/dysmyelopoietic syndrome after different treatment modalities. *Blood*, **71**, 403–414.
48. Committee on the Biologic Effects of Ionizing Radiation (BEIR). (1990) Radiogenic cancer at specific sites. In *Health Effects of Exposure to Low Levels of Ionizing Radiation*, BEIR V Report, pp. 242–351. Washington, DC: National Academy Press.
49. Spiers, A. S. D., Bain, B. J. and Turner, J. E. (1977) The peripheral blood in chronic granulocytic leukaemia: Study of 50 untreated Philadelphia-positive Cases. *Scand. J. Haematol.*, **18**, 25–38.
50. Shepherd, P. C. A., Ganesan, T. S. and Galton, D. A. G. (1987) Haematological classification of the chronic myeloid leukaemias. *Bail's Clin. Haematol.*, **1**, 887–906.
51. Hernberg, S., Savilahti, M., Ahlman, K. and Asp, S. (1966) Prognostic aspects of benzene poisoning. *Br. J. Ind. Med.*, **23**, 204–209.
52. Hammond, D., Sather, H., Nesbit, M. *et al.* (1986) Analysis of prognostic factors in acute lymphoblastic leukemia. *Med. Pediatr. Oncol.*, **14**, 124–134.
53. Yin, S.-N., Li, G. and Lian, Y. (1979) Experimental studies on the effect of Xuezaisham in treatment for benzene-intoxicated rats. *J. Inst. Health*, **8**, 54–58.
54. Smith, A. R. (1928) Chronic benzol poisoning among women industrial workers: A study of the women exposed to benzol fumes in six factories. In *The Journal of Industrial Hygiene*, edited by A. R. Smith, vol. 10, pp. 73–93. New York.
55. Pearson, M. and Rowley, J. D. (1985) The relation of oncogenesis and cytogenetics in lymphoma and leukemia. *Ann. Rev. Med.*, **36**, 471–483.
56. Le Beau, M. M., Lemons, R. S., Espinosa, (III) R. *et al.* (1989) Interleukin-4 and interleukin-5 Map to human chromosome 5 in a region encoding growth factors and receptors and are deleted in myeloid leukemias with a del (5q). *Blood*, **73**, 647–650.

Chemico-Biological Interactions. **In press.**

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 2,923 consecutive patients presenting at 29 hospitals from Aug 2003 to Jun 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 2 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 1960's and 1970's 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 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. Material 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, 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. 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 venipuncture 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 where 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-hour) 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 [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 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 were 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).

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, Texas). 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 Figure 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: 56/52yr; WHO (2008) males/females: 57/52yr. 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). 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 del(7q) and del(5q) in combination were invariably associated with complex karyotypes involving 3 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 $\geq$ 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, $p < .001$) (Figure 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 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 (Figures 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 eosinophils (MDS-Eo) (Figure 4), hematophagocytosis (Figure 5) and stromal degeneration. (Figure 3). Quantitative case-case comparison of clinical features in BZ signal cases are presented in Table 6. Hb levels > 10g/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. 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 tMDS/tAML 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 (Figure 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 multlineage 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 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. Hydroquione (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 plays a predisposing role in the pathogenesis of persistent BM injury and the development of MDS following chronic exposure to BZ.

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References

[1] S.H. Swerdlow, E. Campo, N.L. Harris *et al.* WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue in: Swerdlow SH, Campo E, Harris NL *et al.* WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue. IARC,Lyon, France: 2008.

[2] E. Jaffe, N. Harris, H. Stein, J. Vardiman, World Health Organization Classification of Tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues in: Jaffe E, Harris N, Stein H, Vardiman J. World Health Organization Classification of Tumours. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. IARC Press,Lyon, France:2001.

[3] U. Germing, N. Gattermann, C. Strupp, M. Aivado, C. Aul, Validation of the WHO proposals for a new classification of primary myelodysplastic syndromes: a retrospective analysis of 1600 patients, Leukemia Research 24 (2000) 983-92.

[4] A. Matsuda, U. Germing, I. Jinnai, M. Misumi, A. Kuendgen, S. Knipp, M. Aivado, M. Iwanaga, Y. Miyazaki, H. Tsushima, M. Sakai, M. Bessho, M. Tomonaga, Difference in clinical features between Japanese and German patients with refractory anemia in myelodysplastic syndromes, Blood 106 (2005) 2633-40.

[5] A. Kuendgen, A. Matsuda, U. Germing, Differences in Epidemiology of MDS Between Western and Eastern Countries: Ethnic Differences or Environmental Influence?, Leukemia Research 31 (2007) 103-4.

[6] B. Chen, W.L. Zhao, J. Jin, Y.Q. Xue, X. Cheng, X.T. Chen, J. Cui, Z.M. Chen, Q. Cao, G. Yang, Y. Yao, H.L. Xia, J.H. Tong, J.M. Li, J. Chen, S.M. Xiong, Z.X. Shen, S. Waxman, Z. Chen, S.J. Chen, Clinical and cytogenetic features of 508 Chinese patients with myelodysplastic syndrome and comparison with those in Western countries, Leukemia 19 (2005) 767-75.

[7] R.D. Irons, X. Wang, S.A. Gross, L. Bao, J. Ryder, Y. Chen, H. Chen, H. Sun, J. Zhou, M. Ji, X. Du, H. Fu, G. Lin, Prevalence of MDS subtypes in Shanghai, China: A comparison of the World Health Organization and French American British classifications, Leukemia Research 30 (2006) 769-775.

[8] X.Q. Wang, J. Ryder, S.A. Gross, G. Lin, R.D. Irons, Prospective analysis of clinical and cytogenetic features of 435 cases of MDS diagnosed using the WHO (2001) classification: a

prognostic scoring system for predicting survival in RCMD, International Journal of Hematology (In Press).

[9] A. Cuneo, F Fagioli, I Pazzi, A Tallarico, R Previati, N Piva, GM Carli, M Balboni, G Castoldi, Morphologic, immunologic and cytogenetic studies in acute myeloid leukemia following occupational exposure to pesticides and organic solvents, Leukemia Research 16 (1992) 789-796.

[10] R.B. Hayes, S.N. Yin, M. Dosemeci, G.L. Li, S. Wacholder, L.B. Travis, C.Y. Li, N. Rothman, R.N. Hoover, M.S. Linet, Benzene and the dose-related incidence of hematologic neoplasms in China, J.Natl.Cancer Inst. 89 (1997) 1065-1071.

[11] R.D. Irons, L. Lv, S.A. Gross, X. Ye, L. Bao, X.Q. Wang, J. Ryder, T.W. Armstrong, Y. Zhou, L. Miao, A.T. Le, P.J. Kerzic, W. Ni, H. Fu, Chronic exposure to benzene results in a unique form of dysplasia, Leukemia Research 29 (2005) 1371-80.

[12] T. Armstrong,Y. Zhou,C. Zhang,S. Bowes,Y. Liang,O. Wong,F. Hua, Exposure assessment for a case-control epidemiology study based in Shanghai, China: Summary of methods and results.

[13] S.M. Smith, M.M. Le Beau, D. Huo, T. Karrison, R.M. Sobecks, J. Anastasi, J.W. Vardiman, J.D. Rowley, R.A. Larson, Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series, Blood 102 (2003) 43-52.

[14] G. Chen, W. Zeng, A. Miyazato, E. Billings, J.P. Maciejewski, S. Kajigaya, E.M. Sloand, N.S. Young, Distinctive Gene Expression Profiles of Cd34 Cells From Patients With Myelodysplastic Syndrome Characterized by Specific Chromosomal Abnormalities, Blood 104 (2004) 4210-8.

[15] Z. Qian, A.A. Fernald, L.A. Godley, R.A. Larson, M.M. Le Beau, Expression Profiling of Cd34+ Hematopoietic Stem/ Progenitor Cells Reveals Distinct Subtypes of Therapy-Related Acute Myeloid Leukemia, Proceedings of the National Academy of Sciences of the United States of America 99 (2002) 14925-30.

[16] R.D. Irons, W.S. Stillman, The process of leukemogenesis, Environ.Health Perspect. 104 (1996) 1239-1246.

[17] W.S. Stillman, M Varella-Garcia, RD Irons, The benzene metabolite, hydroquinone, selectively induces 5q31-and-7 in human $CD34^{+}CD19^{-}$ bone marrow cells, Exp.Hematol. 28 (2000) 169-176.

[18] W.S. Stillman, M. Varella-Garcia, J.J. Gruntmeir, R.D. Irons, The benzene metabolite, hydroquinone, induces dose-dependent hypoploidy in a human cell line, Leukemia 11 (1997) 1540-1545.

[19] W.S. Stillman, M. Varella-Garcia, R.D. Irons, The benzene metabolites hydroquinone and catechol act in synergy to induce dose-dependent hypoploidy and-5q31 in a human cell line, Leuk.Lymphoma 35 (1999) 269-281.

[20] M.T. Smith, LP Zhang, M Jeng, YX Wang, WH Guo, P Duramad, AE Hubbard, G Hofstadler, NT Holland, Hydroquinone, a benzene metabolite, increases the level of aneusomy of chromosomes 7 and 8 in human CD34-positive blood progenitor cells, Carcinogenesis 21 (2000) 1485-1490.

[21] M.T. Smith, L.P. Zhang, Y.X. Wang, R.B. Hayes, G.L. Li, J. Wiemels, M. Dosemeci, N. Titenko-

Holland, L.Q. Xi, P. Kolachana, S.N. Yin, N. Rothman, Increased translocations and aneusomy in chromosomes 8 and 21 among workers exposed to benzene, Cancer Res. 58 (1998) 2176-2181.

[22] M.A. Ruiz, L.G. Augusto, J. Vassallo, A.C. Vigorito, I. Lorand-Metze, C.A. Souza, Bone Marrow Morphology in Patients With Neutropenia Due to Chronic Exposure to Organic Solvents (Benzene): Early Lesions, Pathology, Research & Practice 190 (1994) 151-4.

[23] M. Cazzola, L Malcovati, Myelodysplastic syndromes--coping with ineffective hematopoiesis, N Engl J Med 352 (2005) 536-8.

[24] A.J. Barrett, Myelodysplastic syndrome--an example of misguided immune surveillance?, Leukemia Research 28 (2004) 1123-4.

[25] C. Rosenfeld, A List, A hypothesis for the pathogenesis of myelodysplastic syndromes: implications for new therapies, Leukemia 14 (2000) 2-8.

[26] D.H. Biesma, J.G. van den Tweel, L.F. Verdonck, Immunosuppressive therapy for hypoplastic myelodysplastic syndrome, Cancer. 79 (1997) 1548-51.

[27] T. Matsutani, T. Yoshioka, Y. Tsuruta, T. Shimamoto, J.H. Ohyashiki, R. Suzuki, K. Ohyashiki, Determination of T-cell receptors of clonal CD8-positive T-cells in myelodysplastic syndrome with erythroid hypoplasia, Leukemia Research. 27 (2003) 305-12.

[28] T.J. Hamblin, Immunological abnormalities in myelodysplastic syndromes, Seminars in Hematology 33 (1996) 150-62.

[29] Y. Sauntharajah, J.L. Molldrem, M. Rivera, A. Williams, M. Stetler-Stevenson, L. Sorbara, N.S. Young, J.A. Barrett, Coincident Myelodysplastic Syndrome and T-Cell Large Granular Lymphocytic Disease: Clinical and Pathophysiological Features, British Journal of Haematology 112 (2001) 195-200.

[30] T. Shimamoto , T. Iguchi, K. Ando, T. Katagiri, T. Tauchi, Y. Ito, M. Yaguchi, K. Miyazawa, Y. Kimura, M. Masuda, H. Mizoguchi, K. Ohyashiki, Successful Treatment With Cyclosporin A for Myelodysplastic Syndrome With Erythroid Hypoplasia Associated With T-Cell Receptor Gene Rearrangements, British Journal of Haematology 114 (2001) 358-61.

[31] H. Kook, W. Zeng, C. Guibin, M. Kirby, N.S. Young, J.P. Maciejewski, Increased cytotoxic T cells with effector phenotype in aplastic anemia and myelodysplasia, Experimental Hematology 29 (2001) 1270-7.

[32] A.M. Risitano, J.P. Maciejewski, S. Green, M. Plasilova, W. Zeng, N.S. Young, In-vivo dominant immune responses in aplastic anaemia: molecular tracking of putatively pathogenetic T-cell clones by TCR beta-CDR3 sequencing, Lancet 364 (2004) 355-64.

[33] W. Zeng, J.P. Maciejewski, G. Chen, N.S. Young, Limited heterogeneity of T cell receptor BV usage in aplastic anemia, Journal of Clinical Investigation 108 (2001) 765-73.

[34] A.W. Langerak, T. Szczepanski, M. van der Burg, I.L. Wolvers-Tettero, J.J. van Dongen, Heteroduplex PCR analysis of rearranged T cell receptor genes for clonality assessment in suspect T cell proliferations, Leukemia 11 (1997) 2192-9.

[35] A. Jonasova, R. Neuwirtova, J. Cermak, V. Vozobulova, K. Mocikova, M. Siskova, I. Hochova, Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow, British Journal of Haematology 100 (1998) 304-9.

[36] G. Par, D. Rukavina, E.R. Podack, M. Horanyi, J. Szekeres-Bartho, G. Hegedus, M. Paal, L. Szereday, G. Mozsik, A. Par, Decrease in CD3-negative-CD8dim(+) and Vdelta2/Vgamma9 TcR+ peripheral blood lymphocyte counts, low perforin expression and the impairment of natural killer cell activity is associated with chronic hepatitis C virus infection, Journal of Hepatology 37 (2002) 514-22.

[37] G.A. Banat, K. Ihlow, N. Usluoglu, S. Hoppmann, M. Hoeck, H. Pralle, Core-binding factor-beta positive acute myeloid leukaemia cells induce T-cell responses, British Journal of Haematology 123 (2003) 819-29.

[38] Y.S. Hahn, C. Taube, N. Jin, L. Sharp, J.M. Wands, M.K. Aydintug, M. Lahn, S.A. Huber, R.L. O'Brien, E.W. Gelfand, W.K. Born, Different potentials of gamma delta T cell subsets in regulating airway responsiveness: V gamma 1+ cells, but not V gamma 4+ cells, promote airway hyperreactivity, Th2 cytokines, and airway inflammation, Journal of Immunology 172 (2004) 2894-902.

[39] A. Kanehiro, M. Lahn, M.J. Makela, A. Dakhama, M. Fujita, A. Joetham, R.J. Mason, W. Born, E.W. Gelfand, Tumor necrosis factor-alpha negatively regulates airway hyperresponsiveness through gamma-delta T cells, American Journal of Respiratory and Critical Care Medicine 164 (2001) 2229-38.

[40] L. Lv, P. Kerzic, G. Lin, A.R. Schnatter, L. Bao, Y. Yang, H. Zou, H. Fu, X. Ye, S.A. Gross, T.W. Armstrong, R.D. Irons, The TNF-Alpha 238a Polymorphism Is Associated With Susceptibility to Persistent Bone Marrow Dysplasia Following Chronic Exposure to Benzene, Leukemia Research 31 (2007) 1479-85.

[41] R.D. Irons, WS Stillman, DB Colagiovanni, VA Henry, Synergistic action of the benzene metabolite hydroquinone on myelopoietic stimulating activity of granulocyte/macrophage colony-stimulating factor *in vitro*, Proc.Natl.Acad.Sci.USA 89 (1992) 3691-3695.

[42] P. Baines, H. Mayani, M. Bains, J. Fisher, T. Hoy, A. Jacobs, Enrichment of CD34 (My10)-positive myeloid and erythroid progenitors from human marrow and their growth in cultures supplemented with recombinant human granulocyte-macrophage colony-stimulating factor, Exp.Hematol. 16 (1988) 785-789.

[43] H. Quentmeier, WG Dirks, D Fleckenstein, M Zaborski, HG Drexler, Tumor necrosis factor-alpha-induced proliferation requires synthesis of granulocyte-macrophage colony-stimulating factor, Experimental Hematology 28 (2000) 1008-15.

[44] R.D. Irons, WS Stillman, Cell proliferation and differentiation in chemical leukemogenesis, Stem Cells 11 (1993) 235-242.

[45] R.D. Irons, WS Stillman, Impact of benzene metabolites on differentiation of bone marrow progenitor cells, Environ.Health Perspect. 104 Suppl. 6 (1996) 1247-1250.

[46] J.H. Zheng, DW Pyatt, SA Gross, AT Le, PJ Kerzic, RD Irons, Hydroquinone modulates the GM-CSF signaling pathway in TF-1 cells, Leukemia 18 (2004) 1296-1304.

[47] M.B. Jordan, D. Hildeman, J. Kappler, P. Marrack, An animal model of hemophagocytic lymphohistiocytosis (HLH): CD8+ T cells and interferon gamma are essential for the disorder, Blood 104 (2004) 735-43.

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

Table 2. Clonal Cytogenetic Abnormalities by MDS Subtype		
MDS Subtypes	**Total Cases**	**Clonal Abn**
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%)

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 Abnl	46	7.1%

[a]Exclusively in complex karyotypes

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]$p<0.05$

Table 5. Comparison of Hematologic Parameters BZ Signal Cases vs Matched Unexposed Cases										
MDS Cases	**Bone Marrow**						**Peripheral Blood**			
	Cellularity			**Blasts**		**MDS-Eo**	**Neutrophils x10^{9}L**	**Lymphocytes x10^{9}L**	**Hg g/dL**	**Platelets x10^{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)

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

Figure 1a

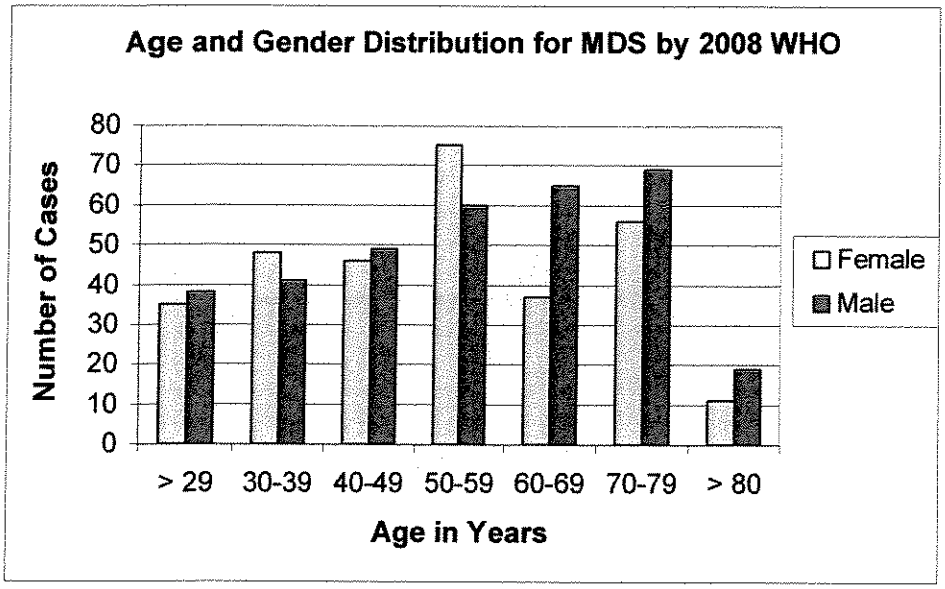


Figure 1b

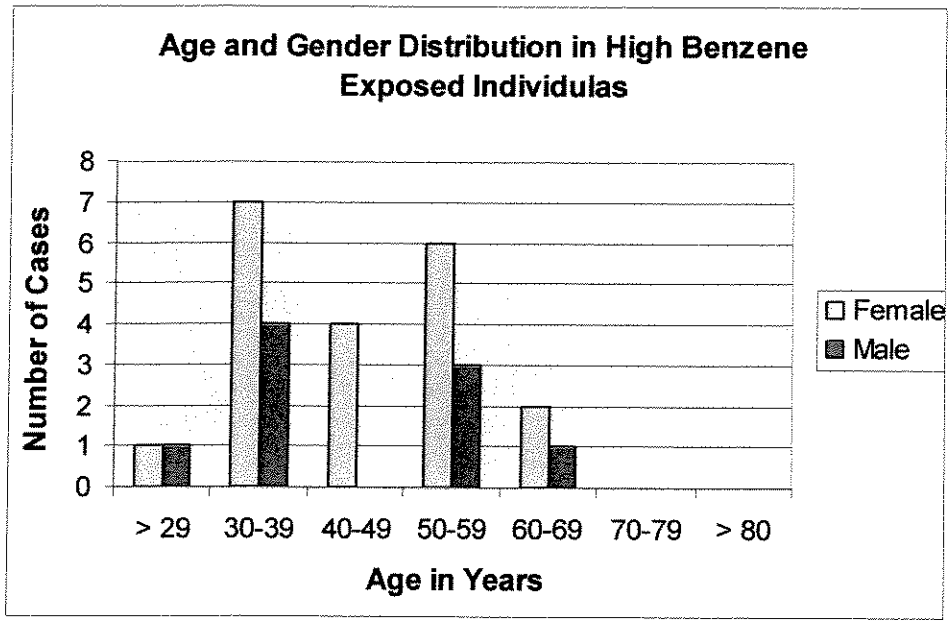
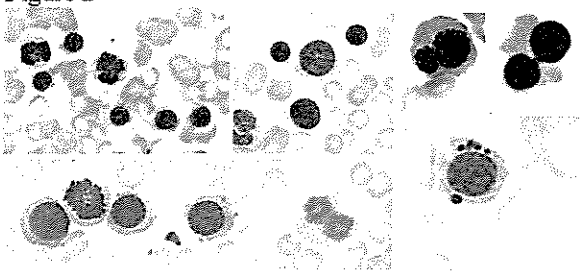


Figure 2



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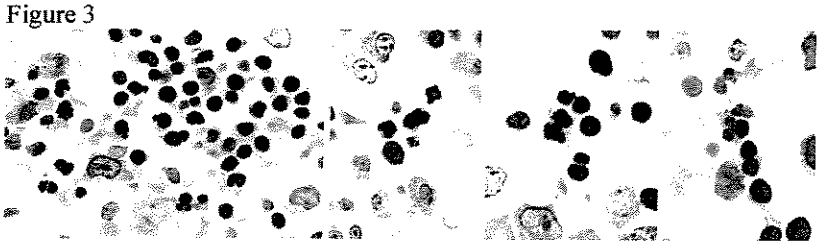

Figure 4

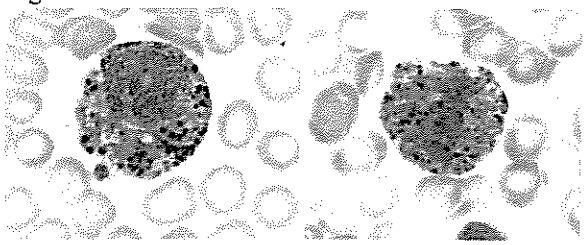

Figure 5

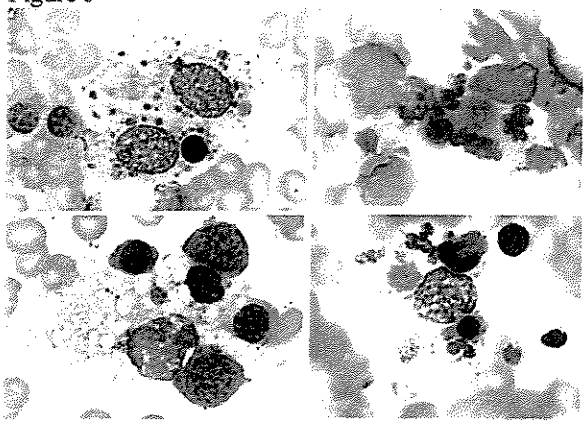

Figure 6

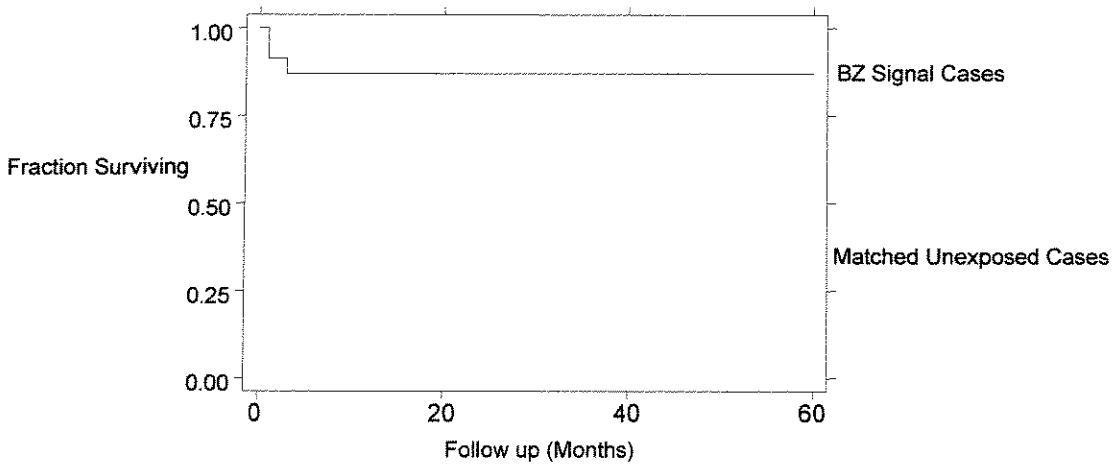
1.00
0.75
0.50
0.25
0.00
Fraction Surviving
0
20
40
60
Follow up (Months)
BZ Signal Cases
Matched Unexposed Cases

Figure Legends

Figure 1a. Age and gender distribution of WHO (2008) cases grouped by decade.

Figure1b. Age and gender distribution of benzene signal cases grouped by decade.

Figure 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 X1000). Reproduced with permission [11].

Figure 3. Dyserythropoiesis and stromal degeneration accompanying MDS-Eo associated with previous high BZ exposure. Core biopsy section. Hematoxylin-Eosin stain (original magnification X1000). Reproduced with permission [11].

Figure 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 X1000). Reproduced with permission [11].

Figure 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 X1000). Reproduced with permission [11].

Figure 6. Kaplan-Meier overall survival analysis for benzene signal cases versus matched unexposed MDS cases.

Myelodysplastic/myeloproliferative neoplasm, unclassifiable

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Definition

Myelodysplastic/myeloproliferative neoplasm, unclassifiable, (MDS/MPN, U) meets the criteria for the MDS/MPN category in that, at the time of initial presentation, there are clinical, laboratory and morphological features that overlap both MDS and MPN. Cases classified as MDS/MPN, U do not meet the criteria for chronic myelomonocytic leukaemia, juvenile myelomonocytic leukaemia or atypical chronic myeloid leukaemia. The finding of a *BCR-ABL1* fusion gene or of rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1* excludes the diagnosis of MDS/MPN, U.

It is important that the designation MDS/MPN, U not be used for patients with a previous, well-defined MPN who develop dysplastic features in association with transformation to a more aggressive phase. However, MDS/MPN, U may include some patients in whom the chronic phase of an MPN was not previously detected, and who initially present in transformation with myelodysplastic features. If the underlying MPN cannot be identified, the designation of MDS/MPN, U is appropriate. If there has been any recent cytotoxic or growth factor therapy, follow-up clinical and laboratory observations are essential to demonstrate that the peripheral blood (PB) and bone marrow (BM) changes are not due to the treatment.

ICD-O code 9975/3

Synonyms

Mixed myeloproliferative/myelodysplastic syndrome, unclassifiable; "overlap" syndrome, unclassifiable.

Sites of involvement

The BM and PB are always involved; spleen, liver and other extramedullary tissues may be involved.

Clinical features

The clinical features of MDS/MPN, U overlap those found in diseases of the MDS and MPN categories [129, 1588, 2311].

Table 4.04 Diagnostic criteria for myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN, U).

- The case has clinical, laboratory and morphological features of one of the categories of MDS (refractory cytopenia with unilineage dysplasia, refractory anaemia with ring sideroblasts, refractory cytopenia with multilineage dysplasia, refractory anaemia with excess of blasts) and <20% blasts in the blood and bone marrow
  and
- Has prominent myeloproliferative features, e.g. platelet count ≥ $450x10^9$/L associated with megakaryocytic proliferation, or WBC count ≥$13x10^9$/L, with or without prominent splenomegaly
  and

  Has no preceding history of an underlying MPN or of MDS, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic or myeloproliferative features, and no Philadelphia chromosome or *BCR-ABL1* fusion gene, no rearrangement of *PDGFRA*, *PDGFRB* or *FGFR1*, and no isolated del(5q), t(3;3)(q21;q26) or inv(3)(q21q26)
  or
- The patient has *de novo* disease with mixed myeloproliferative and myelodysplastic features and cannot be assigned to any other category of MDS, MPN or of MDS/MPN.

Morphology and cytochemistry

These disorders are characterized by proliferation of one or more myeloid lineages that is ineffective, dysplastic or both and simultaneously, by effective proliferation, with or without dysplasia, in one or more of the other myeloid lineages. Laboratory features usually include anaemia of variable severity, with or without macrocytosis and often dimorphic red blood cells on the peripheral smear. In addition, there is evidence of effective proliferation in one or more lineages, either as thrombocytosis (platelet count ≥ $450x10^9$/L) or leukocytosis (white blood cell count ≥$13x10^9$/L). Neutrophils may show dysplastic features and there may be giant or hypogranular platelets. Blasts account for <20% of the leukocytes in the PB and of the nucleated cells of the BM, and a finding of >10% blasts in the PB or BM likely indicates transformation to a more aggressive stage. The BM biopsy specimen is hypercellular and may show proliferation in any or all of the myeloid lineages. However, dysplastic features are simultaneously present in at least one cell line.

Cytochemical findings may be similar to those seen in MDS or in MPN.

Immunophenotype

May be similar to findings in MDS and/or MPN.

Genetics

There is no cytogenetic or molecular genetic finding specific for this group. A Philadelphia chromosome and *BCR-ABL1* fusion gene should always be excluded prior to making the diagnosis of MDS/MPN, U. Cases with rearrangements of *PDGFRA*, *PDGFRB* or *FGFR1* or with isolated del(5q) or t(3;3)(q21;q26) or inv(3)(q21q26) are excluded from this category as well. In difficult cases, the presence of a *JAK2* V617F mutation may help to confirm a haematopoietic neoplasm, though the significance of such mutations in this entity is uncertain. Occasional cases with isolated del(5q) and *JAK2* V617F mutation have been reported to have features that overlap MDS and MPN [1005].

Postulated cell of origin

Haematopoietic stem cell.

Refractory anaemia with ring sideroblasts (RARS) associated with marked thrombocytosis

Definition

In the third edition of the WHO Classification, RARS-T, previously also referred to as essential thrombocythaemia (ET) with ring sideroblasts, was proposed as a provisional entity to encompass patients who have the clinical and morphological features of the myelodysplastic syndrome,

RARS, but who also have marked thrombocytosis associated with abnormal megakaryocytes similar to those observed in the *BCR-ABL1* negative MPN, such as ET or early-stage primary myelofibrosis (PMF) {865, 1077, 2109}. However, some investigators have suggested that RARS-T is not a unique entity but instead represents cases of other subtypes of MDS or well-defined MPN that have acquired ring sideroblasts as a secondary form of dysplasia {1966}. It is not clear whether RARS-T is a distinct entity, one end of the spectrum of RARS, a progression of RARS due to an additional acquired genetic abnormality, or less likely, the occurrence of two rare diseases in the same patient. Therefore, until these questions are more clearly answered, RARS-T remains a provisional entity.

In support of a myeloproliferative component to this neoplasm, the majority of cases reported as RARS-T have shown the *JAK2* V617F mutation, or much less commonly, the *MPL* W515K/L mutation {234, 354, 762, 1835, 1839, 1969, 2081, 2139, 2358}. On the other hand, the few reported cases with this mutation that have been studied for endogenous colony formation *in vitro* have demonstrated a pattern more akin to that of MDS {234, 1835}. Thus it may be that the provisional designation of an MDS/MPN accurately reflects the underlying biology in a substantial proportion of the patients {762}; more study is needed to further clarify this disorder.

Cases with isolated del(5q),t(3;3)(q21;q26) or inv(3)q21q26) are excluded from this category, as are cases with a *BCR-ABL1* fusion gene. In addition, if there has been a prior diagnosis of an MPN without ring sideroblasts, or there is evidence that the ring sideroblasts might be a consequence of therapy or represent disease progression in a patient with features that meet the criteria of another well-defined MPN, this designation should not be used.

ICD-O code 9982/3

Morphology

These cases have features of RARS (anaemia with no blasts in the PB and dysplastic, ineffective erythroid proliferation often with megaloblastoid features, ring sideroblasts ≥15% of the erythroid precursors, and <5% blasts in the BM) and thrombocytosis with a platelet count ≥450x10^9/L associated with proliferation of large atypical megakaryocytes similar to those observed in *BCR-ABL1* negative MPN (See Chapter 2).

The minimum platelet count required for inclusion has been lowered to 450x10^9/L from 600x10^9/L for consistency with the defining criterion for ET, and because several studies have demonstrated that patients with platelet counts lower than 600x10^9/L may have biological features, including *JAK2* V617F mutations, similar to those with counts ≥600x10^9/L {354}. It is important to note that the criteria for RARS-T includes morphologically abnormal megakaryocytes similar to those observed in ET and in PMF. This criterion should aid in distinguishing RARS-T from those cases of RARS commonly reported to have a modest increase in their platelet count. Nevertheless, we recommend testing for *JAK2* V617F when the platelet count is elevated in patients with RARS until the borderline between RARS and RARS-T is more clearly defined.

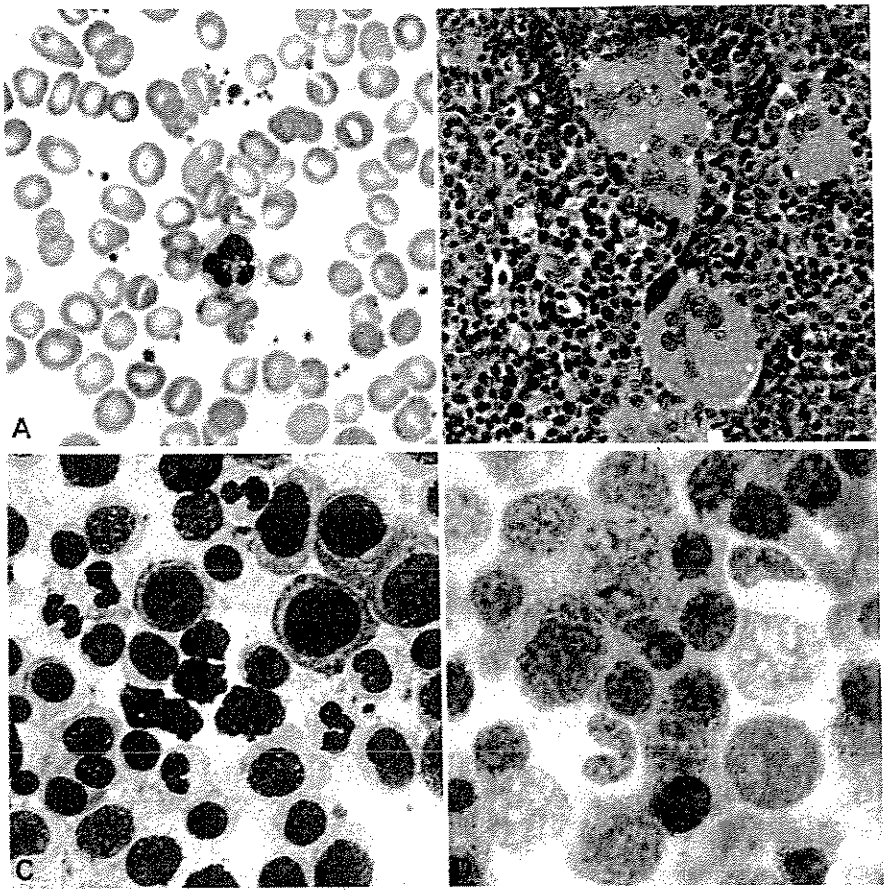

Fig. 4.13 Refractory anaemia with ring sideroblasts and thrombocytosis. This sequence of microphotographs illustrate blood and bone marrow of a 62-year-old man who presented with severe anaemia and a platelet count of 850x10^9/L. **A** Abnormal red cells and thrombocytosis. **B** Erythroid proliferation and abnormal megakaryocytes resembling megakaryocytes seen in ET. **C** Mild dyserythropoiesis. **D** The majority of erythroid precursors were ring sideroblasts.

Genetics

The recent discovery that up to 60% of patients with RARS-T harbour the *JAK2* V617F mutation (an incidence similar to that found in ET and PMF) or less commonly, the *MPL* W515K/L mutation, not only elucidates the reason for the proliferative aspect of RARS-T but also would seem to move it closer to the MPN category {234, 354, 762, 1835, 1839, 1969, 2081, 2139, 2358}. Thus, studies for *JAK2* V617F, and, if indicated, for the *MPL* W515K/L mutation should always be performed in such cases.

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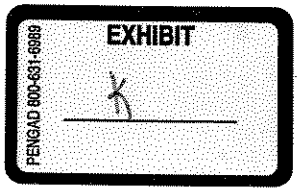

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