Focus: Myelocytic Leukemia
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The American Society for Clinical Laboratory Science, as the pre-eminent organization for clinical laboratory science practitioners, provides dynamic leadership and vigorously promotes all aspects of clinical laboratory science practice, education and management to ensure excellent, accessible cost-effective laboratory services for the consumers of health care.

AMERICAN SOCIETY FOR
CLINICAL LABORATORY SCIENCE
6701 Democracy Blvd, Suite 300
Bethesda, Maryland 20817
(301) 657-2768, (301) 657-2909 (fax)
www.ascls.org/

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ASCLS MEMBER EDITORS
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Susan J. Leclair PhD CLS(NCA)
Department of Medical Laboratory Science
University of Massachusetts Dartmouth
North Dartmouth MA 02747-2715
sleclair@umassd.edu

Continuing Education Editor
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Department of Pathology
619 South 19th Street West Pavillion, P230
U of Alabama at Birmingham
Birmingham AL 35233
gfritsma@path.uab.edu

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Clinical Laboratory Science
Indiana University, 409 Fedor
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Indianapolis IN 46202-5133
broadak@iupui.edu

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TRENDS AND TECHNOLOGY: FALL 2004

Mary Jane Gore
Clinical Laboratory Science Enters New Era

This issue marks the 18th year of publication of Clinical Laboratory Science (Clin Lab Sci) and initiates several changes in both the format of Clin Lab Sci and the manuscript handling process. There will now be three major sections in Clin Lab Sci: Clinical Practice, Research and Reports, and Focus instead of the previous four sections: Clinical Practice, Reports and Reviews, Research, and Focus. In addition, the peer-review process will be converted to a totally electronic process. We believe that this will significantly shorten the time needed to complete reviews as well as simplify the process for authors, reviewers, and editors. These changes are reflected in updated Instructions to Authors and other Clin Lab Sci documents that can be found on the ASCLS Website. Please consult these revised instructions before submitting a manuscript to Clin Lab Sci for possible publication.

With the beginning of a new journal year, there are also editorial and reviewer contributions and changes to note. First of all, we want to thank Dr Isaac Montoya for his outstanding work as Peer Review Editor for the past three years and welcome Dr David Fowler as the new Research and Reports Editor. We also welcome Dr Jesse Guiles, Dr Daniel Hoefner, Linda Kasper, Robin Krefetz, Dr Carol McCoy, and Dr Isaac Montoya as new members of the Clin Lab Sci Review Board.

A heartfelt thank you is due 32 committed Review Board members who contributed their time and expertise to Clin Lab Sci by reviewing manuscripts during 2004. On behalf of Clin Lab Sci editors, authors, and readers, I want to publicly extend our gratitude to each of the reviewers on the following list for their efforts on behalf of Clin Lab Sci during 2004. Their willingness to assist Clin Lab Sci editors and prospective authors has greatly improved the quality of the articles published this past year in Clin Lab Sci.

The Dialogue and Discussion Section is a forum for editorials, short articles, commentaries, and letters to the editor on clinical laboratory science topics and professional issues of general interest to readers including ASCLS activities and position papers. For more information about submissions to the Dialogue and Discussion section contact: Susan Leclair PhD, Editor-in-Chief, Clinical Laboratory Science Editorial Office, Attn: Dialogue and Discussion, PO Box 5399, Coralville, IA 52241-5399. (319) 351-2922, (319) 351-2927 (fax). cls@ia.net

If you are interested in joining the Clin Lab Sci Review Board and reviewing manuscripts submitted for possible publication, please send a letter or e-mail specifying the content areas that you feel comfortable reviewing, e.g., hematology, management, microbiology, etc., and a current curriculum vitae to: Marian Schwabbauer, Clinical Laboratory Science Executive Office, P.O. Box 5399, Coralville IA or cls@ia.net.

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Marian Schwabbauer, Clinical Laboratory Science Executive Editor
WASHINGTON BEAT

New Medicare Screening Test Coverage

KATHY HANSEN, DON LAVANTY

An ongoing activity of the Government Affairs Committee of the American Society for Clinical Laboratory Science (ASCLS) is to respond to changes in laboratory regulations that are proposed by the Centers for Medicare and Medicaid Services (CMS) and other federal agencies. Typically regulations are published in the Federal Register in a proposed form, with a deadline for comments to be submitted. CMS then takes comments under advisement before a revised final regulation is published. Depending upon the volume of comments received, this process can take from a few months to many months before final regulations are published. Such responses to requests for comments are submitted several times each year. ASCLS tries to take every opportunity to express your views in the regulatory process.

A recent example is a proposed rule published on August 5, 2004 in the Federal Register Vol. 69, #150 titled Medicare Program; Revisions to Payment Policies Under the Physician Fee Schedule for Calendar Year 2005. The rules will implement the new cardiovascular and diabetes screening provisions mandated by the Medicare Prescription Drug and Modernization Act of 2003 (MMA).

Section 613 – Diabetes Screening
ASCLS concurs with the CMS definition of “pre-diabetes” as stated in 410.18(a). This definition is consistent with the statements of the American Diabetes Association and the National Institute for Diabetes and Digestive and Kidney Diseases. In response to the request for suggestions to define “a family history of diabetes”, we believe that CMS should use first-degree relatives, i.e., parents, siblings, and children to define family. The genetic links for the inheritance of a predisposition to diabetes have been found to be very direct. Risk factors for Type 1 and Type 2 diabetes have been documented to be inherited from first degree relatives. While we recognize that environment, lifestyle, and ethnicity affect the prevalence of manifesting the disease, the correlation to first degree inheritance still holds.

In 410.18(d) Amount of testing covered, CMS is proposing that individuals diagnosed with pre-diabetes be screened twice per year and all others who qualify be screened once a year. We believe that the screening should be standardized to twice per year for all Medicare recipients for two reasons:

• The process that would be necessary to separate these two populations will be an administrative burden for laboratories. Clinical laboratories will not know whether the patient has pre-diabetes unless that patient has been tested at that laboratory in the past. The ICD-9-CM code being proposed, while correct for this screening, does not indicate that the patient has pre-diabetes and will be the only information the laboratory will have on most of these patients.

• It is our reading of the regulation that CMS is not including patients with risk factors in the population that will be screened twice per year. This is the population that is in most need of careful screening to monitor their potential transition to pre-diabetes and possibly to full disease. The sooner patients at risk are identified as pre-diabetic, the sooner modifications can be made that might avoid the onset of disease.

Section 612 – Cardiovascular Screening Blood Tests
As pertains to 410.17(a), as we stated in our joint comments with AACC in January of this year, we believe that Congress’s intent in MMA was that Medicare only reimburse for scientifically valid screening measures. We realize that the language of MMA states that HHS should also include those tests that
indicate an elevated risk of cardiovascular disease as long as they have been endorsed by the United States Preventive Services Task Force (USPSTF). However, we believe that the body of evidence outlining what constitutes appropriate screening tests for cardiovascular risk has changed since the USPSTF issued its guidance in 2001.

Over the past few years, a significant body of literature has been published indicating that high sensitivity C-reactive protein (hsCRP) is a key measure for assessing an individual's risk of heart disease. (Again, this data was published after the most recent USPSTF study in this area.) Only last year, AHA/CDC issued a Class IIa recommendation stating that hsCRP measurements for risk stratification add important information to the 'classic' cholesterol and HDL measurement. Thus, we urge CMS to include this measure in its initial list of 'approved' screening tests. If not, ASCLS requests that CMS immediately ask USPSTF conduct a formal review of hsCRP as a screening test. In addition, when appropriate, ASCLS will initiate a similar request through the National Coverage Determination Process.

We do not agree with the limit of five years found in 410.17(c) Limitation on coverage of cardiovascular screening tests. We believe that CMS should outline risk factors similar to those delineated for diabetes, such as cigarette smoking, hypertension, physical inactivity, obesity, etc., to identify that portion of the Medicare population that should be monitored more closely. Individuals with increased risk who have not demonstrated hyperlipidemia should be monitored every two years as the statute provides. One mechanism for implementing this recommendation would be to allow the physician to order the proposed screening tests initially. Patients with abnormal results would qualify for appropriate diagnostic (non-screening) tests as medically necessary. Patients with normal results but other risk factors such as family history, high blood pressure, etc. would qualify for screening again in two years. Patients with normal results and no risk factors would no longer qualify for the screening tests.

Again, any abnormal results would qualify the patient for appropriate follow-up diagnostic tests as medically necessary.

What will happen with the ASCLS comments regarding this particular proposed regulation? As of this writing, we don't know, but frequently our suggestions do become part of final regulations. It is ongoing, consistent participation in the process that gives ASCLS a recognized presence in the regulatory process.
Importance of Transplantation History in ABO Discrepancies

CHRISTINA THOMPSON, MARIANELA NEAL, NICOLE PETERS

ABBREVIATIONS: BMT = bone marrow transplantation; PBSCT = peripheral blood stem cell transplantation.

INDEX TERMS: ABO and organ transplant; ABO and stem cell transplant; ABO discrepancy.


Christina Thompson EdD CLS(NCA) MT(ASCP)SBB is Clinical Laboratory Sciences Program Director, Texas A&M University-Corpus Christi, Corpus Christi TX.

Marianela Neal MT(ASCP)SBB is Blood Bank Supervisor, Christus Spohn Shoreline, Corpus Christi TX.

Nicole Peters MT(ASCP) works in the Blood Bank, Christus Spohn Shoreline, Corpus Christi TX.

Address for correspondence: Christina Thompson CLS(NCA) MT(ASCP) SBB, Clinical Laboratory Sciences Program, Texas A&M University-Corpus Christi, 6300 Ocean Dr, Corpus Christi TX 78412. (361)-825-2473, (361) 825-3719 (fax). cthomp@falcon.tamucc.edu

A complete history is important for the solution of blood bank typing problems and, with the increasing numbers of transplantation procedures, a patient’s history may include transplantation at major medical centers with a subsequent return to the local community hospital. If the recipient and donor are different ABO blood types, both stem cell and organ transplantation demonstrate challenging considerations for transfusion support both during and after the procedure. Two cases are presented to demonstrate the importance of a complete history for the solution of ABO typing problems.

Table 1. Case 1 – current and historical ABO typing

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A cells</th>
<th>B cells</th>
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<tbody>
<tr>
<td>Current sample</td>
<td>3+</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>Historical results</td>
<td>Neg</td>
<td>4+</td>
<td>3+</td>
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Case 1
A 53-year-old male with a history of multiple myeloma was admitted to the hospital with shortness of breath and a cough. A request for two units of packed red blood cells was received by the blood bank. A review of the patient’s history showed diabetes and a stem cell transplant a year ago. Previous blood bank typing revealed a type B positive with no discrepancies. These records also showed that the patient had several previous admissions and had received transfusions of compatible B positive blood and components without complications. Because of the difference in the current blood type, A positive, and the previous type, the patient was recollected and given a second ID wrist band for confirmation.

Table 1 sample matched the current results and typed as A positive. Current and historical typing results are included in Table 1.

Case 2
A 57-year-old man was admitted to the hospital unconscious and in septic shock. The patient’s blood pressure at admission was 60/0 and laboratory results were: Hgb – 11.8 g/dL, Hct – 0.335 L/L, and WBC 52 x 10⁹/L. Two units of packed red blood cells were ordered to treat the combination of anemia and shock. Blood type results showed an A positive with a mixed field reaction in the Anti-A. The ‘mixed field’ results with anti-A sera showed one large agglutinate with ‘free cells’ in the background. Discussion with the family revealed the patient had been admitted to a hospital in another city two weeks prior for a liver transplant. A call to the transfusion service revealed that the patient’s original blood type was A positive and he had received 11 units of O positive blood, 14 units of fresh frozen plasma type A or AB, pooled cryoprecipitate, and type O pooled platelets.
DISCUSSION
Case 1 demonstrates ABO typing problems associated with bone marrow transplantation (BMT) and peripheral blood stem cell transplantation (PBSCT). Support for ABO mismatched allogeneic transplantations depends on whether the transplantation introduces a novel ABO antigen (major incompatibility), a novel ABO antibody (minor incompatibility), or both a novel ABO antigen and antibody (both major and minor incompatibility). In BMT and PBSCT, transfusion problems can be caused by antibodies present before transplantation or by antibodies developed after transplantation.1-8 Since recipient derived immunoglobulins present before transplantation may persist for weeks or months, even in the presence of engraftment from the donor, a major incompatibility (novel antigen) may cause a positive direct antiglobulin test (DAT) and allo-immune hemolytic anemia.2 Tasaki reported the presence of recipient antibody in a patient one year after transplantation.8 This patient with unexplained hemolysis demonstrated the new blood type and a negative DAT; however, recipient antibody was present in an eluate performed on the red cells.

Red cell transfusion for BMT or PBSCT with major incompatibility (novel antigen) should initially be type O with a change to the donor type when the recipient’s original antibodies disappear. If hemolysis is suspected, a DAT and an elution should be performed. The elution should be performed even if the DAT is negative and the eluate tested with A, B, and O cells to demonstrate the presence of ABO antibodies as well as other red cell antibodies.1-3,8

Minor ABO mismatched BMT or PBSCT (novel antibody) may be associated with immediate or delayed hemolysis. Immediate hemolysis is a result of donor plasma present in the transplant and delayed hemolysis is the result of donor lymphocyte induced antibody against the recipient’s antigens.3 Delayed hemolysis may be more common in PBSCT than in BMT because more lymphocytes are present in the transplant.4 As in the case of major incompatibility, if delayed hemolysis is suspected, a DAT and elution should be performed and tested with A, B, and O cells.1-3,7 A relatively high return of host-type lymphopoiesis and/or host-type hematopoiesis can occur following allogeneic bone marrow transplants and are referred to as “mixed chimeras.” Mixed chimeras have a two-cell population—one from the recipient and one from the donor—and demonstrate a mixed cell blood typing reaction. However, these patients don’t appear to have other immunohematologic complications.2

The patient in Case 1 was originally B positive and, after myeloablation, had received PBSCT from an A positive donor. This case is an example of major and minor incompatibility (novel antigen and novel antibody). Initial red cell transfusions should be type O with a change to the donor type when recipient antibodies disappear. After a year, the patient’s blood type had changed to the donor type, A positive. If the patient had any indication of immune hemolysis or the presence of anti-A in the reverse type or eluate, type O cells would be recommended for transfusion. There was no indication of hemolysis in this case. The patient had a negative direct antiglobulin test and elution. In addition, the reverse type demonstrated a weak anti-B with no anti-A. Type A blood was transfused to the patient with no complications.

Case 2 demonstrates problems associated with an ABO mismatched organ transplant. Although ABO identical organs demonstrate the best survival, group O organs are often transplanted to recipients with other ABO types. ABO mismatch with organ transplantation presents different problems than BMT or PBSCT since all incompatibilities are minor incompatibilities involving novel antibodies. Donor lymphocytes in the new organ or in tissue transplanted with the new organ may continue to produce ABO or other allo-antibodies and may cause hemolysis if incompatible with the recipient’s red blood cells. These donor lymphocytes and the potential for hemolysis may persist for weeks.9-11 The frequency and severity of graft antibodies generally increase in patients treated for immunosuppression with cyclosporine only and with the amount of lymphoid content present in the donor organ.10,11 Because of the potential for hemolysis, type O red blood cells are indicated for transfusion before, during, and immediately after ABO incompatible organ transplant.1,11 Donor antibodies and hemolysis have been detected over a year after transplantation. Therefore, if hemolysis is suspected, a DAT and eluate should be performed.

In Case 2, the patient had a negative DAT and eluate and there was no indication of hemolysis. However, since the patient had received a liver transplant just two weeks prior to this admission, the possibility still existed that delayed hemolysis could develop due to antibodies produced by donor lymphocytes. The transfusion facility chose to crossmatch and transfuse type O red cells. The patient received two units of packed red blood cells with no complications.

These cases demonstrate the importance of a complete history from patients for resolution of ABO typing problems. Obtaining the history has become more difficult because...
patients receive treatment from a variety of specialists and transplantation services and may return to the local community hospital for treatment of complications. The transfusion facility may need to contact previous physicians, medical facilities, or the patient’s family, as well as obtain information from current and previous admissions. Although it may be difficult to obtain a complete history, these two cases demonstrate the necessity of complete investigation to solve typing problems and to provide the optimum blood component for transfusion.

REFERENCES
OBJECTIVE: The aging population will likely have a major impact on laboratory utilization. Utilization data will be necessary for laboratory managers to make informed decisions concerning staffing patterns and services offered.

DESIGN: In a retrospective non-descriptive study, the relationships among age groups, hospital type, diagnosis, and the numbers and types of laboratory tests performed were investigated.

SETTING: Half of the participants were from a private hospital, Touro Infirmary, and half were from a large public hospital, The Medical Center of Louisiana at New Orleans. Both facilities are located in New Orleans, Louisiana.

PATIENTS: Laboratory records from a random sample of 250 inpatients age 21 to 64, a sample from 250 inpatients age 65 to 84, and a sample from 250 inpatients age 85 and over with at least one of five admission or discharge diagnoses were analyzed.

INTERVENTIONS: Twenty-five records from each of the five diagnostic categories for each of the three age groups and two hospital types were analyzed, yielding a total sample of 750 records.

MAIN OUTCOME MEASURES: Laboratory tests for each inpatient stay were counted and categorized for analysis. The one-way ANOVA was used to test the degree of concordance between age groups and numbers of tests ordered and between age groups and types of tests ordered across hospital types.

RESULTS: Data analysis showed statistically significant differences in the total number of laboratory tests ordered for the three age groups regardless of facility ($p < 0.008$). The age group with the highest number of total laboratory tests ordered was the group aged 65 to 84 (48.64 mean tests per patient). Across the total sample, more tests were ordered at the public facility than the private facility (51.75 and 32.42 mean tests per patient, respectively). Statistically significant differences in orders between the two facilities were noted in chemistry, hematology, and toxicology ($p < 0.001$). When analyzing numbers of tests by age group and facility, no statistically significant differences were noted in any laboratory category. Analysis of disease and laboratory test categories, regardless of facility, showed statistically significant differences in numbers of tests ordered in microbiology, cytology, histology ($p < 0.001$), and blood bank ($p < 0.001$). When analyzing numbers of tests by disease category and facility, significant correlation was noted in toxicology ($p < 0.001$).

CONCLUSION: This research allowed comparisons in laboratory utilization between a private and a public hospital among different age groups. Differences were noted in both volume and type of laboratory tests ordered on patients with specific diagnoses in the two facilities. Although comorbidity was not well controlled for, the study does suggest that clinical laboratories may undergo changes in utilization as our nation's population ages.


INDEX TERMS: clinical laboratory; diagnosis; geriatrics; laboratory tests.


Joette Beregi Taylor MHS CLS (NCA) was a clinical chemist at Touro Infirmary, New Orleans LA at the time this article was written.

Address for correspondence: Joette Beregi Taylor MHS CLS (NCA), 14050 Peairs Road, Zachary LA 70791. (225) 654-6117, (225) 654-4696 (fax). jberegi@worldnet.att.net
As a new century begins, the United States faces a fast-approaching situation that many other nations worldwide have been dealing with for some time—a graying population. Since the beginning of the last century, the number of persons under the age of 65 has increased three-fold, while the number of persons age 65 and over has increased eleven-fold. In the last 35 years, the population of those 65 and older has increased by approximately 82%. With the majority of the baby boomer generation over 50 years of age, it is projected that by the year 2030, 21% of the U.S. population will be age 65 or older. Part of this group, those individuals age 85 and older, comprises the fastest growing segment of our population. It is expected that by 2030, these oldest individuals will be close to nine million in number (Figure 1).

With the extension of life expectancy, the healthcare industry must strive to expand the quantity of life, as well as to maintain quality of life. Living longer theoretically exposes individuals to disabilities and chronic illnesses that burden our healthcare system. In both 1998 and 1999, the rate of hospital stays nationwide was more than three times higher for individuals age 65 and over as compared to those age 45 to 64, and over four times higher compared to those age 15 to 44. For those age 85 and over, their rate of hospital stays is almost six times higher than those age 45 to 64, and almost eight times higher than those age 15 to 44. In addition to the number of hospital stays, the elderly population’s average length of stay in the hospital is longer than any other segment of the population. These same statistics point to five conditions that most often affect the population age 65 and over, resulting in long lengths of stay in hospitals: heart disease, pneumonia, cerebrovascular disease, malignant neoplasms, and fractures.

One reason given by physicians for ordering laboratory tests on patients is monitoring. Generally, the longer a patient stays in the hospital, the more the patient’s health is monitored. Another factor that appears to influence the number of tests ordered is the type of hospital, with more laboratory tests ordered in teaching hospitals than in private hospitals. With an increase in the number of older Americans and an expected increase in the prevalence of chronic disease and hospital stays, laboratorians might question how these trends will impact the hospital-based clinical laboratory. Clinical laboratories provide the largest portion of quantitative data that is used in the treatment of patients; therefore, it is imperative that hospitals have access to the most complete and appropriate laboratory services in order to treat patients efficiently while thriving in the competitive healthcare marketplace. To thrive, however, laboratories must be prepared to focus their efforts and expand their services strategically. In an age when laboratories are rarely considered profit centers of hospitals, the laboratory workforce is decreasing nationwide. While technology continues to expand the possibilities of laboratory services, administrators and laboratory managers need access to information that can impact the organization and operation of clinical laboratories.

In recent years, only a few studies have focused on specific diseases and their volume and cost impacts on the clinical laboratory. No studies were found that specifically focused on the impact of a geriatric population on the laboratory, both in volume and in the types of tests utilized in providing care for this population. Previous research has shown laboratory costs associated with certain diseases and laboratory usage in teaching versus private facilities, but these studies did not investigate whether or not patient age played a role in the use of clinical laboratory tests. This information is vital to hospital administration planning efforts.

For the purposes of this study, teaching hospital shall be defined as a facility that is university-based, in which the majority of tests are ordered by interns or residents. Private hospital shall be defined as a community-based hospital, in which the majority of tests are ordered by attending physicians. Laboratory test shall be defined as an ordered procedure classifiable into one of the following categories: chemistry, hematology/coagulation, immunology, microbiology, histology, cytology, toxicology, and blood bank. Length of stay shall be defined as the number of days a patient remains in the hospital, from the date of admission to the date of discharge.

In this study, the researcher explored whether differences existed in numbers and types of laboratory tests ordered for younger and older segments of the population who have one or more of five conditions. The researcher also explored whether differences existed in laboratory tests for these popu-

![Figure 1. Number of persons in US in millions](image)
lations in public versus private hospitals. The research was accomplished by comparing laboratory records from patients of different age groups from both a public and a private hospital located in the same urban geographic area. The following research questions were posed in this study: 1) Do differences exist in numbers of clinical laboratory tests ordered for an adult population under the age of 65, for an adult population age 65 to 84, and for an adult population age 85 and over with specific diagnoses for inpatient stays at public and private hospital facilities? 2) Do differences exist in the types of clinical laboratory tests ordered for these three populations across the two types of hospital facilities? 3) Do differences exist in comorbidity and length of stay for these three populations across the two types of hospital facilities? 4) Does a difference exist in total number of tests ordered between the two types of facilities? 5) Do differences exist in types of laboratory tests ordered between the two types of facilities?

MATERIALS AND METHODS

Research design
A retrospective, non-descriptive design (chart review) was used to examine the relationships among age groups, hospital types, diagnoses, and numbers and types of clinical laboratory tests ordered. Two facilities were used to obtain data, one a private, not-for-profit facility staffing over 300 beds (Facility 1), and the other a teaching hospital servicing two Schools of Medicine with two sites that staff approximately 850 total beds (Facility 2). For purposes of this study, the facility’s two sites were considered one teaching hospital. The sample consisted of 750 inpatient laboratory reports equally divided between the two facilities. Laboratory records eligible for inclusion were those associated with individuals who were 21 years of age or older and were patients at either of the two facilities within a three year period from August, 1999 through July, 2002.

Objects of measurement
The objects of measurement for this study consisted of 750 laboratory reports from patient records. Individuals, both male and female, associated with these patient records, were treated as inpatients at one of the two facilities included in the study within the three year period. Each patient was 21 years of age or older upon admittance to the facility and had at least one admission or discharge ICD-9 diagnosis being studied. Based on a search of journal articles and national healthcare statistics, the researcher chose these diagnoses for this study due to prevalence among a population age 65 and over. These diagnosis categories include the following:

1. Heart disease—including acute myocardial infarction, coronary atherosclerosis, ischemic heart disease, cardiac dysrhythmias, and congestive heart failure (ICD-9-CM codes 391-392.0, 393-398, 402, 404, 410-416, and 420-429);
2. Pneumonia—including infectious (viral, bacterial, and fungal) and aspiration pneumonias (ICD-9-CM codes 480-486, 487.0, and 507);
3. Cerebrovascular disease—including stroke, Alzheimer’s disease, and cerebral aneurysm (ICD-9-CM codes 430-438);
4. Malignant neoplasms—including all organ systems (ICD-9-CM codes 140-208 and 230-234); and
5. Fractures—including all body sites (ICD-9-CM codes 800-829).

These diagnoses are based on the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM).8 The diagnosis categories were made broad enough as to ensure an adequate sample size for the study.

Patients meeting the inclusion criteria were divided into three subgroups: those between the ages of 21 and 64 at the time of admission to the hospital (n = 250), those between the ages of 65 and 84 at the time of admission (n = 250), and those age 85 and over at the time of admission (n = 250). Within each of the three subgroups from each of the two facilities, approximately 25 patient records from each of the five diagnosis categories were randomly chosen for the study. This yielded a sample of 75 patient records per diagnosis (375 records per facility). Patients having more than one diagnosis from those included in this study were grouped according to the primary diagnosis coded for their stay. All secondary diagnoses, including those from the five groups in this study and all other diagnoses, were recorded by number as comorbid conditions.

Measurement
For each inpatient stay selected, the number and type of clinical laboratory tests, along with certain individual tests, were recorded. These individual tests were those that could have a high impact on a geriatric hospital population. Tests were grouped into one of the following categories:

1. Chemistry—including endocrinology and osmolarity. Individual tests and groups of tests recorded included basic metabolic panel, comprehensive metabolic panel, electrolytes, lipids, hepatic function panel, glucose, blood urea nitrogen, creatinine, calcium, creatine kinase isoenzymes, troponin isoenzymes, thyroid stimulating hormone, hemoglobin A1C, albumin, prealbumin, and serum iron.
2. Hematology/Coagulation—including urinalysis and hemoglobin electrophoresis. Individual tests included complete blood count with differential, urinalysis, activated partial thrombin time, prothrombin time, and fibrin split products.

3. Immunology—including complements components and protein electrophoresis. Individual tests included hepatitis B antigens and antibodies, hepatitis C antibody, human immunodeficiency virus, rheumatoid factor, serum protein electrophoresis, and antinuclear antibodies.

4. Microbiology—including parasitology, mycology, and virology. Individual tests included bacterial cultures for wounds, blood, urine, sputum/tracheal, and acid-fast bacilli; Clostridium difficile toxin; and general viral cultures.

5. Cytology—Individual tests included the papanicolaou stain.

6. Histology/Surgical Pathology—Individual tests included the hematoxylin and eosin stain and frozen sections.


8. Toxicology—Individual tests included digoxin, vancomycin, and phenytoin.

Each category included any molecular testing which would have fallen into these areas. Point-of-care and respiratory testing (arterial blood gases) were not included in this study.

Procedures
Approval from the Institutional Review Board of the sponsoring institution, as well as approval from the Institutional Review Board of each hospital was obtained prior to data collection. The sample was chosen randomly from reports generated by the facilities’ medical records systems. These reports were generated based on the above-mentioned inclusion criteria of the sample and on the diagnoses of the patients and therefore included all possible patients who met the criteria.

Those participants’ laboratory encounters were then accessed through the laboratories’ information systems. Tests were recorded in appropriate categories according to patient age group, type of hospital, patient diagnosis, and type of test requested for each patient hospital stay. All results were recorded on a hand-written form and then transferred to a computer spreadsheet application.

Once data were collected for each patient stay, any information that could potentially identify the patient was removed and replaced with a number that was in no way associated with the patient’s true identity. Strict patient confidentiality was adhered to. Obviously duplicate tests and canceled requests were not included in this study.

An independent clinical laboratory scientist from each facility performed an interrater reliability study by completing laboratory data collection forms on ten percent of the sample from each facility. These two individuals were trained by the researcher. Patient reports involved in this study were chosen at random, and reliability was calculated as a percentage (agreement divided by agreement plus disagreement).

Data analysis
Data were analyzed using both descriptive and inferential statistics. Descriptive statistics were used to describe the profiles of the subjects, such as gender, age, diagnosis, comorbid conditions, and length of hospital stay. The one-way ANOVA was used to test the degree of concordance between age groups and numbers of tests ordered and between age groups and types of tests ordered across hospital types. Data analysis was performed using SPSS software version 11.0.

RESULTS
A sample population of 375 patients from each hospital was randomly selected from all possible candidates who met the study qualifications. All laboratory data were collected and categorized by the researcher for each patient’s total stay with the qualifying ICD-9 diagnosis.

Interrater reliability
Ten percent of the data from each facility was analyzed for interrater reliability. The interrater reliability was 98.2% at the private facility and 96.3% at the public facility.

Facility comparisons
Gender distribution varied little between the two facilities, with 62.1% female patients at the private facility (Facility 1) and 56.8% females at the public facility (Facility 2). Mean ages were slightly higher at Facility 1 among all three age groups, with the biggest difference noted in the 21 to 64 year olds (50.9 years at Facility 1 and 44.9 years at Facility 2). Length of stay differences between the two facilities were statistically significant with 13.31 days at Facility 1 and 8.86 days at Facility 2 (p 0.003). Statistically significant differences in length of stay were also found among the five disease categories, irrespective of facility (p 0.033). There were no statistically significant differences in lengths of stay among the three age groups, irrespective of facility (p 0.435). Differences in comorbidity between the two facility types were not statistically significant (p 0.093) (Table 1).
Test categories
The mean number of total laboratory tests per patient was 42.09 for both facilities combined. By individual facility, patients at Facility 1 had 32.42 tests ordered for their stay, and those at Facility 2 had an average of 51.75 tests ordered for their stay. Among the eight laboratory categories, larger numbers of tests were ordered at Facility 2 than Facility 1 with the exceptions of microbiology and histology. Significant differences were seen in chemistry ($p < 0.001$), hematology ($p < 0.001$), and toxicology ($p < 0.001$) (Table 2). Among the individual tests in the chemistry category, more comprehensive metabolic profiles were ordered at Facility 1 than Facility 2 (mean of 1.54 at Facility 1 and 0.80 at Facility 2) while basic metabolic profiles were utilized more often at Facility 2 than Facility 1 (6.67 and 4.44, respectively). In addition, cardiac en-

Table 1. Facility comparisons

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</tr>
</thead>
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<td>(public)</td>
</tr>
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<td>Male (%)</td>
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<td>Female (%)</td>
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<td>Mean age years (85 and over)</td>
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<td>89.26</td>
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<td>Mean total length of stay (days)</td>
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</tr>
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<td>Pneumonia</td>
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<td>Cerebrovascular disease</td>
<td>14.28</td>
<td>8.73</td>
</tr>
<tr>
<td>Neoplasms</td>
<td>15.81</td>
<td>12.21</td>
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<td>Mean additional ICD-9 codes</td>
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Table 2. Mean tests by laboratory category

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</thead>
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<td>26.82</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Hematology</td>
<td>10.05</td>
<td>13.95</td>
<td>&lt;0.001*</td>
</tr>
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<td>Immunology</td>
<td>0.96</td>
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<td>Microbiology</td>
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<td>Cytology</td>
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<td>0.32</td>
<td>0.020</td>
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<td>Histology</td>
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<td>1.19</td>
<td>0.651</td>
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<td>Blood Bank</td>
<td>1.75</td>
<td>2.30</td>
<td>0.064</td>
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<tr>
<td>Toxicology</td>
<td>1.19</td>
<td>2.98</td>
<td>&lt;0.001*</td>
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* Statistical significance at alpha = 0.006

Table 3. Age group and mean laboratory test analysis

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<th>Facility 2</th>
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<td>Age 65-84</td>
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<td>Age 85 and over</td>
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<td>Hematology (total)</td>
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<td>13.95</td>
<td>0.614</td>
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<td>13.83</td>
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<td>Age 65-84</td>
<td>11.26</td>
<td>16.55</td>
<td></td>
</tr>
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<td>Age 85 and over</td>
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<td>0.778</td>
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<td>Age 65-84</td>
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</tr>
<tr>
<td>Age 85 and over</td>
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<td>0.59</td>
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</tr>
<tr>
<td>Microbiology (total)</td>
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<td>Age 21-64</td>
<td>4.32</td>
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<tr>
<td>Age 65-84</td>
<td>4.46</td>
<td>3.26</td>
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</tr>
<tr>
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<td>3.62</td>
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</tr>
<tr>
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<tr>
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<tr>
<td>Age 85 and over</td>
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<td>1.08</td>
<td>0.95</td>
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<tr>
<td>Blood Bank (total)</td>
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<td>0.744</td>
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<td>1.92</td>
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<td>Toxicology (total)</td>
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<td>2.98</td>
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<tr>
<td>Age 85 and over</td>
<td>1.28</td>
<td>2.10</td>
<td></td>
</tr>
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Computed using alpha = 0.006
* facility/age group
zymes were ordered more than three times more often at Facility 2 than Facility 1. Among the individual tests in the hematology category, mean numbers of tests ordered were higher at Facility 2 for all tests with the exception of fibrin split products, which were equal at 0.04 tests.

**Age**

Among the three age groups, differences were noted in the numbers of tests ordered. The mean numbers of tests were higher for the 65 to 84 year age group in many categories with the exceptions of cytology and histology, with statistically significant differences found in hematology ($p < 0.004$). When analyzing numbers of tests by age group and facility, no significant differences were noted (Table 3).

**Disease categories**

Among the five disease categories, the laboratory was utilized in various ways. Tests for patients with heart disease were most often associated with chemistry (22.24 tests per patient), while microbiology and immunology tests were often associated with patients with pneumonia (5.94 and 1.49 tests per patient, respectively). A majority of toxicology orders were seen in patients with cerebrovascular disease (2.89 tests per patient), and neoplasm was most often associated with chemistry and hematology (22.09 and 14.23 tests per patient, respectively). Cytology and histology tests were most often ordered on patients with neoplasms (0.61 and 4.17 tests per patient, respectively), and the blood bank was most often utilized in cases involving fractures (2.84 tests per patient).

Analyzing disease and test categories, statistically significant differences in ordered tests were noted in microbiology, cytology, histology ($p < 0.001$), and

---

**Table 4. Disease category and mean laboratory test analysis**

<table>
<thead>
<tr>
<th>Test Category</th>
<th>Facility 1</th>
<th>Facility 2</th>
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<tr>
<td>Chemistry (total)</td>
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<td>Pneumonia</td>
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<td>26.29</td>
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<tr>
<td>Cerebrovascular disease</td>
<td>14.59</td>
<td>26.89</td>
<td></td>
</tr>
<tr>
<td>Neoplasms</td>
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<td>30.65</td>
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<tr>
<td>Fractures</td>
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<td>Fractures</td>
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<tr>
<td>Cerebrovascular disease</td>
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<td>Neoplasms</td>
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<td>Fractures</td>
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<td>Fractures</td>
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<td>Cytology (total)</td>
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Statistical significance at alpha = 0.006

* facility/disease category
blood bank ($p \ 0.001$). When analyzing tests by disease category and facility, statistically significant differences in ordered tests were noted in toxicology ($p \ 0.001$) (Table 4).

**DISCUSSION**

The aim of this study was to determine the effects of hospital type and age on laboratory utilization in patients with specific diagnoses. Even though only two facilities were used in the study, the researcher was able to make comparisons between a private hospital of moderate size and a larger, public teaching hospital.

The significant difference in lengths of stay between the private hospital and the public hospital was surprising. Previous research has shown no significant difference in lengths of stay between private and public facilities, but in this study, the average length of stay was 13.31 days at the private facility and 8.86 days at the public facility, with the average length of stay for the entire sample being 11.08 days.6 It was expected that the figure would be higher than the national average of 5.2 days due to the disease categories that were chosen for examination. The 13.31 day average stay at Facility 1, however, was unexpectedly high. One possible explanation could be comorbidity, which was not well controlled for in this study. Like past research, the lengths of stay were slightly longer at both facilities for patients age 65 to 84. The average stay for this group was 11.02 days, which is also higher than the national average of 6.5 days for this age group. In addition, this study also found that patients diagnosed with neoplasms had slightly longer lengths of stay at both facilities than the other four disease categories.

No significant difference was found in the numbers of additional diagnoses for each patient between the two facilities. One of the weaknesses of this study was an inability to control for comorbidity. The study does include the number of additional ICD-9 diagnoses for each patient (in addition to the qualifying diagnosis), but the types and severity of these additional diagnoses were not included. Perhaps future research can include this information, as comorbidity has been shown to affect length of stay, and thus, utilization of hospital resources, including the laboratory.

Previous research showed that the laboratory was utilized more frequently in public hospitals, and that was also the finding in this study.6 The average patient at the private facility (Facility 1) had 32.42 laboratory tests ordered for his/her stay, as opposed to 51.75 tests at the public (Facility 2). This study also found that the total number of laboratory tests ordered on patients age 65 to 84 was significantly higher than the other two age groups, regardless of facility type. Surprisingly, this study showed that the lowest number of tests ordered was for patients age 85 and over. Their average of 35.36 tests was lower than the 42.25 tests for the patients age 21 to 64. One possible explanation for this finding could be less aggressive actions taken by physicians in managing patient care for those age 85 and over. No significant differences were found in total tests ordered among different disease categories, although as in past studies, the highest numbers of tests were ordered at both facilities on patients with neoplasms.

As expected, chemistry and hematology were the most often utilized departments in the laboratory, with 19.86 and 12.00 tests per patient, respectively. Of the eight main test categories, chemistry, hematology, and toxicology showed a significant difference in the number of tests ordered across the two facilities. In the cases of chemistry and hematology, these findings can probably be explained by the sheer volume of tests ordered in these two categories. In the case of toxicology, the difference may be explained by the disparity in orderable in-house tests available at the facilities. Facility 1 offered only a qualitative overdose procedure, which could be sent out for confirmation of those analytes found to be positive. At Facility 2, individual procedures were offered for the drugs of abuse, along with confirmations for those analytes. Toxicology was the only department in which this disparity in orderable tests was found in this research.

When age was studied in relation to the types of tests being ordered regardless of facility type, the researcher found that the largest number of tests by department were ordered on patients age 65 to 84 in six of the eight categories: chemistry, hematology, immunology, microbiology, blood bank, and toxicology. Of the six, only hematology showed a significant difference in the number of tests ordered among the three age groups. The difference in chemistry, although not significant, was substantial. In the other four areas, the volume of tests ordered was simply not large enough from which to draw any conclusions. When age groups were analyzed in conjunction with facility, no statistically significant differences were found in the numbers of tests ordered by category. The high utilization of the clinical laboratory by those patients age 65 to 84 cannot be explained with one answer. Nationwide, this age group has longer lengths of stay, higher incidence of chronic disease, and larger numbers of comorbid conditions, all of which probably play a part in higher laboratory utilization. In both cytology and histology, the largest numbers of tests were ordered on those patients age 21 to 64. In this study, these
two departments were almost exclusively linked to neoplasms. Perhaps more aggressive measures were taken to treat the younger age group with malignancies, and thus cytology and histology were more widely utilized in this group. When analyzing types of tests ordered on patients with specific diagnoses, significant differences were found in several areas of the laboratory, including cytology, histology, and blood bank. As expected, cytology and histology were most often associated with malignant neoplasms. In the blood bank, the largest numbers of tests were ordered on those patients diagnosed with fractures. The researcher can speculate that this finding is tied to surgical procedures that are often necessary to correct fractures. When facility is factored into the analysis of tests ordered on patients with specific diagnoses, significance was found only in the area of toxicology; however, again, one should not draw many conclusions from this finding because of the disparity in available tests at the two facilities.

The most disappointing results of this research are associated with the individual tests that were categorized for each of the eight main areas of the laboratory. Very few values stand out when reviewing the utilization of these individual tests in the two facilities. The comprehensive metabolic profile was ordered almost twice as much at Facility 1, despite the fact that twice as many chemistry tests were ordered at Facility 2. In addition, cardiac enzymes were ordered more than twice as often at Facility 2 than Facility 1. The researcher expected to find a higher number of these tests at Facility 1 where the length of stay was longer for patients with heart disease, but perhaps the finding can be explained by Facility 2’s status as a teaching hospital. If future research is conducted in the area of laboratory utilization, it should perhaps include a much smaller number of individual tests in each category, if any, for analysis.

CONCLUSION
Differences were found in both volume and type of laboratory tests ordered on patients with specific diagnoses in two types of inpatient facilities. After extensive literature review, the researcher believes that this was the first study that specifically looked at the impact of age on the entire hospital laboratory and its main departments. These results do seem significant enough to warrant interest in the utilization of clinical laboratory resources in the coming years. Perhaps a future study could include point-of-care testing and could better control for comorbidity. Additionally, research in this area should include studying rural versus urban community hospitals and a study of the number and type of clinical laboratory scientists who will be necessary to efficiently operate our nation’s hospital laboratories.

REFERENCES
Factors Contributing to the Retention of Clinical Laboratory Personnel

KATHY DOIG, SUSAN BECK

OBJECTIVE: To identify factors contributing to retention of clinical laboratory practitioners.

DESIGN: A paper survey addressing retention was distributed to a potential of 4000 clinical laboratory professionals.

SETTING: The survey was distributed to subjects by their laboratory manager to be completed at the worksite or home.

PATIENTS OR OTHER PARTICIPANTS: 599 usable surveys were received from non-supervisory individuals employed in clinical laboratory science (CLS) for five years or more.

INTERVENTIONS: Surveys were mailed to laboratory managers in March 2003 with directions to distribute to practitioners with five or more years of work experience.

MAIN OUTCOME MEASURES: Percentages of respondents agreeing and disagreeing with Lickert-type opinion items were determined. The means, ranges, and standard deviations were calculated for the number of hours of continuing education, years of experience, percentage of time spent on tasks, and years in the current job. The means for job satisfaction were calculated and compared statistically based on respondents' job function, satisfaction with salary, job independence, sense of appreciation, and responsibility for continuing education. Open-ended responses were tabulated and categorized.

RESULTS: Committed practitioners believe their work is important and find it challenging. Those who are most satisfied with their jobs believe they make a good salary ($p = 0.000$), have work independence ($p = 0.000$), and feel that their work is appreciated ($p = 0.000$). Job satisfaction does not differ for CLTs vs. CLSs. Salaries comparable to nurses and appreciation from physicians, nurses, and hospital administrators are cited by respondents as the most important factors to retaining laboratory staff.

CONCLUSION: Committed practitioners believe that salaries comparable to nurses are needed to improve retention of staff. Respondents said that being appreciated by hospital administrators, nurses, and physicians would also contribute to improved retention.

ABBREVIATIONS: ASCLS = American Society for Clinical Laboratory Science; ASCP = American Society of Clinical Pathology; CE = continuing education; CLMA = Clinical Laboratory Managers Association; CLS = clinical laboratory science; CLSs = clinical laboratory scientists; CLTs = clinical laboratory technicians.

INDEX TERMS: clinical laboratory manpower; clinical laboratory techniques; job satisfaction; medical technology; retention.


Kathy Doig PhD CLS(NCA) CLSp(H) is at Michigan State University, E Lansing MI.

Susan Beck PhD CLS(NCA) is at The University of North Carolina at Chapel Hill, Chapel Hill NC.

Address for correspondence: Kathy Doig PhD CLS(NCA) CLSp(H), Medical Technology Program, Michigan State University, 322 N. Kedzie Hall, E Lansing MI 48824-1031. (517) 353-7800 x 8, (517) 432-2006 (fax). doig@msu.edu

For the clinical laboratory profession, the first years of the 21st century have been marked by a shortage of qualified personnel. While the Bureau of Labor Statistics projects an annual need for 9000 laboratorians through 2008, the number of graduates of clinical laboratory technician (CLT) and clinical laboratory science (CLS) programs is only half of that. The current personnel shortage has exceeded the shortage of the early 1990s and it has a slightly different flavor. The average age of laboratorians is now believed to be in the late 40s (unpublished data, 2004) and more of the workforce is closer to retirement than it was a decade ago. Impending retirements will exacerbate the shortage and future-oriented laboratory managers have reason to be more concerned about this personnel shortage than they were in 1990. One approach to addressing the shortage is to attract new individuals to the laboratory professions. This task falls more heavily on faculty in educational programs than on laboratory managers and, even if full classes of CLT and CLS
students are recruited, it will not provide the number of graduates needed to alleviate the shortage.

For laboratory managers, the more immediate challenge is to retain staff, particularly the younger professionals, while recruiting qualified professionals to their institutions from those entering or already in the workforce. The benefits of retaining staff include:

- reduced training time, higher quality work, and hence, greater productivity,
- established and effective relationships among staff members that contribute to better working conditions and further encourage retention,
- cost savings through lower recruiting and training costs, and
- improved recruiting as prospective employees recognize a quality work environment.

**LITERATURE REVIEW**

In addition to the wage and vacancy surveys conducted every two years by the American Society for Clinical Pathology (ASCP) that document the shortage of laboratory professionals, the literature contains many reports addressing employment satisfaction and retention in the clinical laboratory. Factors contributing to job satisfaction of healthcare professionals include worker input to decisions, a safe and clean work environment, satisfaction with the work, good management, fair salary, and benefits. In 1989, Karni and Feikert summarized the research findings from 1970 to 1985 on clinical laboratory scientists’ (CLSs) job satisfaction. Limited opportunity for upward mobility and low pay were frequently mentioned as causes of dissatisfaction. From their study of laboratory managers, Karni and Feikert concluded that job satisfaction for CLSs required more opportunities for career advancement, increased salaries, a variety of duties, and control over one’s work methods and pace.

Several reports have included estimates of the level of job satisfaction in laboratory personnel. A study by Harmening in 1994 reported that over 75% of practitioners in a ten-year retrospective study of medical technologists were satisfied with their jobs.

A survey of women in the laboratory profession in 1992 reported that 78% were ‘satisfied’ and 9% were ‘extremely satisfied’ with their careers as laboratorians. In the mid-90s, a survey by Maher found that 87% of laboratorians were at least somewhat satisfied with their positions. In Maher’s survey, however, more than 50% of respondents reported that they had considered a career change and more than 60% would not advise young people to enter the profession.

Studies on the retention of laboratory personnel have focused on the causes of employee attrition and strategies to promote retention. A report by Hallam in 1990 listed low salaries, burn-out, and stress as the major reasons laboratory personnel left the profession. In a study of 1905 CLS graduates from the University of Minnesota, about 10% were inactive and 20% were retired, but an additional 40% had left the field, most often for family responsibilities. Other frequently cited reasons for leaving were returning to school, low wages, inadequate advancement opportunities, and lack of recognition.

Most articles on employee retention stress the importance of improving salaries; however, salary data for laboratory personnel document slow and minute progress in this area. Guilles and Lunz reported that, in 1992, salaries for CLSs were lower than salaries of nurses, physical therapists, occupational therapists, and teachers. According to the Bureau of Labor Statistics 2002 National Occupational Employment and Wage Estimates, the estimated mean salaries for clinical laboratory technologists and technicians were $43,670 and $30,330 respectively. Comparison with other healthcare professions indicates that salaries in the clinical laboratory are still lower than many healthcare professions including nursing ($49,840) dental hygienists ($57,790) and nuclear medicine technologists ($52,260). A study by Estry showed that salaries for CLTs and CLSs just kept pace with inflation between 1979 and 1989. A comparison of current salary figures with those 1989 salaries indicates that this is still the case. There have been no real gains in salaries for CLTs and CLSs since 1979 other than inflationary adjustments.

The relationship between job satisfaction and employee retention was analyzed by Lunz in a report of a longitudinal study of CLSs. This study categorized respondents’ commitment to a laboratory career using a seven-item survey and most respondents demonstrated moderate commitment. The study also surveyed the respondents’ satisfaction with employment benefits and found that those with the highest career commitment were also the most satisfied with their benefits. However, even those who were most committed were not very satisfied with their benefits. Blau analyzed this same data from a different perspective. They separated employment benefits into two groups: basic, e.g., sick leave, retirement, life insurance; and career enrichment, e.g., continuing education, flexible work schedules, reward for advanced degrees. They were able to show that, though related, the satisfaction with these types of benefits can be distinguished. Analogous to Herzberg’s distinction between hygiene and motivation factors, basic benefit satisfaction may keep employees from leaving their jobs, but career enrichment satisfaction is needed to get employees to commit to an organization.
RESEARCH AND REPORTS

This current study was conducted to focus on factors contributing to retention of laboratory personnel in the clinical laboratory at this time. CLT and CLS practitioners who had five or more years of experience were selected and surveyed to assess their views of their work, the reasons they stay in the profession, and the factors that they think are important for employee retention. Professional fundraisers have known for years that donors who have contributed money in the past are likely to give money again. So, fundraisers pay a great deal attention to donors who contribute on a regular basis. Similarly, surveying practitioners who have made a commitment to their careers in CLS may lead to a better understanding of how to instill commitment, thus leading to better retention.

MATERIALS AND METHODS

The researchers prepared a survey, list of definitions, and cover letter for clinical laboratory practitioners with questions based on a review of the literature on retention of laboratory staff. It included a set of 30 Likert–style statements designed to ascertain the opinions of the practitioners about their work and jobs (Table 1). Statements were phrased in the positive and negative to insure that subjects read each question carefully. Respondents were asked to indicate their views using the scale SA = strongly agree, A = agree, D = disagree, SD = strongly disagree.

The survey included demographic questions on practitioners’ geographic location, type of work facility, size of institution, primary job function, gender, ethnicity, highest degree, certification, and hours of continuing education attended each year. To group geographic locations, the American Society for Clinical Laboratory Science (ASCLS) regions were used. Participants were asked to indicate their level of satisfaction with their choice of a clinical laboratory career and the percentage of time they devoted to various tasks such as test performance and attending meetings. To assess factors related to retention of laboratory personnel, practitioners were also asked to indicate why they left their last job, what factors keep them in the profession, and their career goals. For each of these questions, the participants could chose from a list of options provided on the survey and write in additional comments. Finally, participants were asked to write their answer to the question, “What factors do you think are most important in retaining qualified clinical laboratory practitioners in the laboratory today?”

The survey, cover letter, and definitions were reviewed by an advisory board comprised of laboratory managers, practitioners, and educators. The survey distribution model was to send the survey to laboratory managers with the request that they distribute the surveys to five practitioners who had been working in the laboratory field for five years or more. This same distribution model was used to pilot test the surveys, cover letters, and definitions. The managers selected for pilot testing were a convenience sample of individuals known to the researchers. The results of the pilot study were reviewed and the survey was revised based on these suggestions. The survey and cover letters were approved by the University Committee on Research Involving Human Subjects of Michigan State University, E Lansing MI.

The managers selected for the study were identified from the mailing list of the Clinical Laboratory Managers Association (CLMA). They were selected by choosing every sixth name from the zip code sorted list. To maximize the likelihood that the survey recipient would be a laboratory manager, individuals whose place of employment or job title suggested they were not managing a laboratory were deleted. Eight hundred of the managers were selected for the final mailing with the potential for responses from 4000 practitioners. The surveys were sent in March 2003, with instructional cover letters and postage-paid return envelopes addressed to the researcher. Two weeks after sending the manager packets, a follow-up reminder postcard was sent to all managers receiving the survey packets.

DATA ANALYSIS

SPSX 11.5 was used to analyze the data collected in this study. Practitioners were defined as respondents with a primary job title of CLT or CLS and five or more years of experience. The frequency of the practitioners’ responses to each item was tabulated. For the 30 Lickert-type items addressing practitioners’ opinions on their work, the percentage of responses in each category (strongly agree, agree, disagree, and strongly disagree) was tabulated. To analyze the data, the percentage of responses in the agree and strongly agree categories were combined into one ‘agree’ category and the percent of responses in the disagree and strongly disagree categories were combined into one ‘disagree’ category. The means, ranges, and standard deviations were calculated for the number of hours of continuing education, years of experience, percentage of time spent on tasks, and years in the current job.

Job satisfaction was measured on a scale on which 1 = very satisfied, 2 = somewhat satisfied, 3 = somewhat dissatisfied, and 4 = very dissatisfied. The means for job satisfaction were calculated and compared for respondents based on their job function, satisfaction with salary, job independence, sense of appreciation, and responsibility for CE. Job function was based on the respondents’ self-identification as a CLT or CLS. Satisfaction with salary was based
Table 1. Practitioners’ opinions on statements related to their work and careers (n = 599)

<table>
<thead>
<tr>
<th>#</th>
<th>Statement</th>
<th>% Agree†</th>
<th>% Disagree‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>I believe my work is important.</td>
<td>99.8</td>
<td>0.2</td>
</tr>
<tr>
<td>19</td>
<td>My work does not add much to patient care.</td>
<td>3.2</td>
<td>96.8</td>
</tr>
<tr>
<td>16</td>
<td>My job is different and interesting every day.</td>
<td>67.9</td>
<td>32.1</td>
</tr>
<tr>
<td>3</td>
<td>My work tends to be repetitive and boring.</td>
<td>24.9</td>
<td>75.1</td>
</tr>
<tr>
<td>9</td>
<td>I feel lucky to be able to do the work that I do.</td>
<td>81.3</td>
<td>18.7</td>
</tr>
<tr>
<td>28</td>
<td>I feel stuck doing the work I do.</td>
<td>29.1</td>
<td>70.9</td>
</tr>
<tr>
<td>23</td>
<td>I’m proud of the quality of work in our lab.</td>
<td>94.3</td>
<td>5.7</td>
</tr>
<tr>
<td>27</td>
<td>I worry about the quality of the work in our lab.</td>
<td>20.4</td>
<td>79.6</td>
</tr>
<tr>
<td>13</td>
<td>I feel that the work I do is valued and appreciated.</td>
<td>66.0</td>
<td>34.0</td>
</tr>
<tr>
<td>24</td>
<td>The work I do is rarely appreciated by others.</td>
<td>43.4</td>
<td>56.6</td>
</tr>
</tbody>
</table>

Independence

| 4  | I have a great deal of independence in the work that I do.               | 78.4     | 21.6        |
| 12 | I do not have independence in my job.                                    | 21.6     | 78.4        |
| 18 | I have responsibility for the day-to-day decisions I encountered in my work. | 87.8     | 12.2        |
| 8  | My supervisor makes most of the day-to-day decisions that arise in my work. | 36.2     | 63.8        |

Environment – people and place

| 11 | My work environment is comfortable and safe.                            | 84.9     | 15.1        |
| 22 | My workplace is not comfortable and has safety problems.                | 13.8     | 86.2        |
| 25 | It is a pleasure to work with my co-workers.                            | 85.7     | 14.3        |
| 10 | My co-workers are difficult to work with and create a negative environment. | 20.5     | 79.5        |
| 21 | My job requires me to interact with other healthcare workers a great deal of the time. | 58.7     | 41.3        |
| 7  | I rarely interact with healthcare workers outside the clinical laboratory. | 40.7     | 59.3        |

Career development and rewards

| 30 | I have opportunities for career advancement and promotions in my current job. | 14.5     | 85.5        |
| 2  | The opportunities for career advancement and promotion in my laboratory are very limited. | 93.6     | 6.4         |
| 5  | My employer is interested in my professional development.                | 55.2     | 44.8        |
| 17 | My employer does not care about my professional growth.                  | 44.6     | 55.4        |
| 15 | I make a good salary.                                                    | 48.5     | 51.5        |
| 6  | My salary does not adequately compensate me for my education and work.   | 79.2     | 20.8        |

Responsibility for continuing education

| 26 | I take the responsibility for arranging and funding my continuing education. | 51.7     | 48.3        |
| 14 | Providing and funding continuing education is the responsibility of my employer. | 75.0     | 25.0        |
| 20 | I try to stay up to date on the current legislation and regulations that apply to the clinical laboratory. | 74.2     | 25.8        |
| 29 | I rely on the laboratory management to stay up to date on current legislation and regulations that affect the clinical laboratory. | 72.9     | 27.1        |

# = order of statement on original survey
†Agree = % of respondents circling “strongly agree” and “agree”
‡Disagree = % of respondents circling “strongly disagree” and “disagree”
on agreement or disagreement with statement 15 (Table 2), job independence was based on respondents’ agreement or disagreement with statement 4, sense of appreciation was based on responses to statement 13, and responsibility for one’s own continuing education was based on agreement or disagreement with statement 26. F tests were used to assess differences in responses among groups and the level of significance was set at a \( p \) value of 0.01.

Participants’ written responses to the question, “What factors do you think are most important in retaining qualified clinical laboratory practitioners in the laboratory today?” were tabulated and grouped into major categories by the researchers.

RESULTS
Response
A total of 809 surveys were returned which represents a 20% response rate if 4000 surveys were given to practitioners. Because the distribution model depended on the laboratory managers’ assistance, it is not possible to know whether or not 4000 practitioners actually received the survey. There were 145 CLTs and 454 CLSs who met the definition of practitioner in this study (a primary job title of CLT or CLS and five or more years of experience). Respondents with five or more years of experience who identified their primary job as supervisor (118) or director (35) were not included in the practitioner sample; however, their responses to selected survey questions were analyzed and compared to the practitioners.

Demographic information
Practitioners came from all geographic regions of the country. The highest percentage of practitioners (17.6%) came from the ASCLS Region IV (MI, IN, OH, KY) and the lowest percentage of practitioners (2.2%) came from ASCLS Region VIII (CO, ID, MT, UT, WY). In the other ASCLS regions, the percentage of practitioners ranged from 9.7% to 15.1%.

<table>
<thead>
<tr>
<th>Table 2. Job Satisfaction based on respondents’ job function, satisfaction with salary, job independence, sense of appreciation, and responsibility for CE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
</tr>
<tr>
<td>Job function: CLT or CLS</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Satisfaction with salary based on statement 15: “I make a good salary”</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Job independence based on statement 4: “I have a great deal of independence in the work that I do”</td>
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<td></td>
</tr>
<tr>
<td>Sense of appreciation based on statement 13: “I feel that the work I do is valued and appreciated”</td>
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<tr>
<td></td>
</tr>
<tr>
<td>Responsibility for CE based on statement 26: “I take the responsibility for arranging and funding my continuing education”</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

\* 1 = very satisfied; 2 = somewhat satisfied; 3 = somewhat dissatisfied; 4 = very dissatisfied

† Significant at \( p = 0.01 \)
Most of the practitioners worked in hospitals or medical centers (80.4%). The next largest percentage of practitioners worked in physician office or group practice laboratories (8.4%) or reference laboratories (5.7%). A small percentage of the practitioners indicated that they worked in academic health centers (1.0%), HMOs (0.5%), or in an educational program (0.5%).

To assess institution size, practitioners were asked for the annual volume of tests performed in their clinical laboratory. Most practitioners (32.8%) were from institutions with test volumes between 100,001 - 500,000. Eighty-nine (16.6%) of the practitioners worked in institutions with test volumes between 500,001 - 1,000,000, 14.7% worked in institutions with test volumes greater than 1,000,000 and 14.9% worked in institutions with volumes less than 100,000 tests annually. Twenty-one percent indicated that they did not know the test volume in their institution.

The practitioners were primarily female (86.0%) and Caucasian (91.3%). The ethnic group selected by the second highest percentage of practitioners was Asian (4.0%). Only 2.4% of the practitioners were African American and a small percentage were Hispanic (1.5%) and Native American (0.5%).

Sixty-eight percent of practitioners listed the baccalaureate degree as their highest degree. The associate degree was the highest degree for 24.7% of the practitioners and 2.9% of the practitioners indicated they had received a Master's Degree. Practitioners were graduates of certificate CLT programs (6.6%), associate degree CLT programs (22.3%), hospital-based CLS programs (26.8%), and university-based CLS programs (36.6%). A small percentage of the practitioners (4.7%) qualified for certification with work experience. The majority of the practitioners held CLS/MT certification (68.2%) and 8.1% of those practitioners held additional credentials including CLT, supervisor, director, and specialist. Twenty-seven percent of the practitioners indicated that CLT/MLT was their only credential and 0.5% held the CLT/MLT credential and a second credential as a supervisor or specialist.

Practitioners averaged 19.5 years of paid experience with a range of 5 to 44 years. They reported a mean of 11 years working in their current positions with a range of less than 1 to 39 years. Practitioners were asked whether or not continuing education (CE) was required for their current jobs and 59.3% indicated that it was. The annual number of hours of CE reported by the practitioners ranged from 0 to 120 with a mean of 13.8 hours per year and a median of 10 hours per year.

Practitioners’ opinions of their work and careers
Practitioners’ responses to 30 Lickert-type questions on their work and careers are shown in Table 1. Statements addressing the same issue including those worded both positively and negatively are grouped together. The statement number indicates the order in which the statements appeared on the survey.

The five groupings are practitioners’ attitudes toward 1) the work they perform, 2) autonomy and independence in their jobs, 3) the work environment, 4) career development and compensation, and 5) continuing education.

Job satisfaction
Most practitioners indicated that they were either very satisfied (36.8%) or somewhat satisfied (44.5%) with their choice of a clinical laboratory career. Three percent of the practitioners indicated that they were very dissatisfied and 15.6% were somewhat dissatisfied. The level of job satisfaction of the respondents was compared based on their job function (CLT or CLS), satisfaction with salary, job independence, sense of appreciation, and responsibility for CE. The means, standard deviations, and significance levels are listed in Table 2. Job satisfaction was significantly higher for practitioners who indicated that they made a good salary, had independence in their jobs, and felt that their work was appreciated. There were no differences in satisfaction based on job function or responsibility for continuing education.

Tasks performed
The mean percent of time that respondents spent in a variety of activities at work is listed in Table 3. For comparison, the responses of the supervisors and directors were analyzed and included. CLT and CLS practitioners spent the majority of their time (84%) performing tests and reporting results. Supervisors and directors spent more time in meetings and administrative functions.

Retention
Retention of clinical laboratory personnel was addressed in this study by collecting information on why practitioners left their last job, why they stay in their current jobs, the factors that they consider most important in retaining laboratory personnel, and their career plans for the next five years.

Why they left
When asked for the major reason they left their last job, approximately 24% of the practitioners indicated that this question was not applicable to them, which may mean that they haven’t changed jobs. Of the people who responded to this question, the reasons they listed for leaving their last job were:
Why they stay
Practitioners were asked about the factors that keep them in the clinical laboratory profession. Respondents could circle more than one factor and could write in additional factors. Their responses, in rank order were:

- interesting work (65.2%),
- security (52.3%),
- like my colleagues (45.8%),
- good location (41.5%),
- challenging work (38.0%),
- good benefits (36.8%),
- good salary (35.6%),
- reluctance to change jobs (34.4%),
- flexible hours (30.5%),
- lack of other opportunities (25.9%),
- the work is easy for me (25.0%),
- opportunity to learn new things/professional development (24.7%),
- works well with childcare needs (12.5%),
- good administration/management (11.7%),
- adequate staffing levels (9.0%),
- other (5.0%),
- mobility (4.7%), and
- advancement opportunities (1.7%).

### Table 3. Percent of time spent on tasks in work setting

<table>
<thead>
<tr>
<th>Task</th>
<th>CLT n= 145</th>
<th>CLS n = 454</th>
<th>Supervisor n = 118</th>
<th>Director n = 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Performing tests and reporting results (includes QC and QA within the laboratory)</td>
<td>84.5</td>
<td>84.1</td>
<td>51.5</td>
<td>13.1</td>
</tr>
<tr>
<td>Attending meetings with laboratory personnel in your facility</td>
<td>3.0</td>
<td>2.6</td>
<td>5.9</td>
<td>10.2</td>
</tr>
<tr>
<td>Attending meetings with non-laboratory personnel in your facility</td>
<td>1.1</td>
<td>1.0</td>
<td>3.6</td>
<td>10.8</td>
</tr>
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<td>Teaching, e.g., new employees, students, residents, continuing education sessions</td>
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<td>1.8</td>
<td>2.8</td>
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<td>Financial resource management, e.g., budgets, test cost analysis, reimbursement requirements, materials/inventory management</td>
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<td>2.1</td>
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<td>7.5</td>
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</tbody>
</table>

* Phlebotomy, laboratory information systems, compliance activities, billing, ordering, customer service, safety proficiency testing, troubleshooting, and procedure writing.
How to retain others

The open-ended question, “What factors do you think are most important in retaining qualified clinical laboratory practitioners in the laboratory today?” generated 1900 suggestions from the 599 practitioners. The practitioners’ written responses were summarized and grouped into major categories. The major categories of comments and the percent of respondents who made suggestions in each category were:

- salary commensurate with education, experience/equivalent to nursing (77.1%),
- recognition from other healthcare professionals/hospital administration (30.4%),
- benefits - comparable to nursing (19.0%),
- adequate staffing/reduced stress (18.3%),
- flexible/better hours (17.1%),
- good working conditions (12.4%),
- advancement opportunities/career ladder (12.0%),
- good management (11.0%),
- continuing education/professional development (10.6%),
- challenging/interesting/variable/responsible work (9.3%),
- praise/appreciation from laboratory management (7.8%),
- good/qualified co-workers (7.3%), and
- involvement in decision making (2.6%).

Practitioners gave specific examples of ways in which salaries needed to be improved. The most common suggestion was to establish parity with nurses but perceived inequities within the laboratory also were mentioned. These included equal pay for CLTs performing the same job tasks as CLSs and differential pay for credentialed individuals vs. those trained on-the-job. Elimination of salary ceilings was also suggested by long-term employees.

The practitioners who listed improvements in benefits as a retention factor gave examples such as increased numbers of vacation days with longevity, better healthcare benefits, job sharing options, employer-paid continuing education, and on-site day care. The latter was mentioned in connection to the employer’s expectation for over-time and odd schedules. Other issues relative to scheduling were also mentioned frequently. These included flexibility in scheduling, but also the desire for no weekend or holiday work. Some practitioners commented that the lack of scheduling flexibility was tied to overall inadequate staffing.

Where they are going

To assess the likelihood of retaining this group of practitioners in the clinical laboratory workforce, practitioners were asked to identify their career goals for five years in the future. They were asked to select all the goals from a list on the survey that applied to them and write in additional goals.

The practitioners’ goals for five years in the future were:

- same position with additional skills and experience (55.4%),
- further education in the laboratory field (19.4%),
- retirement (16.1%),
- laboratory supervisory/management position (14.8%),
- leave laboratory field entirely (13.7%),
- further education in a non-laboratory field (12.9%),
- technical position in a different institution (8.7%),
- quality assurance position (3.9%),
- research position (3.9%),
- information systems position (3.5%),
- other (3.4%),
- clinical trials/pharmaceutical industry position (2.7%), and
- sales or marketing (2.5%).

The practitioners who selected ‘other’ wrote that their goals included forensics, regulatory inspector, working part time in the laboratory, and teaching. The 13.7% of practitioners who said they were leaving the field entirely listed a number of reasons including changing to another health profession, meeting family obligations, going into business, and teaching. Some said they were not sure, but they wanted to find something with better salary and advancement opportunities.

DISCUSSION

The practitioners in this study felt strongly about the value of their work. The Lickert statement with the highest percent of agreement was #1, “I believe my work is important”. This strong sense of the value of the clinical laboratory may have helped these practitioners persevere in the profession when other factors such as salary, over-work, or lack of appreciation caused them to question their commitment. Maintaining a sense of the importance of one’s work can be difficult in large, computerized, highly automated laboratories in which practitioners do not collect samples, report results verbally to attending staff, or otherwise interact with providers or patients. As one respondent observed, “Working in our lab has become like working in a factory. At times I feel like a robot packaging answers to be zapped through the computer to another planet. Surely work could be made more fulfilling.”

In this study, over 78% of the practitioners agreed with two statements indicating that they had independence in their work. It was interesting to note that those who felt they had independence in their work were also significantly more satisfied with their choice of CLS as a career than those who did not feel they worked independently. This is consistent with Blau’s work on career enrichment factors and Karni and Feikert’s recommendations for improving job satisfac-
Also in written comments, practitioners adamantly assert microbiology with a passion. It makes me consider changing jobs even though I enjoy band) who didn’t go to school are making $13.00 an hour…

We work very hard but factory employees (such as my husband) who worked in the lab with a supervisory position in chemistry. My responsibilities wereCLTs. CLT and CLS practitioners spend approximately the same amount of time doing tests (84%), although there are distinctions in the types of tests that each group performs.

Practitioners' written comments addressed other salary issues including intra-laboratory salary inequities, salary ceilings, lack of annual raises, and lack of recognition for additional education or responsibilities. CLT respondents expressed dissatisfaction when the pay scale differentiates based on education and preparation but the job requirements are not clearly different. One respondent said, “As an MLT in a rural hospital, I have the same job responsibilities as an MT. However, I get paid less.” Another wrote, “I am an MLT-C with a supervisory position in chemistry. My responsibilities are greater than some of the MTs with bachelor degrees but my salary is much less than theirs…It's very frustrating.”

Salary ceilings were described as a cause of dissatisfaction by respondents in comments such as “Pay ceilings have always been very limited with insufficient reward for experience or longevity” and “Many techs reach salary max within ten years; there’s no further reward.” Additional dissatisfaction was mentioned when non-credentialed individuals are paid comparably to those with professional credentials as expressed by one respondent, “I work beside co-workers that are not med techs and they make more salary than I do with 23 years of experience and Master’s degree. This is just not fair!”

In spite of the general concern about appreciation by other healthcare workers and salaries, the majority of respondents felt lucky to do the work they do. Over 80% were satisfied with their choice of CLS as a career. This percent is relatively unchanged from the 1994 study by Harmening. There were no significant differences in job satisfaction for respondents who indicated that they were CLTs and those who were CLSs. CLT and CLS practitioners spend approximately the same percent of their time performing and analyzing laboratory tests (84%), although there are distinctions in the types of tests that each group performs.

Practitioners spent an average of almost 14 hours each year in continuing education (CE) activities, which is consistent with NCA re-certification requirements for continued competence. Slightly over half of the respondents felt that they were
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responsible for providing and funding their own CE and approximately 75% thought this was the employers’ responsibility. Practitioners demonstrated that they value CE by their level of participation in CE activities, but they have mixed feelings about who is responsible for providing and funding the CE. Practitioners who felt that they were responsible for their own CE might be expected to be more committed to the profession and more satisfied with their choice of CLS as a career. This was not the case, however, because there were no differences in levels of satisfaction among respondents who said they took responsibility for their own CE and those who didn’t. It is likely that the respondents’ experiences of arranging and funding CE varies greatly from one institution to another making comparisons on this factor difficult. For example, an individual might be willing to pay to attend a CE program but has an employer who does not approve his or her time off to attend. This employee could have a sense of responsibility for CE and be dissatisfied.

In response to structured and open-ended questions on why they stay in their current jobs, the practitioners in this study expressed their strong commitment to the work of the clinical laboratory. Approximately 65% of the respondents stay in the field because the work is interesting. Other important factors in retaining these practitioners were job security, good relationships with colleagues, and a good location. Job security was probably very important because of the current economic climate in which many people are unemployed or underemployed. Approximately 37% of the respondents indicated that they considered ‘good benefits’ important in retention. As one respondent observed, “My salary has not kept me where I am. It is the benefits.” Another noted, “Our hospital has a very nice paid-time off policy. To move to another job would result in losing the five to six weeks of paid time off I’ve accumulated. That’s a real benefit I do not want to lose.”

At the bottom of the list of factors that respondents considered important for the retention of laboratory personnel were “praise/appreciation from lab management”, “good/qualified co-workers”, and “involvement in decision making”. It is doubtful that these are truly considered unimportant because several were cited in prior studies as important to retention. It is more likely that these are factors that are commonly present and hence may be taken for granted. For example one respondent wrote, “My manager has always listened to my concerns – no matter what they are – she has always made me feel appreciated and invaluable. Knowing I have the support of my manager and co-workers really goes a long way.” Involvement in decision making was probably not a concern for this group of respondents because 88% agreed with the statement “I have responsibility for the day to day decisions I encounter in my work.” However, when one of these factors was missing, the respondents expressed their concern. The lack of good/qualified co-workers was mentioned by several respondents in comments such as “There are problems with less (or minimally) competent employees being retained due to shortages in the field. This also adds additional burden to the competent employees” and “I would rather work short staffed than have our manager hire ‘a warm body’. The poor work being turned out is a reflection on us all and gets very frustrating, especially when you are constantly covering mistakes.”

The major reason that these practitioners left their last job was relocation. This is not unexpected given that one of the attractions of a career in CLS is that one can move and find work in another setting. This is also consistent with Harmening’s study in which 35% of the respondents said that the major reason for seeking new employment was geographic relocation. Some turnover due to relocation is inevitable. While this may leave one employer with an open staff position, it may benefit an employer in a new location. However, this becomes a high risk time for the retention of an individual in the clinical laboratory profession. Some individuals may leave one location with the intention of looking for a laboratory position in the new location, but during the job search they may find something outside the laboratory profession.

The majority of those surveyed plan to stay in their current positions and many respondents were thinking about future education in a laboratory field, a new position in a laboratory, a new role as a supervisor, or a new laboratory-related career in quality assurance, research, or information systems. This is good news, showing that most of these practitioners of five years or more are either satisfied where they are or are looking for career development within the profession. A survey of less tenured employees may show a higher percentage considering leaving the profession.

Still of concern is the number of people who may be leaving the profession due to retirement or dissatisfaction. Approximately 16% indicate that they plan to retire and approximately 14% said they planned to leave the field entirely. So, in this group of practitioners, an estimated 30% will be leaving the profession. Replacing almost one-third of the clinical laboratory workforce is a daunting prospect and will only support the status quo, not the predicted needs for the future. While automation and productivity improvements can compensate for some of the future personnel needs, it is clear that more people need to enter and remain in the laboratory profession.
LIMITATIONS
In this study, practitioners were defined as CLTs and CLSs with five or more years of experience. Of the 809 surveys received, approximately 600 practitioners met these criteria. The survey had the potential of reaching 4000 practitioners; however, this depended on the participation of laboratory managers. The actual response rate may be higher, but this cannot be known because the number of surveys actually given to practitioners is unknown. Although the overall response rate of 20% appears low, it is comparable to other national unsolicited surveys of the clinical laboratory population. The demographic information reported in this study, which is consistent with other descriptions of the profession, and the broad geographic distribution of the participants contribute to the validity of the results. This population of CLT and CLS practitioners with five or more years of experience was selected to represent those who have made a commitment to the profession. The findings should not be generalized to the total population of laboratory professions. For example, although over 80% of the respondents were satisfied with their choice of CLS as a career, it is likely that job satisfaction results would have been different if the survey population included all clinical laboratory practitioners. Those who are most dissatisfied with their choice of CLS as a profession might be expected to leave before five years of employment.

RECOMMENDATIONS TO MANAGERS
What can managers learn from the results of this study? Committed laboratory professionals stay because they like the work they do and they know the value of that work for patients. They wish others, especially physicians, nurses, and administrators, understood the importance of their work and their professional skills. Laboratory managers should emphasize, highlight, and display the value of laboratory work, both to laboratory staff and to the other healthcare providers and administrators in their own institution. For example, laboratory managers could:
• promote greater involvement of laboratory professionals in hospital-wide issues and problems,
• target other healthcare professionals within the institution during national medical laboratory week rather than the public,
• undertake efforts to educate institutional administrators about the value and quality of laboratory services and the education of laboratory professionals, and
• find examples of laboratory services that made a difference in a patient’s life or in the functioning of the institution. Then promote it and celebrate it within the laboratory and institution-wide.

Salary is an indicator of how important the individual is to the institution and it contributes to job satisfaction and retention. Laboratory managers should work to improve salaries and eliminate the disparity between the salaries of laboratory employees and healthcare workers with similar education. Often compensation specialists collect information on salaries of laboratory professionals at comparable institutions when making salary adjustments. This study indicates, that in the view of practitioners, the comparison group should be the healthcare providers in their own institution, particularly nurses. Laboratory managers must make the case within their own institutions that comparison of laboratory salaries across institutions may attract job seekers in the short term, but it does not contribute to retention within the clinical laboratory over the long term. Laboratory managers should also work with their administrators to develop strategies to minimize salary ceilings. Within the laboratory, managers should address intra-laboratory pay scales and ensure that they are commensurate with job responsibilities and that job responsibilities are commensurate with education.

Good benefits and job security are important retention factors for laboratory professionals. If an institution has a good benefits package, laboratory managers should emphasize that in communications with employees. A benefits package that is not comparable to other healthcare workers in the same institution or not competitive with other institutions increases the likelihood of attrition. As with salary, comparability of benefits to other hospital employees is important in communicating the institution’s appreciation for and value of the employees.

A good relationship with co-workers is an important factor in employee retention and so the time that laboratory managers take to address problem employees and hire good employees is well spent. In addition to good working relationships, this study reinforced the importance of independence and autonomy in job satisfaction. Employers should look for ways to give employees more control over their time and work.

Some employee turnover is inevitable, but employers can help retain individuals in the laboratory profession by giving departing employees information about institutions in their new location and contacts with laboratory managers in that area. Finally, laboratory managers must help with the recruitment of young people into the laboratory profession by supporting the recruitment efforts of educational programs and serving as clinical sites for students.

CONCLUSION
The findings in this study are consistent with prior reports addressing job satisfaction and retention of staff. Two major
factors were identified in this study that contribute to dissatisfaction with careers in the clinical laboratory and undermine retention efforts. The first is salary. Although salaries have risen with inflation, laboratory employees feel that salaries are not commensurate with their education and experience, and they are not comparable to other healthcare providers with similar education. Second, laboratory professionals feel their work is not appreciated by administrators, physicians, and nurses. These factors along with other causes of dissatisfaction create a cycle of attrition, staffing shortages, and further dissatisfaction (Figure 1). Efforts to retain laboratory staff members by addressing the root problems of salaries and appreciation by healthcare providers outside of the laboratory are needed to stop this cycle. Laboratory managers and professional associations should incorporate these findings into their strategic planning in order to address the shortage of laboratory professionals now and in the future.

This study was supported by a grant from the Education and Research Fund of the American Society for Clinical Laboratory Science and mailing list donation from the Clinical Laboratory Managers Association.

ACKNOWLEDGEMENTS
The authors express appreciation to Advisory Board members: Cheryl Caskey, George Mavros, Michelle Montgomery, Kay Paff, and Lindsay Suber; Staff of the Medical Technology Program, Michigan State University for assistance with mailing and processing of surveys.

REFERENCES
Advances in Understanding the Biology and Genetics of Acute Myelocytic Leukemia

SHIRLYN B MCKENZIE

Note: This article is also a continuation of the series on Neoplastic Hematologic Disorders that appeared in the previous issue of Clinical Laboratory Science (Fall 2004).

Acute myelocytic leukemia (AML) is a malignant neoplasm of hematopoietic cells characterized by an abnormal proliferation of myeloid precursor cells, decreased rate of self-destruction and an arrest in cellular differentiation. The leukemic cells have an abnormal survival advantage. Thus, the bone marrow and peripheral blood are characterized by leukocytosis with a predominance of immature cells, primarily blasts. As the immature cells accumulate in the bone marrow, they replace the normal myelocytic cells, megakaryocytes, and erythrocytic cells. This leads to a loss of normal bone marrow function and associated complications of bleeding, anemia, and infection. The incidence of AML increases with age, peaking in the sixth decade of life. In the United States, there are about 10,000 new cases of AML and 7,000 deaths in those with an AML diagnosis per year. Current molecular studies of AML demonstrate that it is a heterogeneous disorder of the myeloid cell lineage.

This paper will discuss the most recent understanding and research of the cellular origin of AML and associated common genetic mutations that fuel the neoplastic process. Also discussed are how these advances have impacted the classification, selection of therapy, and definition of complete remission in AML. Promyelocytic leukemia will be discussed in detail as this AML subtype reveals how our understanding of the biology and genetics of the disease has led to targeted therapy that results in a cure in up to 80% of patients.

ABBREVIATIONS: AML = acute myelocytic leukemia; APL = acute promyelocytic leukemia; ATRA = all trans retinoic acid; CBF = core binding factor; FAB = French-American-British; HDAC = histone deacetylase; ITD = internal tandem duplications; MDS = myelodysplastic syndrome; MLL = mixed lineage leukemia; NB = nuclear body; NCOR = nuclear corepressor; NK = natural killer; PML = promyelocytic leukemia; PTD = partial tandem duplications; RA = retinoic acid; RAR = retinoic acid receptor; TK = tyrosine kinase; WHO = World Health Organization.

INDEX TERMS: acute myelocytic leukemia; clonal genetic mutations; hematopoietic stem cells; lineage commitment; PML-RARA; promyelocytic leukemia.


Shirlyn B McKenzie PhD CLS (NCA) is Professor and Chair of the Department of Clinical Laboratory Sciences at the University of Texas Health Science Center at San Antonio, San Antonio TX.

Address for correspondence: Shirlyn B McKenzie PhD CLS(NCA), Department of Clinical Laboratory Sciences, UTHSCSA, 7703 Floyd Curl Dr, San Antonio TX 78229-3900. (210) 567-8860. mckenzie@uthscsa.edu

Shirlyn B McKenzie PhD CLS(NCA) is the Focus: Myelocytic Leukemias guest editor.

Focus Continuing Education Credit: see pages 57 to 59 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES
1. Explain the cancer stem cell hierarchical model and how it applies to acute myelocytic leukemias (AMLs).
2. Correlate cytogenetic and molecular genetic findings in the diagnosis and prognosis of AML.
3. Compare and contrast class I and class II mutations in AML and give examples of each.
4. Explain the functions of the PML/RARA fusion protein in promyelocytic leukemia (PML).
5. Propose what will occur at the molecular level when ATRA is given to patients with PML and correlate with clinical findings in the patients.
6. Assess how advances in our understanding of the biology and genetics of hematopoietic neoplasms has affected the classification of these disorders.
7. Compare and contrast hematologic remission, cytogenetic remission, and molecular remission.

CELLULAR ORIGIN OF AML
Cancer is a clonal disease in that it is initiated by mutations in a single cell. The mutated cell produces progeny that form the
tumor. The mutations occur in genes that control cell proliferation, survival, or differentiation and act as dominant genes. These mutated genes are known as oncogenes. Their normal cellular counterparts are called proto oncogenes. Cancer can also result from mutations in tumor suppressor genes. These genes are recessive genes. In AML, the progeny of the original tumor cell are functionally heterogeneous. This raises two confounding questions: What is the target cell where the original mutation occurred and which tumor cells have the capacity to sustain or re-initiate the tumor? Understanding which progenitor cell is the target mutated malignant cell (leukemic stem cell) helps in understanding the cellular processes that are affected by the mutation and may help identify specific therapy that targets the mutated gene or protein.

Two models have been proposed to answer the above questions.1 The first proposes that each tumor cell has the capacity to renew the tumor. The second model is based on a stem cell hierarchical framework in which the target cell is a cancer stem cell that may give rise to cells capable of reinitiating the tumor and cells that have limited capacity to differentiate. The extent to which the target cell progeny retain the functional and morphological properties of stem cells depends on the extent to which the target cell can differentiate. For example, if the leukemic cell progeny can differentiate into more mature cells such as in chronic myelocytic leukemia (CML), the more mature cells are not able to renew and appear morphologically different from the leukemic stem cell. In CML, the BCR-ABL gene translocation is in an early stem cell that has both lymphoid and myeloid differentiation capacity. This helps explain the fact that this disease may progress to either myeloid or lymphoid blast crisis.

Even though most AML patients go into hematologic remission with therapy, less than 50% are cured. This suggests that although therapy is effective at killing most leukemic cells, it does not kill the leukemic stem cell. It also suggests that the leukemic blasts identified by morphology and flow cytometry do not have the same cell markers as the leukemic stem cell since leukemic blast cells are not detected during complete remission.2 If the cancer stem cell model is accurate, then the target mutated cell is probably at the top of the hierarchy and represents a minority of cells, as in the hematopoietic stem cell theory. Stem cells have a high self-renewal capacity and if dysregulated could provide unrestrained self-renewal (cancer cell). Stem cells are also long-lived so it would be easier for these cells to accumulate the number of mutations needed to develop into a cancer stem cell than it would be for more differentiated cells.

Support for the cancer stem cell theory comes from studies in mice.3 One measure of self-renewal is the ability of a transplanted cell to form progeny. Primitive hematopoietic precursor cells have the following cell phenotype: CD34+, CD38+, CD71-, Thy1-. When transplanted into mice the CD34+, CD38- AML cell fraction initiated an AML-like disease. Yet these cells made up only 1/100 to 1/1000 of the leukemic cells found in the mice. CD34+, CD38+ cells, and CD34- cells did not initiate tumors, but constituted the bulk of the leukemic cells. Analysis of different AML FAB subtypes revealed that, despite morphological differences of the predominant immature hematopoietic cell, the repopulating cells are mostly quiescent and found in the CD34+, CD38- population. This suggests that the uncommitted progenitor cells in AML are the targeted mutant cells. The heterogeneity of AML may be explained by the occurrence of different mutations in the target mutant cell. The expression of their leukemic gene product influences lineage commitment and the degree of cellular differentiation. The growing body of knowledge of genetic mutations in hematopoietic neoplasms has led to a new classification of these disorders, which is largely based on specific abnormal cell karyotypes and gene mutations by the World Health Organization (WHO) classification.

GENETIC MUTATIONS IN AML
Over 300 recurring chromosome translocations have been identified in leukemia. About a third of these have been cloned and characterized giving implications as to their causal role in leukemia.4 In most cases these translocations result in expression of a chimeric fusion protein. The fusion proteins result in abnormal functions of cell self-renewal, proliferation, differentiation, and/or apoptosis.

Cytogenetic analysis of AML
Cytogenetic analysis is considered the single most important factor in determining prognosis in AML. Therefore it is recommended that karyotyping be done on every patient at diagnosis.5

Clonal cytogenetic mutations can be identified in up to 50% of patients with de novo AML.6 These mutations have not all been defined at the molecular level. However, they currently are used to define the overall survival risk status and to make treatment decisions. The Southwest Oncology Group and Eastern Cooperative Oncology Group studied the outcomes of 609 patients with AML after induction therapy. They identified four cytogenetic risk status groups: favorable, intermediate, unfavorable, and unknown (Table 1).7 Seventy-one

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of promyelocytic leukemia (PML), sometimes referred to as acute promyelocytic leukemia (APL), with the t(15;17) mutation is promising when treated with all-trans retinoic acid (ATRA). Thus, cytogenetic analysis is a very important parameter in diagnosis and treatment of AML.

Although 50% of AML patients have no identifiable karyotype abnormalities, molecular mutations may be present. Examples of this are the partial tandem duplication of the mixed lineage leukemia (MLL) gene and length mutations of FMS-like tyrosine kinase 3 (FLT3) detected by molecular methods but not by cytogenetics. AML that is secondary (occurs in a patient with previous myelodysplastic syndrome [MDS] or myeloproliferative disorders [MPD]) or that is therapy-related, may show cytogenetic abnormalities distinctly different from those of primary AML.

**Molecular genetics of AML**

It is a well-accepted concept by hematologists that, for development of the full neoplastic process of myeloproliferation and de-differentiation in AML, two broad, cooperating mutations are necessary, Class I and II. Class I mutations give a proliferative and/or survival advantage to the mutated myeloid precursors (Table 2). These mutations have no effect on differentiation. They result in constitutive activation of tyrosine kinase receptors or downstream effectors. Class II mutations complement Class I mutations and impair myeloid differentiation (Table 2). By interfering with terminal differentiation and apoptosis, Class II mutations also provide a survival advantage. Class II mutations involving core binding factors (CBF) are the most common in acute leukemia but this mutation alone is not sufficient to cause leukemia. Mouse models show that leukemia is accelerated in animals with CBF mutations if chemical mutagens are given. This supports the need for additional mutations for the leukemic process to develop.

**Common Class I mutations**

Tyrosine kinases (TKs) are normally involved in regulation of hematopoesis (cell proliferation, migration, differentiation, and survival) as well as other cell functions. Several growth factor receptors have TK activity (TK receptors). These TK receptors have an extracellular domain that binds ligands, a transmembrane domain, and an intracellular tyrosine kinase domain (Figure 1). When a growth factor binds the extracellular domain of the receptor, the TK becomes active and transfers phosphate from ATP to tyrosine residues on intracellular proteins. Phosphorylation is a common mode of regulating the activity of intracellular proteins.
Many TK genes are proto oncogenes. When mutated, they are constitutively activated and can induce uncontrolled cellular proliferation, inhibit differentiation and apoptosis, and decrease adhesion. Recently, a second role for TKs in cancer was identified. Tumors that express these mutated proteins are resistant to chemotherapy and irradiation. There are at least four mechanisms through which the TKs can become constitutively activated. These include chromosome translocation, truncation, over expression, and activating mutations. The mutations remove the inhibitory domains or induce a configuration that activates the TK. The first TK oncogene identified in a hematopoietic neoplasm was BCR/ABL found in CML.

The FLT3 gene codes for a receptor with TK activity that is involved in the proliferation and differentiation and/or survival of hematopoietic stem cells. The FLT3 ligand (FL, also known as stem cell factor/SCF) is expressed in bone marrow stroma in both membrane-bound and soluble forms. Binding of a ligand to the normal FLT3 receptor enhances the effect of colony stimulating factor on hematopoietic progenitor cells in synergy with other colony stimulating factors. The receptor is involved in activating several signal transduction pathways as well as tyrosine phosphorylation. It is expressed on immature hematopoietic precursors (CD34+).

FLT3 is the most common mutated gene in AML. It is highly expressed in both AML and ALL. Up to 41% of AML cases have activating alleles of FLT3. FLT3 internal tandem duplications (ITD) are most common. Missense or in-frame deletion of critical residues, most often Asp 835, is less common and associated with a higher WBC count and higher blast count than FLT3 (ITD). When mutated, FLT3 protein is a constitutively activated cell receptor through autophosphorylation (growth factor independent). This results in a proliferation and survival advantage to the mutated cell. It is believed that FLT3 mutations work together with other gene mutations to cause AML. The FLT3 mutation by itself causes a myeloproliferative phenotype but does not result in AML. The FLT3 mutations are present in patients with t(15;17), t(8;21), inv(16), and 11q23 rearrangements.

Most studies reveal that the duration of remission and overall survival are shorter in patients with the FLT3 mutation than in those who lack the mutation. The occurrence of FLT3(ITD) was found to be lower in children than in adults with AML. However this may be related to the smaller normal cytogenetics subgroup in children (15% to 30%). It is found in all FAB subtypes of AML.

### Class II mutations
Core binding factors, CBF, are transcription factors with two subunits. One subunit binds DNA (CBFα) and the other is a non-binding DNA subunit (CBFβ). The CBFs are involved in several hematopoietic cell pathways and are essential for normal hematopoietic development. In mice, knockout of both CBFα and CBFβ genes results in blocked hematopoiesis.

A gene encoding a CBFα, known as RUNX1, CBFA, or AML1 and another encoding a CBFβ subunit, CBFB, are

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<td>Class I mutations found in AML that confer a proliferative or survival advantage to leukemic cells:</td>
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<td>c-Kit</td>
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<td>RAS</td>
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<td>TEL-PDGFRβ</td>
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| Class II mutations found in AML that confer a survival advantage to leukemic cells by interfering with differentiation or apoptosis: |
| AML1-ETO      |
| CBFB-MYH11    |
| CEBPα         |
| AML1 mutations |
| MLL rearrangements |

---

**Figure 1.** A tyrosine kinase receptor has an extracellular domain that binds ligand, a transmembrane domain, and the intracellular tyrosine kinase domain. When a ligand binds to the receptor, it activates the kinase which in turn phosphorylates proteins, a common way to regulate protein function. When mutated, these kinases may be constitutively activated.
frequently mutated in leukemia, particularly in acute lymphoblastic leukemia. The AML1 gene appears to influence cell maintenance and expansion or survival of hematopoietic stem cells or more differentiated progenitor cells. In t(8;21), one of the most frequent genetic mutations in AML, the AML1 gene on chromosome 8 is fused to the ETO gene (also called MTG8 or CBF2T1) on chromosome 21. The AML1-ETO fusion protein inhibits normal function of CBF by repressing transcription, thus inhibiting myeloid differentiation. The mutation leads to an acute leukemia with FAB morphology. It is associated with a long-term CR and disease free survival if treated with high dose cytarabine. The t(8;21) mutation can be detected by karyotyping, fluorescent in situ hybridization (FISH), and polymerase chain reaction (PCR). Another frequent mutation involving a CBF is pericentric inversion of chromosome 16, inv16(p13q22). In this mutation, there is a CBFB/MYH11 fusion. Although the molecular pathogenesis of this mutation is similar to t(8;21), it leads to a different leukemia morphology (FAB classification, M4eos). Patients with CBF mutations are usually less than 60 years of age and enter a complete remission with standard chemotherapy.

Target mutant cell

In one study, the PML clone with the PML/RARA fusion gene arose in CD34+ and CD38+ progenitors (more differentiated myeloid cells). CD34 was expressed at the granulocyte-monocyte precursor stage but was absent on the pro-myelocyte. There was no involvement of the CD34+ CD38-cells (primitive hematopoietic progenitor cells).

The immunophenotype of PML cells is characterized by homogeneous expression of CD33, heterogeneous expression of CD13, HLA-DR-, CD34+, CD11b-, CD15-, CD9+, and absence of lymphoid antigens (except CD19). In some studies, there is atypical expression of both lymphoid and myeloid markers in PML blasts that suggest the mutation occurred in an undifferentiated stem cell. Some harbor the PML/RARA fusion in cells that express CD34 and CD56.

FOCUS: MYELOCYTIC LEUKEMIA

Translocation of the mixed lineage leukemia (MLL) gene (ALL1 or HRX) is found in both ALL and AML. It is located in the chromosome band 11q23 with a region of about 100 kb of DNA. It is associated with leukemia in infants as well as in mixed lineage leukemia. The wild type gene is thought to affect the proliferation and commitment of the hematopoietic stem cell to progenitor cells. Forty genes have been identified that can partner with MLL producing a fusion gene. The mutated gene is found in t(11q23) abnormalities, a common mutation point in AML and in 90% of patients with trisomy 11 (+11). MLL oncogenic fusion proteins increase the growth potential of cells. Molecular analysis reveals duplications of the MLL gene in about 10% of AML patients with normal karyotypes. It was the first molecular mutation identified in AML patients with a normal karyotype. The significance of this mutation is not understood as partial tandem duplications (PTD) of MLL can be identified in the blood and bone marrow of healthy individuals.

BIOLOGY AND GENETICS OF PROMYELOCYTIC LEUKEMIA (PML)

Promyelocytic leukemia is the best understood AML. It is the first AML in which a specific molecular mutation was identified, the cellular pathway affected was identified, and a targeted molecular therapy was developed. PML is characterized by chromosomal translocations involving 17q21. This leads to rearrangements of the gene that codes for RARα (RARα), a nuclear hormone receptor involved in modulating myelopoiesis. The most common fusion partner (found in >95% of PML cases) is PML on chromosome 15 (t[15;17][q22;q12-21]) forming the PML/RARA fusion gene and protein. This mutation results in two recombinant chromosomes, 15q+ and 17q-. The PML protein is a nuclear protein that accumulates in distinctive subnuclear domains together with several other proteins. These domains are called the PML nuclear bodies (PML-NBs). PML protein localization in the NBs is thought to be essential for PML to modulate transcription. The fusion protein PML/RARA, can disrupt and delocalize PML protein from the NBs in the promyelocytic leukemic cells and thus, affect transcription of target genes.
(natural killer, NK cell), CD19 (B-cell marker), and CD2 (T-cell marker) but are CD38-. The CD34 cells with lymphoid markers are associated with the hypogranular variant of PML (FAB classification, M3v).\textsuperscript{32}

The association of lymphoid markers with myeloid markers raises the debate of lineage infidelity or lineage promiscuity. In the lineage infidelity model, the coexpression of myeloid and lymphoid markers on the same cell may be due to a deregulation of the lineage-associated genes during leukemic transformation.\textsuperscript{33} In the lineage promiscuity model, the coexpression of these markers may reflect the immunophenotype of the mutated progenitor cell and perpetuated in the cell’s progeny.\textsuperscript{34} More recent studies of murine bone marrow indicate that myeloid and lymphoid genes are both expressed on pluripotent progenitor cells prior to commitment of the cell to one lineage or the other.\textsuperscript{35,36} When cells become committed to the myeloid lineage, there is progressive silencing of the lymphoid and NK cell genes. On the other hand, when progenitor cells become committed to the lymphoid cell line, there is progressive silencing of the myeloid genes. Further, a study of leukemic transformation as a result of fusion genes involving MLL showed that the leukemic phenotype is dependent on the targeted mutated cell.\textsuperscript{37} If the hematopoietic stem cells and multipotential progenitors were targeted, the result was AML, ALL, and biphenotypic leukemia. However if the committed myeloid progenitor cells or committed lymphoid progenitors were targeted, the result was the formation of only myeloid or lymphoid colonies respectively. Although the typical immunophenotype of PML blasts are CD34-, the majority of CD34+ cells in these cases, harbor the PML/RARA fusion gene suggesting the mutation occurs in an earlier progenitor cell. Thus, in at least some cases, it appears that the mutated target cell in PML is the noncommitted hematopoietic progenitor cell.

The target mutated cell in PML may affect the biological characteristics and clinical presentation of PML. In patients with hypergranular PML (FAB classification M3), the BCR1 PML breakpoint is common. These cases most often express the wild-type FLT3 and the PML cells lack lymphoid markers. In the hypogranular variant of PML (FAB classification M3v), the BCR3 PML breakpoint is more common, the cells most often have activating mutations of FLT3 and the PML cells co-express lymphoid antigens. These findings suggest that the variants of PML are determined by the progenitor targeted and the related genetic mutations.

Genetic mutations
As discussed above, the genetic mutation diagnostic of PML is PML/RARA fusion gene and protein, a mutant of one of the retinoic acid receptors. This is a class II mutation that affects the ability of the cell to differentiate. The RARA part of the fusion protein retains both the DNA binding domain and the carboxy terminal E domain. This mediates interaction between the ligand, retinoic acid (RA), and the retinoid acid receptor (RAR) which is needed for high-affinity binding at the retinoid response elements.

The normal activity of PML protein (growth inhibitor and regulator of apoptosis) is disrupted in the fusion PML/RARA protein. PML-NBs are involved in transcriptional regulation. PML does not bind DNA directly but may regulate transcription through interactions with other proteins. Recently it has been found that tumor suppressor proteins also are located in the NBs, including p53. PML can activate transcription of p53 in an NB-dependent process. P53 is activated post-translationally by acetylation. Although PML does not have acetyltransferase activity, it may be important in the stability of p53-acetylation complex. PML may also regulate p53 phosphorylation. When PML is fused with RARA, the NBs are disrupted and PML function is deregulated which may give the leukemic PML cell a survival advantage.\textsuperscript{38}

The following activities have been ascribed to the PML/RARA fusion protein:

- At physiologic levels of retinoic acid (RA), PML/RARA fusion protein complexes with nuclear co repressors (NCOR) and histone deacetylase (HDAC) with a higher affinity than wild-type RARA. This complex interferes with the transcription of downstream retinoid target genes. In addition, DNA methyltransferases are recruited and methylate key promoters which represses transcription of these target genes. This is thought to mediate the block in differentiation beyond the promyelocyte stage.
- PML/RARA forms a dimer with the wild type PML protein causing structural disruption of the PML nuclear body. This may interfere with signal transduction, apoptosis, and DNA transcription.

In rare cases the promyelocytic leukemia zinc finger (PLZF;11q23) fuses with RARA to form PLZF-RARA (t11;17)(q23;q21).\textsuperscript{38} Promyelocytic leukemia zinc finger is a DNA binding transcription repressor and regulates apoptosis and cell proliferation. In PML patients with this translocation the prognosis is poor and there is no response.
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to ATRA treatment. Several other genes may fuse with RARA resulting in PML including nucleophosmin (NPM), t(5;17)(q35;q12-21), nuclear matrix associated (NuMA), t(11;12)(q13;q12-21) and STAT5b, del(17q).40-42 In mice, expression of the reciprocal fusion occurs when there are other non-random chromosomal aberrations. In mice, expression of the reciprocal fusion RARA/PML in addition to PML/RARA results in more mice developing PML but does not alter the latency of PML development. This suggests that additional mutations are necessary for the development of PML. (About 80% of human patients with PML express the reciprocal fusion RARA/PML in addition to PML/RARA.) The most common recurring secondary abnormalities in humans with PML are +8 or partial trisomy of 8q, and ider(17)t(15;17). The PML/RARA mutation may cause genetic instability resulting in the secondary mutations. The significance of these secondary mutations is not known as they are not associated with prognosis and do not predict response to therapy with ATRA.

Immunophenotyping

The clinical consequences of untreated PML are life threatening. Thus rapid identification of patients with this disease is important so that targeted therapy with ATRA can begin as soon as possible. Currently, morphology is the primary means of diagnosis. Morphology shows good correlation with the t(15;17) karyotype but morphology alone may miss the M3v variant of PML. The results of cytogenetics and molecular testing are not available for days. Thus, immunophenotyping may be helpful in providing timely results in cases in which morphology is equivocal. One study showed that morphology, cytochemistry with myeloperoxidase, and immunophenotyping using anti-PML-RARA antibodies is definitive and provides timely information for an accurate diagnosis. Another study suggests using immunophenotyping for rapid discrimination using heterogeneous expression of CD13, the existence of a single major blast cell population, and a CD34/15 phenotype.44

Therapy

A new treatment strategy for acute leukemia is molecular targeting of genes by agents that induce the cell to differentiate and undergo apoptosis. The first such agent, ATRA, was introduced for treatment of PML in 1986. Treatment with pharmacologic doses of ATRA is combined with chemotherapy. Complete remission is achieved in 90% to 95% of patients who receive ATRA with chemotherapy. However, up to 30% of patients relapse and become resistant to ATRA. At pharmacologic concentrations of ATRA, the following occurs:46

• ATRA binds to mutated RAR, causes degradation of the PML/RARA protein, and restores the cell’s ability to differentiate.
• ATRA induces relocalization of PML in the nucleus and restores the natural structure of PML-NBs. This may restore the PML-NB related-function, including transcription of the p53 tumor suppressor gene.
• In the presence of pharmacological doses, binding of ATRA to PML/RARA results in a conformational change in the fusion protein. This causes nuclear corepressors to dissociate from the repressive complex and coactivator is recruited. This results in renewal of transcription of target genes and subsequent cell differentiation that ends with apoptosis.

More recently arsenic trioxide has been used in treatment of PML, particularly in patients with relapse PML. Clinical remission can be achieved for at least 18 months but it is not clear if its use increases the five year survival rate. It has also been used in post-remission therapy to prevent disease recurrence. Arsenic can be carcinogenic or have antitumor effects. Arsenic induces apoptosis and degrades the PML-RARA oncoprotein. It also causes PML to localize in the nucleus where it is degraded by proteosomes. Arsenic inhibits transcription of the telomerase gene. Telomerase is expressed in most cancer cells but not somatic cells after birth. Telomerase is important to maintain the length of chromosome ends that would shorten after cell division without it. When the telomeres reach a critical short length, the cells stop dividing and become senescent. Most cancer cells that lack telomerase have slow growth and death rates. Thus, therapy aimed at inhibiting telomerase is being investigated as a possible target therapy not only in PML but also in other cancers.

In one study, arsenic trioxide was given to patients who relapsed after achieving remission with ATRA and chemotherapy. The patients were divided into two groups. One group was given ATRA plus arsenic and the other was given
only arsenic. In both groups there was an 80% remission rate. Thus, arsenic may be another promising therapy for PML.

**REVISE IN CLASSIFICATION OF AML**
The specific genetic mutations in AML have an impact on clinical behavior of the disease. Some mutations are specific for a particular type of AML and assist in classification while others have prognostic or treatment implications. AMLs with genetic abnormalities, distinct clinical features, and characteristic morphology are now classified as separate categories within the WHO classification system. Except for PML, these new AML categories do not correlate with the FAB classification, a morphology based classification system. However, morphology is still used to help initially identify AML that then is confirmed with genetic analysis. There are four main groups of AML in the WHO classification:

1. AML with recurrent cytogenetic translocations.
2. AML with myelodysplasia-related features.
3. Therapy-related AML and MDS.
4. AML not otherwise categorized.

The categories with distinct genetic aberrations are described in Table 3. These AMLs are excluded from the FAB classification. Patients with the t(8;21), inv(16), or t(15;17) genetic aberrations are diagnosed with AML regardless of the bone marrow or peripheral blood blast count. Severe multilineage dysplasia, prior therapy, and/or prior MDS are poor prognostic factors in AML. It is suggested that these AMLs probably have a common pathogenesis. AMLs secondary to alkylating-therapy are associated with specific cytogenetic abnormalities (3q-,-5,-5q,-7,-7q,+8,+9,11q12p-), and complex karyotypes. AML secondary to therapy with topoisomerase II inhibitors show cytogenetic abnormalities similar to those found in de novo AML and should be considered as distinct from alkylating-therapy AMLs. The most common abnormalities associated with topoisomerase II inhibitors are 11q23, t(8;21), inv(16), and t(15;17).

**DEFINING COMPLETE REMISSION IN AML**
As clinical research into new therapy for AML patients progresses and techniques to detect minimal residual disease (MRD) improve, the criteria for clinical remission must be redefined. The recommendations of the International Working Group are an attempt to standardize the design and report of clinical trials by revising guidelines for assessing patient response to therapy. Cytochemical and phenotypic analysis should be done on all AML or suspected cases of AML. Cytogenetics performed at diagnosis is important in directing therapy and for prognosis. After therapy or with disease progression, karyotypes often vary from karyotypes at diagnosis. Immunophenotyping will help differentiate AML from ALL. Molecular studies are important in the development of targeted therapies and, in the case of PML-RARA, in delivering targeted therapy.

The goal of therapy in AML is to achieve a complete remission. In AML, there are three types of remission: hematologic, cytogenetic, and molecular. In hematologic remission, peripheral cell counts are normal, the differential count is normal, and the bone marrow has less than 5% blasts. The number of blasts in the peripheral blood is not significant if the bone marrow blast count is less than 5% blasts. In cytogenetic remission, all cells examined (usually 20) have a normal karyotype. In molecular remission, there is no evidence of a mutation at the molecular level. Molecular remission is considered most sensitive as it will identify abnormal cells at a sensitivity level of 1 in 10,000 to 1 in 100,000 cells. The recommended criteria for defining these various types of remission are listed in Table 4. In cytogenetic remission, it is important to define the criteria for a normal karyotype including the number of metaphases required and technique used, i.e., fluorescence in situ hybridization is more sensitive than conventional banding. Molecular remission criteria also must be defined according to the molecular markers studied and the sensitivity of the assay used.

**SUMMARY**
Advances in biology and genetics have led to new insights into our understanding of AML. The molecular target for the original genetic mutation appears to be the primitive hematopoietic stem cell. The heterogeneity of AML is probably related to the specific mutation in the target cell. Two muta-

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**Table 3.** Specific categories of AML with distinct genetic aberrations according to the WHO classification

- AML with t(8;21)(q22;22), AML1(CBFA/ETO)
- APL t(15;17)(q22;q11-12)
- AML with abnormal eosinophils, inv(16)(p13;q22) or t(16;16)(p13;q22), CBFB/MYH11
- AML with 11q23(MLL) abnormalities
The laboratory’s role in identifying leukemic blasts by morphology and cytochemistry and differentiating ALL from AML by immunophenotyping, remains important for initial diagnosis. However, as the molecular basis for these malignancies are identified, the laboratory’s role will expand to include more molecular testing for diagnosis and for identifying molecular remission following treatment.

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FOCUS: MYELOCYTIC LEUKEMIAS

Chronic Myelocytic Leukemia – Part I: History, Clinical Presentation, and Molecular Biology

TIM R RANDOLPH

DATA SOURCES: Current literature.

DATA SYNTHESIS: Chronic myelocytic leukemia (CML) was initially described in 1845 and is considered one of the first leukemias to be discovered. Diagnosis of CML was dramatically improved with the discovery of the Philadelphia chromosome by Nowell and Hungerford in 1960. However, the rudiments of our understanding of the molecular cause of CML began in 1973 when Janet Rowley discovered that the Philadelphia chromosome is a reciprocal translocation between chromosomes 9 and 22. The leukemogenic mechanisms of CML were hypothesized 20 years later when it was discovered that the t(9;22) translocation produced a fusion gene involving the BCR gene from chromosome 9 and the ABL protooncogene from chromosome 22. Multiple breakpoints in BCR produce fusion genes that are translated into chimeric protein products of different lengths that are associated with different leukemic subtypes.

CONCLUSION: Although CML has a rich history of interest to hematologists, it also represents a leukemogenic paradigm to the molecular biologist. Nearly all malignancies result from a series of mutagenic events, which culminate in full malignant transformation. However, it appears that CML results from a single mutagenic event involving the t(9;22) translocation leading to the development of the BCR/ABL fusion gene and the corresponding fusion protein. The successful transcription and translation of the BCR/ABL fusion protein led researchers to carefully study its involvement in leukemogenesis. The BCR/ABL fusion protein exhibits increased and constitutive tyrosine kinase activity that differs depending on which BCR breakpoint is expressed, resulting in varying clinical presentations.

ABBREVIATIONS: ABL = Ableson oncogene found in a strain of mouse leukemia virus; ALL = acute lymphocytic leukemia; BCR = breakpoint cluster region; CML = chronic myelocytic (myelogenous) leukemia; FAB = French-American-British; FAK = focal adhesion kinase; GEF = GDP-GTP exchange factor; JAK-STAT = janus kinase-signal transducers and activators of transcription; PI-3 Kinase = phosphoinositide-3 kinase; RAC GAP = RAS-like GTPase GTP activator; WHO = World Health Organization.

INDEX TERMS: BCR/ABL; chronic myelocytic leukemia; Philadelphia chromosome; t(9;22); tyrosine kinase inhibitor.


Tim R Randolph MS CLS (NCA) is an assistant professor in the Department of Clinical Laboratory Science, Doisy School of Allied Health Professions, Saint Louis University Health Sciences Center, St Louis MO.

Address for correspondence: Tim R Randolph, Department of Clinical Laboratory Science, Doisy School of Allied Health Professions, Saint Louis University Health Sciences Center, 3437 Caroline St, St Louis MO 63104-1111. (314) 977-8518, (314) 977-8503 (fax). Randoltr@slu.edu

Shirlyn B McKenzie PhD CLS(NCA) is the Focus: Myelocytic Leukemias guest editor.

LEARNING OBJECTIVES: Following careful study of this review, the reader will be able to:
1. Discuss the first scientific description of CML;
2. Discuss the history of the Philadelphia chromosome to include the discovery of the “minute” chromosome 22 and the t(9;22) reciprocal translocation;
3. Describe the clinical and laboratory features of CML;
4. Sketch the t(9;22) translocation that produces the Philadelphia chromosome;
5. Describe the molecular biology of the four primary BCR/ABL fusion genes to include the four discrete breakpoints and the resulting gene arrangements;
6. Discuss the leukemogenic mechanisms in CML involving the BCR/ABL fusion protein; and
7. Compare three different versions of the fusion protein and discuss disease associations.

Chronic myelocytic leukemia (CML) is a myeloproliferative disorder that engenders scientific interest among groups as diverse as clinical hematologists, clinical laboratory professionals, molecular biologists, and oncologists. To the clini-
cal hematologist, CML represents a common hematologic disorder requiring careful scrutiny of both clinical and diagnostic information to make informed therapeutic decisions to maintain quality patient care. To the laboratory professional, CML is known to be the first malignancy directly linked to a genetic mutation, creating a reliable diagnostic tool. Molecular biologists are intrigued by the effect of a single mutagenic event on signal transduction pathways leading to malignant transformation. Since leukemogenic transformation in CML is sufficiently accomplished by the t(9;22) translocation, the multistep mechanisms of carcinogenesis necessary in most other forms of cancer are not required in CML creating a carcinogenic paradigm to the oncologist.

**LEUKEMOGENIC AND THERAPEUTIC PARADIGM**

Nearly all malignancies, regardless of type, are thought to be the result of a series of mutations in a progenitor cell that causes the cell to lose control of growth, differentiation, and apoptotic mechanisms resulting in full malignant transformation. When a cell line has received sufficient mutations to alter phenotype, but insufficient mutations to produce full malignant transformation, the cell line is said to be dysplastic or pre-malignant. The list and sequence of mutations commonly identified in a given cancer type seems to vary significantly between patients diagnosed with the same malignancy. This makes diagnosis and prognosis based on genetic abnormalities difficult for most malignancies. For a particular cancer type, patients will express different lists of mutations that appear at different stages during the progression of their disease. However, there are exceptions to this model and two such exceptions are hematopoietic malignancies, namely AML/M3 (also known as acute promyelocytic leukemia) and CML. In both cases it appears that a single mutation is sufficient to produce full leukemic transformation.

In AML/M3 the mutation is the t(15;17) translocation involving the PML/RARα genes, and in CML it is the t(9;22) translocation forming the Philadelphia chromosome that produces the BCR/ABL fusion gene. The phenomenon of full leukemogenic transformation from a single mutation makes both AML/M3 and CML leukemogenic paradigms. In addition, the discovery of single mutation transformations and their impact on leukemogenesis resulted in the development of tailored, single agent therapies targeting the products of these mutations, creating a therapeutic paradigm.

**HISTORICAL ORIGIN OF CML**

The first scientific description of CML is credited to John Hughes Bennett in Edinburgh in 1845. However, patients with vague but similar symptoms can be found in the French literature as early as 1825. It is possible that some of these earlier patients may have also suffered from CML. For example, Velpeau reported a case of a 63-year-old woman who, at autopsy, was found to have an enormous spleen and whose blood was “thick like gruel such that one might have asked if it were not rather laudable pus, than blood.” Later in 1839 the French microscopist Paul Donne described a 44-year-old woman who presented at autopsy with an enlarged spleen and whose blood seemed “semipurulent under the microscope with more than half of the cells appearing to be white globules.” Nevertheless, Bennett’s description was more complete and scientific in nature thereby earning him credit as the first description of CML. Bennett became interested in the disorder when his mentor, Dr David Craigie, observed two patients admitted to the Royal Infirmary in Edinburgh with unusual blood consistency and a splenic tumor. The first patient was observed in 1841 but was dismissed as unusual until 1844 when a 28-year-old man presented with similar symptoms. John Bennett was given permission to perform an autopsy and study the pathology of this second case. His report entitled “Case of hypertrophy of the spleen and liver in which death took place from suppuration of the blood” was published in the *Edinburgh Medical and Surgical Journal* in October 1845.

In the same year, Robert Virchow, a 24-year-old graduate of the Berlin Army Medical School, observed his first case of CML while studying the pathology of phlebitis. A 50-year-old woman was admitted to the Charite’ Hospital in Berlin complaining of fatigue, nosebleeds, swelling of the legs and abdomen, and died within four months. Virchow noted the enlarged spleen and liver, but also described blood vessels full of material resembling pus. In 1847 Virchow suggested the term “leukamie” for the disorder, meaning white blood, but it did not achieve universal approval because many physicians agreed that “blood was never white.” In 1852, Bennett recommended the term “leucocytia” for the disorder, meaning increased white blood cells, which was better accepted, especially in light of the 37 cases Bennett had described to date. In 1856, Virchow was credited with concluding that the disorder was not the result of an infectious process but rather was caused by the tissue that produced the white cells. He also categorized two types of chronic leukemia, splenic and lymphatic, which we now know as leukemia and lymphoma, respectively.

Today we understand CML as a malignant clonal disorder of the pluripotent hematopoietic stem cell, resulting in proliferation of predominantly immature myeloid cells. However, CML also affects cells of the monocytoid, eryth-
The stem cell origin of the disorder is reflected in the concomitant thrombocytosis and the eventual production of blasts that are frequently not of the myeloid line. The low LAP confirms abnormal myeloid function suggesting a malignant origin. A bone marrow analysis is often unnecessary to establish the diagnosis, but when performed will generally reflect the peripheral findings. The bone marrow will be hypercellular with an elevated M:E ratio corresponding to the elevated WBC count and the left shift. An increase in megakaryocytes is responsible for the thrombocytosis, and the combination of bone marrow suppression by the leukemic myeloid cells and the production of fibrotic tissue produce the anemia.

PHILADELPHIA CHROMOSOME (Ph1)

The discovery of the Philadelphia chromosome had a major impact on CML diagnosis. The Philadelphia chromosome was discovered by Nowell and Hungerford in 1960 at the University of Pennsylvania in Philadelphia. Although experimentation in chromosomal analysis began in the 1940s, it wasn’t until the 1950s that the technology was applied to neoplastic disorders and leukemias. Nowell and Hungerford described a minute acrocentric chromosome, termed the Philadelphia chromosome, that was initially observed in two male patients, followed by seven other patients all with CML. Blood cells from the CML patients were cultured on a microscope slide tilted in a culture bottle to provide a gradient of cell numbers, oxygen tension, and oxidation/reduction potential. As the cells matured they fell off the slide and settled in the bottom of the jar. Colchicine was added to stop cell division in metaphase and the cells were swollen in a hypotonic medium. The chromosomes were then photographed and observed for deviation from normal.

The Philadelphia chromosome was identified as a “minute” chromosome and was subsequently observed in about 90% of patients with CML. The name was later shortened to Ph1 to indicate the first chromosome in what researchers expected to be a string of associations between consistent chromosomal abnormalities and malignancies. However, for more than a decade the Philadelphia chromosome remained the only chromosomal lesion consistently associated with a specific neoplastic disease.

The Philadelphia chromosome was first thought to be chromosome 21 but when banding techniques were introduced it was discovered that the Philadelphia chromosome was actually a deletion of chromosome 22. It wasn’t until 1973 when Janet Rowley, from University of Chicago, was able to show that the Philadelphia chromosome was not simply a deletion of the long arm of chromosome 22, but rather a reciprocal translocation between chromosomes 9 and 22. Rowley separated the red blood cells...
from the white blood cells in CML patients and cultured the WBCs both with and without phytohemagglutinin (PHA), a lymphocyte mitogen. Cells in PHA were used as the normal control because PHA stimulates cell division. In contrast, only leukemic cells could proliferate in the absence of PHA. The t(9;22) translocation was resolvable using standard staining and fluorescent staining techniques. As can be seen in Figure 1, chromosome 22 is broken just below the centromere while chromosome 9 is broken near the distal end of the long arm. The small piece from chromosome 9 is translocated to chromosome 22 creating a significantly shortened chromosome 22, previously described as a “minute” by Nowell and Hungerford. In contrast, chromosome 9 is lengthened due to the removal of a small segment from the distal end and the addition of a large segment from chromosome 22. The Philadelphia chromosome translocation is now known to occur as t(9;22)(q34;q11).

The value of the Philadelphia chromosome as a diagnostic tool became immediately obvious when it was observed in the karyotypes of approximately 90% to 95% of the CML cases tested. When molecular hybridization techniques were introduced and applied to Philadelphia negative CML cases, it was found that many of these exhibited the translocation at the molecular level.7 Apparently, the original translocation may be followed by another translocation, either between chromosomes 9 and 22 or other chromosomes, that redistributes sufficient genetic material to restore the chromosomes to nearly the original length. The slight difference in genetic material between karyotype negative and karyotype positive Philadelphia chromosome samples in this scenario is not resolvable by karyotype analysis. Therefore, taken together, nearly all patients with CML were found to have the translocation producing the Philadelphia chromosome identified either at the chromosomal or molecular level.

In addition, the Philadelphia chromosome is found in 5% of children with ALL, 15% to 30% of adults with ALL and 2% of patients with newly diagnosed AML.8,9 The most obvious explanation for these findings is that many of these patients may represent the blast crisis phase of a previously undiagnosed CML. However, increasing numbers of investigators were finding cases of apparently de novo acute leukemia that are Philadelphia chromosome positive. This phenomenon can best be explained by the discovery that the site of breakage on chromosome 9 occurs at different places, which alters the product of the translocation and thus the leukemogenic mechanism.

PATHOPHYSIOLOGY OF CML

The pathophysiology of CML is a very interesting story that has become clearer in recent years. The origin of the malignant transformation in CML is understood to be the Philadelphia chromosome. This connection is supported by three pieces of evidence. First, nearly all patients with CML have the 9;22 translocation. Second, in most cases of CML, Philadelphia chromosome is the only genetic lesion detected. Last, Philadelphia chromosome is rarely detected in malignancies that do not have a CML origin. The cause of the 9;22 translocation is not known, but an increased incidence has been associated with radiation and benzene exposure. The link to radiation was made when the number of cases of CML increased 100 fold in survivors of the nuclear bombing of Hiroshima and Nagasaki during World War II. A significant increase was also noted among radiologists prior to lead shielding and in workers exposed to benzene. There are about two new cases of CML each year per 100,000 people, accounting for about 15% of leukemias in adults. CML affects middle-aged men more frequently, with a male to female ratio of 3:2 and a median age at diagnosis of 53 years. About 40% of CML diagnoses are made in asymptomatic patients solely from laboratory observations of abnormal blood counts and differentials.
As stated earlier, CML follows a triphasic clinical course with the chronic phase lasting approximately four years, the accelerated phase between six to eighteen months and the blast crisis phase terminating in death in less than eight months. The chronic phase is characterized by a high WBC count, a left shift, thrombocytosis, and a mild to moderate normocytic/normochromic anemia. The WBC is usually greater than $100 \times 10^9/L$ with a typical range of $200-500 \times 10^9/L$. WBC counts as high as $1.0 \times 10^{12}/L$ have been reported. The left shift reveals all stages of myeloid differentiation with a noticeable increase in promyelocytes, metamyelocytes, and myelocytes. Basophilia and eosinophilia are also common.

Approximately half of the patients will present with thrombocytosis and a mild to moderate anemia producing a hemoglobin value of between 9 and 13 g/dL. A bone marrow analysis is usually not necessary for diagnosis but when performed will exhibit hypercellularity, an M:E ratio of between 10:1 to 50:1, a notable left shift, and occasional presence of fibrotic tissue. Blasts may be increased but must be less than 30% in the bone marrow to be classified as chronic leukemia by FAB criteria and less than 20% by WHO criteria. Most patients present with minimal symptoms but usually exhibit hepatosplenomegaly, resulting from extramedullary hematopoiesis in the liver and spleen.

The accelerated phase of CML is marked by an increasing WBC and basophil count, a decreasing platelet and RBC count and, most notably, an increase in circulating blasts. Increasing blasts in the presence of immature myeloid cells indicate the transformation from chronic leukemia to acute leukemia. The bone marrow shows an increased number of blasts with suppression of erythroid and megakaryocytic proliferation, which is responsible for the presence of peripheral blasts, anemia, and thrombocytopenia in the blood. The promyelocytes, myelocytes, and metamyelocytes observed in the chronic phase are more likely to be released into circulation as compared to the blasts that accumulate in the accelerated phase.

Blasts possess the necessary homing receptors to a greater degree than do their more mature counterparts, which facilitate retention in the bone marrow resulting in cellular accumulation. The increasing number of blasts and the proliferation of fibrotic tissue contribute to bone marrow suppression that produces the anemia and thrombocytopenia characteristic of the accelerated phase of CML. Symptoms of fever, night sweats, weight loss, and splenomegaly are exacerbated in the accelerated phase. Additional chromosomal mutations are observed in this stage of CML and are largely responsible for the transformation from the chronic to the acute clinical picture. The accelerated phase of CML lasts approximately six to eighteen months with 30% of patients dying prior to entering blast crisis.

Prior to tyrosine kinase inhibitors, once a patient enters blast crisis, interventions were futile and death imminent. Both the symptoms and the peripheral blood abnormalities intensify. However, in about one fourth of CML patients, the blast crisis phase occurs without the typical transition through the accelerated phase. The differential reflects the increasing number of blasts and the worsening anemia and thrombocytopenia. Patients develop bleeding symptoms from the thrombocytopenia, bone tenderness from the expanding bone marrow and gouty arthritis from uric acid build-up, as cell turnover increases. The bone marrow reflects the increasing blasts, and the stage is marked by a bone marrow blast count of >30% by FAB criteria. About 25% to 35% of patients that enter blast crisis produce ALL, while about 65% to 75% result in AML. Less than 10% of cases result in acute leukemias of other lineages. Historically, death would occur in less than eight months after entering blast crisis, generally from bleeding, infection, or bone marrow aplasia. Patients with ALL blast crisis have a higher complete remission rate (60%) as compared to patients with AML blast crisis (20-30%), but the duration of remission is less than one year. The hopelessness associated with blast crisis is changing with the advent of targeted molecular therapy involving tyrosine kinase inhibitors.

An atypical form of CML has been observed in children. This atypical form is termed juvenile CML by the FAB group and is omitted in the WHO classification system. It accounts for between 1% and 5% of childhood leukemias and generally affects children under five years of age. The WBC count is usually between $15 \times 10^9/L$ and $100 \times 10^9/L$ at diagnosis with a mean of $30 \times 10^9/L$. There is a slow increase in blasts and the children develop skin rashes and infections. Juvenile CML progresses faster than adult CML producing death in about two years.

**MOLECULAR BIOLOGY OF t(9;22) IN CML**

Our understanding of the molecular biology of the t(9;22) translocation has contributed to the current theories of leukemogenesis in CML. Once the ABL oncogene was mapped to the long arm of chromosome 9 it was quickly confirmed that the t(9;22) translocation brought together the ABL oncogene to an unknown area of chromosome 22. It was later discovered that the breakpoints that occurred on chromosome 22 clustered within a limited region on the long arm that was subsequently termed breakpoint cluster region (BCR).
fore, the t(9;22) translocation creates a BCR/ABL fusion gene that is transcribed into a chimeric BCR/ABL mRNA and translated into a hybrid protein.\(^8\) As can be seen in figure 1, the 9;22 translocation interrupts the ABL oncogene on chromosome 9 and the BCR gene on chromosome 22. The ABL gene is a murine viral oncogene that is 230 kilobases in length and contains 11 exons with two splice sites (Figure 2a). The ABL gene normally codes for a 145 kilodalton nuclear protein, called p145, that possesses tyrosine kinase activity. In contrast, the BCR gene complex is composed of at least four separate genes termed BCR1, BCR2, BCR3, and BCR4. BCR1 is the most common BCR gene involved in the 9;22 translocation and is illustrated in the upper panel of Figure 2a. BCR1 is approximately 100 kilobases in length and divided into 20 exons with two splice sites. The gene normally codes for a 160 kilodalton protein (p160) that is constitutively expressed in many cell types, but strongly expressed in hematopoietic cells.

**NORMAL PROTEIN PRODUCT OF THE ABL GENE (p145)**

P145 is the gene product of the normal c-abl protooncogene, and is a nuclear protein with non-receptor, tyrosine kinase activity. The p145 protein has been shown to migrate between the nucleus and cytoplasm.\(^{13,14}\) This activity has been linked to cellular growth control by being associated with several growth factor receptors like epidermal growth factor (EGF), platelet derived growth factor (PDGF), and colony stimulating factor (CSF). The protein p145 is found in Drosophila and functions in the regulation of normal cell proliferation. It is also highly conserved in vertebrates and strongly expressed in hematopoietic cells.

**NORMAL PROTEIN PRODUCT OF THE BCR GENE (p160)**

The normal function of p160 protein, transcribed from the BCR gene, is less well understood. It is constitutively expressed in many cell lines and strongly expressed in hematopoietic cells. The important functional domains are illustrated in Figure 3a. The coiled-coil motif is essential for polymerization with other proteins. The dimerization domain (DD) facilitates the formation of protein dimers. The tyrosine residue at position 177 is

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FOCUS: MYELOCYTIC LEUKEMIAS

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Figure 2a. Normal BCR and ABL genes

**Chromosome 22**

Normal BCR1 gene

1 1' 2 3 4 5 6 7 8 9 10 11 1 2 3 4 5 17 18 19 20

 minor bcr

 major bcr

 micro bcr

Figure 2b. BCR-ABL fusion gene (p210)

Two most common fusion genes in CML
(Creations from the major BCR
(Breakpoints #1 and #2)

**Chromosome 22**

BCR head

1 1' 2 3 4 5 6 7 8 9 10 11 1 2 3 4 5 6 7 8 9 10 11

**Chromosome 9**

ABL tail

1 1' 2 3 4 5 6 7 8 9 10 11 1 2 3 4 5 6 7 8 9 10 11

ABL = Ableson oncogene; BCR = breakpoint cluster region.
of particular importance in that it is an essential contact point for the binding of signal transduction proteins. The next functional domain to the right is the serine/threonine kinase domain that serves as the catalytic domain for phosphorylation activity. The GEF domain stands for GDP/GTP exchange factor and functions to compete with other GTP binding proteins for GTP, which is used as the phosphate donor for phosphorylation reactions. The RAC GAP domain controls the rate of GTP hydrolysis and is sometimes called RAS-like GTPase. The RAS GAP domain functions by converting active RAS proteins to their inactive form, when bound to GDP. So both proteins appear to function in the phosphorylation of other proteins involved in signal transduction pathways that serve to stimulate and regulate cell growth.

BCR/ABL FUSION GENES

As can be seen in Figure 2b, the 9;22 translocation brings together the 5' portion of the BCR gene with the 3' end of the ABL gene. It has been shown that there exists at least four primary versions of the BCR/ABL translocation resulting from four distinct breakpoints in the BCR gene. These four breakpoints create three different protein products, p210 (Figure 3b), p190 (Figure 3c), and p230 (Figure 3d), which may account for some of the differences in leukemogenesis between CML, Philadelphia chromosome positive de novo ALL, and chronic neutrophilic leukemia (CNL), respectively. The four known breakpoints on the BCR1 gene are illustrated in Figure 2a by the upward arrowheads. The most common breakpoint region in the BCR 1 gene is called major BCR (M-BCR) and is located in the middle of the BCR 1 gene. Major BCR contains five exons (labeled 1-5) and two of the four known breakpoints. These five exons within M-BCR correspond to exons 12-16 of the BCR1 gene.

The majority of breakpoints that occur in the BCR gene are between exons 2 and 3 (arrowhead #1) or between 3 and 4 (arrowhead #2). There are only two known breakpoints in the ABL gene occurring either between exons 1 and 1’ or between exons 1’ and 2. The fact that there are two breakpoint options in the ABL gene is irrelevant because RNA splicing always results in the lead exon being number 2 (Figure 2a). Therefore, the genetic contribution of the ABL gene remains constant. The two common breakpoint possibilities in M-BCR, coupled with the only possibility in ABL, results in the two transcripts illustrated in Figure 2b. The upper fusion gene represents the first 13 exons of the BCR and the last 10 exons of ABL, while the lower fusion gene represents the first 14 exons of BCR and the same 10 exons from the ABL gene. In both cases the protein product is a 210 kilodalton protein that is either 902 or 927 amino acids in length. This is the protein product associated with classical CML.

An alternative breakpoint region on the BCR1 gene is termed minor BCR (m-BCR), and is located 5' of the major BCR. This breakpoint is associated with the majority of cases of Philadelphia positive ALL and in rare cases of CML that tend to produce a monocytosis. In this case, only exon 1 is joined to the same 10 exons of the ABL gene, translating into a smaller 185/190 kilodalton protein shown in the upper panel of Figure 2c. The last breakpoint region on BCR occurs between exon 19 and 20 creating a longer fusion protein, 230 kilodaltons in size, illustrated in the lower panel of Figure 2c. This version is rarely observed in CML, but when identified seems to produce a version of CML called chronic neutrophilic leukemia (CNL), that is characterized by an abundance of more mature neutrophils and thrombocytosis. Other fusion products of the t(9;22) translocation have been described but rarely occur.

BCR/ABL FUSION PROTEIN

The understanding of the composition of the BCR/ABL fusion protein and the functions of the corresponding wild-type BCR and ABL proteins, allows us to predict the function of the fusion protein and its ultimate role in leukemogenesis. The ABL moiety of the fusion protein contributes to the transforming capability of the protein in at least three ways. First, and most important, the ABL moiety exhibits alterations in the normal function of the SH2 and SH3 domains that control the tyrosine kinase activity of the SH1 domain (Figure 3b). It is well
established that tyrosine kinase functions to add phosphate groups to other proteins. In signal transduction pathways designed to control cell proliferation, increased phosphorylation promotes proliferation, and dephosphorylation inhibits proliferation. In the wild-type ABL protein, the SH2 domain normally up regulates tyrosine kinase activity, and the SH3 domain down regulates tyrosine kinase activity. The t(9;22) translocation event has created a fusion gene and a resultant protein product that has lost the amino terminus of the ABL gene designed to regulate tyrosine kinase activity and has gained BCR genes that will affect the tyrosine kinase activity of the SH2 and SH3 domains. The result is constitutive tyrosine kinase activity.

Second, the breakpoint in the 5' end of the ABL gene occurs in the myristoylation domain. The loss of genetic material in the myristoylation domain of ABL results in an altered binding affinity for F-actin. This may contribute to a reduction in adherence of CML cells to bone marrow stromal elements resulting in a reduction of contact inhibition and the premature release of immature myeloid cells into circulation. In vitro studies have confirmed that primary CML cells adhere poorly to bone marrow stroma. Antisense oligonucleotides to BCR-ABL and interferon-alpha have both been shown to reverse the loss of adhesion of CML progenitor cells to bone marrow stroma and fibronectin resulting in a reduction in proliferation.

Third, loss of the myristoylation domain may also interfere with apoptotic mechanisms. The myristoylation domain normally confers nuclear localization properties to the wild-type ABL protein allowing it to shuttle between the nucleus and cytoplasm of the cell. The loss of this domain may be responsible, at least in part, for the lack of nuclear localization observed for the fusion protein. The BCR/ABL fusion protein is restricted to the cytoplasm due mainly to the constitutive activation of the tyrosine kinase. Wild-type ABL located in the nucleus has apoptotic properties, while BCR/ABL localized to the cytoplasm has anti-apoptotic functions.

The leukemogenic contribution of BCR to the fusion protein centers on the coiled-coil motif and the serine/
threonine domain. The t(9;22) translocation preserves these two domains and transposes them to the 5’ end (head) of the fusion gene. The coiled-coil motif of BCR stimulates SH2 and inhibits SH3 resulting in continuous tyrosine kinase activity. The tyrosine at position 177 (Y177) in the coiled coil motif is crucial to the binding of adaptor proteins like Grb-2 that serve to initiate signal transduction pathways. The serine/threonine kinase domain of BCR participates in leukemic transformation by retaining kinase activity, and by the activation of several signal transduction pathways involving SH2 proteins, the most important of which is the RAS pathway.20

The two remaining BCR domains, GEF (GDP-GTP exchange factor) and RAC GAP (RAS-like GTPase), may also contribute to the transforming function of the fusion protein. GEF domain binds GTP to facilitate phosphorylation, the primary function of the kinases. The RAC GAP domain normally functions to help regulate kinase activity. Therefore, the loss of this domain results in a loss of phosphorylation control within the serine/threonine domain.

Taken together, these aberrant cellular functions within both the BCR and ABL domains of the fusion protein collaborate to produce the clinical picture associated with the chronic phase of CML. The continuous activation of tyrosine kinase, together with the altered binding affinity of the fusion protein for membranes and DNA, and the activation of signal transduction pathways involving oncogenes like RAS, are sufficient to produce full transforming capabilities. It is thought that the BCR-ABL fusion protein activates the same signal transduction pathways normally activated by cytokines that control growth and differentiation. Therefore, the cells bearing the BCR-ABL fusion protein are behaving as though they are receiving constant cytokine signals stimulating proliferation at the expense of differentiation.27 In contrast, one potential explanation for the acute leukemia phenotype associated with the p190 fusion protein (Figure 3c) and the chronic presentation associated with the p210 fusion protein involves the increased tyrosine kinase activity produced by the p190 fusion protein.28,29

Progression of CML from the chronic phase to the accelerated and blast crisis phases generally involves additional genetic mutations. Cells dividing more rapidly than normal and containing genetic lesions, as do the myeloid cells in the chronic phase of CML, are more prone to additional genetic mutations as compared to normal cells. In addition, chemotherapy increases the rate of genetic mutations. Therefore, given the mutagenic predisposition of the 9;22 translocation and chemotherapeutic interventions, the additional mutations needed to progress from the chronic phase to the accelerated and blast crisis phases will usually occur. Some of the additional mutations responsible for progression to the accelerated and blast crisis phases of CML can be identified by karyotype analysis, while others require molecular techniques for identification. Monosomy of chromosomes 7, 17, or Y, trisomy 8, 17, 19, and 21, an additional Philadelphia chromosome, and the 3;21 translocation that is sometimes encountered in acute leukemias, are examples of compounding chromosomal lesions. Additional genetic mutations, resolvable at the molecular level include p53, RB1, c-MYC, RAS, and AML-EVI-1.20 Mutations in these genes have produced proteins associated with malignant transformation in many other cancer systems.

PROPOSED LEUKEMOGENIC MECHANISM
The most widely accepted and significant leukemogenic mechanism attrib-
uted to the fusion protein involves the constitutive stimulation of tyrosine kinase. The tyrosine kinase activity affects a variety of signal transduction pathways. Various regions of the fusion protein bind and activate several adapter proteins, five of which are illustrated in Figure 4 as, from left to right, BAP-1, GRB2, CBL, SHC and CRKL. These adapter proteins normally bind the same regions of the wild-type ABL and BCR proteins as occurs with the fusion protein. However, binding of the adapter proteins to the fusion protein dramatically alters their normal activation cycle. As stated earlier, the coiled coil motif and the serine/threonine kinase domain at the amino terminus of BCR, up regulates the SH2 domain and inhibit SH3 domain of the ABL moiety, occupying the carboxy terminus of the fusion protein. This participates in constitutive stimulation of the tyrosine kinase activity of the fusion protein. The serine/threonine kinase domain of the BCR moiety may also contribute additional kinase activity to increase the overall rate of phosphorylation.

Increased phosphorylation of adaptor proteins stimulates several signal transduction pathways, maintaining them in the "on" position. The most important of these signal transduction pathways is the RAS pathway. The constitutive tyrosine kinase activity produced by the fusion protein results in an increased activation of RAS, a known oncogene, creating proliferation that is independent of cytokine control. It has been shown in vitro that leukemogenic transformation can be prevented in cells expressing BCR-ABL tyrosine kinase activity by inhibiting RAS pathways. This aberrant activation also appears to protect against the pathway of natural cell death called apoptosis. The result is malignant transformation of myeloid cells that are prone to accelerated division, reduction in apoptosis, and failure to fully differentiate.

In a similar way, the constitutive tyrosine kinase activity phosphorylates adaptor proteins that up regulate other signal transduction pathways like JAK-STAT (Janus kinase-signal transducers and activators of transcription) and PI-3 kinase (phosphoinositide-3 kinase). These pathways are thought to induce cell proliferation and, when inhibited, prevent the growth of cells expressing the BCR-ABL fusion gene. Therefore, a larger number of myeloid precursors are engaged in cell division and fewer succumb to apoptosis. The combination of reducing apoptosis, increasing proliferation, and incomplete differentiation, results in an increase in immature WBCs in the bone marrow.

The phosphorylation activity of the fusion protein also activates the FAK (Focal Adhesion Kinase) pathway. The FAK pathway seems to decrease cellular adhesion to bone marrow stroma in vitro. CML cells that are unbound, or free in suspension, tend to divide more readily than cells that are bound to another cell or to a molecular matrix normally found in the bone marrow. The normal process that produces a reduction in proliferation from cells binding to each other or to matrices is called contact inhibition. The expression of integrins on the cell surface facilitates adhesion. Therefore, a reduction in integrins minimizes adhesion thus reducing contact inhibition. A reduction in inhibition is effectively stimulating division. A lack of adhesion to molecular matrices in the bone marrow also facilitates the premature release of CML cells from the bone marrow into circulation. This process allows the immature myeloid cells that are accumulating in the bone marrow to be released into circulation, increasing the peripheral WBC count and producing the typical left shift associated with CML. In addition, the premature release of these myeloid cells reduces the crowding and choking of the normal bone marrow element effectively minimizing...
the anemia and thrombocytopenia that is typically associated with acute leukemias.

An understanding of the role of the BCR-ABL gene product in the leukemogenesis of CML has led researchers to develop designer drugs to specifically target the fusion protein. These tyrosine kinase inhibitors are replacing the conventional chemotherapeutic approach to the treatment of CML. Although patient responses are very encouraging, these designer drugs are not without side effects. Some patients have experienced drug resistance and adverse events. In addition, molecular techniques used to monitor patient responses to tyrosine kinase inhibitor therapy, appear to predict outcome and guide therapeutic decisions. Therapeutic approaches involving tyrosine kinase inhibitors and the prognostic value of molecular monitoring will be discussed in Part II of this review.

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FOCUS: MYELOCYTIC LEUKEMIAS

Chronic Myelocytic Leukemia – Part II: Approaches to and Molecular Monitoring of Therapy

TIM R RANDOLPH

DATA SOURCES: Current literature.

DATA SYNTHESIS: Chronic myelocytic leukemia (CML) was initially described in 1845 and is considered one of the first leukemias discovered. Effective approaches to therapy were not instituted until arsenic was first administered in 1865. Since then, four major therapeutic milestones have been achieved: the development of alkylating agents like busulphan and 6-thioguanine in 1953, alpha interferon in 1983, bone marrow transplantation in 1986, and tyrosine kinase inhibitors in 1998. The discovery that the protein product of this fusion gene expresses constitutive tyrosine kinase activity prompted the synthesis of a designer drug, imatinib mesylate, which binds the fusion protein and neutralizes the tyrosine kinase activity. Molecular methods of detecting BCR-ABL transcripts are showing promise in confirming drug resistance and predicting patient outcomes in response to imatinib mesylate therapy. Evidence of drug resistance can guide physicians in selecting alternative therapeutic approaches early in the course of the disease to potentially rescue non responders. Although the success of clinical trials has been dramatic, drug resistance and disease relapse are issues to be considered.

CONCLUSION: The discovery that the BCR/ABL fusion protein exhibits increased and constitutive tyrosine kinase activity led investigators to develop an inhibitor to this activity. The synthesis of imatinib mesylate, currently marketed as Gleevec™ or Glivec®, is in stage III clinical trials and has proven to be the most successful antileukemic drug to date. As in CML, an understanding of the leukemogenic mechanisms involved in other leukemias will provide the groundwork for the development of therapeutic interventions tailored to the specific molecular defects identified, eventually rendering obsolete the shotgun approaches to massive cell killing produced by chemotherapy.

ABBREVIATIONS: ABL = Ableson oncogene found in a strain of mouse leukemia virus; ASH = American Society of Hematology Annual Meeting; BCR = breakpoint cluster region; CCR = complete cytogenetic response; CHR = complete hematologic response; CML = chronic myelocytic leukemia; INF = interferon; MCR = major cytogenetic response; MMR = major molecular response; RT-PCR = reverse transcriptase-polymerase chain reaction; SCT = stem cell transplant.

INDEX TERMS: BCR/ABL; chronic myelocytic leukemia; Philadelphia chromosome; t(9;22); tyrosine kinase inhibitor.


Tim R Randolph MS CLS(NCA) is an assistant professor in the Department of Clinical Laboratory Science, Doisy School of Allied Health Professions, Saint Louis University Health Sciences Center, St Louis MO.

Address for correspondence: Tim R Randolph, Department of Clinical Laboratory Science, Doisy School of Allied Health Professions, Saint Louis University Health Sciences Center, 3437 Caroline St, St Louis MO 63104-1111. (314) 977-8518, (314) 977-8503 (fax). Randoltr@slu.edu.

Shirlyn B McKenzie PhD CLS(NCA) is the Focus: Myelocytic Leukemias guest editor.

Focus Continuing Education Credit: see pages 57 to 69 for learning objectives, test questions, and application form.

LEARNING OBJECTIVES: Following careful study of this review, the reader will be able to:
1. Describe the first documented therapy for CML.
2. Discuss the chemotherapeutic approach to CML treatment.
3. Discuss one advantage and one disadvantage of alpha interferon and bone marrow/stem cell transplants in the treatment of CML.
4. Discuss the therapeutic approach to CML involving tyrosine kinase inhibitors to include:
   a. molecular target of tyrosine kinase inhibitors.
   b. function of the tyrosine kinase inhibitors.
   c. effectiveness of therapy.
   d. drug resistance and adverse events.
   e. alternative therapeutic approaches in patients with drug resistance.
EVOLUTION OF THERAPEUTIC APPROACHES TO CML
The approach to CML therapy has evolved over the years and is on the threshold of a potential cure for many patients. The first documented therapy that was effective for CML was arsenic, first administered in 1865 by a German physician named Lissauer. In this patient, arsenic therapy was shown to reduce the WBC count, diminish the splenomegaly, and improve anemia, all contributing to the amelioration of symptoms. This therapeutic approach was continued until 1903 when radiotherapy was instituted. In 1912, benzene was introduced in conjunction with radiotherapy as a treatment for CML. An alternative form of radiotherapy using radioactive phosphorous was initiated in 1938.

Nitrogen mustards, an agent of chemical warfare, were administered to CML patients in 1947.5, 6 These experiments with nitrogen mustards led to the development of other alkylating agents, such as busulfan which was introduced in 1953, and ushered in the modern era of chemotherapy. Long-term survival was improved for the first time and busulfan combined with 6-thioguanine became the mainstay of treatment for the next 35 years. Alpha interferon was introduced in 1983 as a therapeutic approach that not only increased survival, but it also induced Philadelphia chromosome negativity and reduced progression to blast crisis. This therapy was supplemented with bone marrow transplantation in 1986 for patients under 55 years of age and became the mainstay until recently. The future direction of therapy lies in tyrosine kinase inhibitors and other molecules that block key steps in the leukemogenic signal transduction pathways leading to full transformation.

CML THERAPY IN THE MODERN ERA
Until recently, the standard approach to CML therapy was to reduce the tumor burden with tumor reducing chemotherapy. Although CML precursors were engaged in cell division to a greater extent than normal myeloid stem cells, they were not active enough to be significantly affected by aggressive chemotherapy, as is used in acute leukemias. The more commonly used drugs to treat CML were busulfan, hydroxyurea, 6-mercaptopurine, and 6-thioguanine. Hematologic remissions were achieved in 75% of patients treated with busulfan or hydroxyurea but these remissions only lasted two to three years. Although hydroxyurea is preferred over busulfan due to lesser toxicities and prolonged survivals, (56 months vs. 44 months respectively), neither drug produced a clinically acceptable rate of cytogenetic response or slowed disease progression toward blast crisis.10, 11

The advent of alpha interferon (INF-α) improved outcomes in CML patients when used as initial therapy by inducing cytogenetic remissions and increasing survival rates from 35% to 55%. These findings earned INF-α the position of first-line therapy in CML patients who were not eligible for allogeneic bone marrow transplantation. INF-α reduces myeloid cell numbers by stimulating a cell-mediated, anti-tumor host immune response. INF-α also induces apoptosis in the leukemic clone and restores integrin-mediated adhesion to collagen. Binding of CML progenitors to bone marrow stroma induces natural proliferation inhibition mechanisms. INF-α has improved the rate and duration of hematologic remissions to between 60% to 80%, produced major cytogenetic responses (MCR) (<35% Ph1+ metaphases detected) in 25% of cases and produced complete cytogenetic remission (CCR), defined as Ph1 negativity, in 10% to 20% of the cases.

Dramatically improved response rates can be achieved when cytarabine (Ara-C) is administered with INF-α. At 18 months post therapy, the INF-α with Ara-C arm of the IRIS (International Randomized INF vs. STI571) study revealed a hematological remission rate of 93%, an MCR of 34%, and a CCR of 15%. However, some patients experienced severe side effects, developed drug resistance, and succumbed to relapses at rates similar to chemotherapy. Most important, the majority of patients achieving CCR following interferon therapy retained the BCR-ABL gene and its products as detected by molecular methods like RT-PCR and fluorescent in situ hybridization (FISH).15

As techniques improved, bone marrow and stem cell transplants have become a viable form of therapy, especially in younger patients in the chronic phase of CML, due to the curative potential of the treatment. Relapses occur in 15% to 30% of transplanted patients but relapses become infrequent beyond the five-year survival threshold. Long-term survivals were reported between 50% and 80% with disease free survival rates between 30% and 70%. Normal hematopoietic progenitors exist in the CD34(+) cell pool and can be mobilized from autologous and allogeneic donors for stem cell transplants. To be considered a good candidate for transplantation, patients need to be less than 50 years old, in the chronic phase of the disease, and within one year of diagnosis. The patient would receive ablative chemoradiotherapy, followed by transplant. Unfortunately, the combination of limited numbers of HLA compatible related donors and age limitations of CML patients, results in only 15% to 20% of CML patients qualifying for transplantation. If HLA matched unrelated donors are considered, the candidacy rate for transplantation increases to 30%.16
In contrast, autologous transplants usually result in relapse within one year, due to residual disease in the patient, or in the bone marrow preparation. If bone marrow transplant is performed in the chronic phase of the disease, the five-year survival rate is 50%. This statistic drops to 30% if performed in the accelerated phase, and to 15%, if performed in blast crisis phase. Stem cell transplant remains the treatment of choice for young patients (<40 years) with CML who have HLA-identical siblings. The three-year survival rates for unrelated donors matched for HLA-A, HLA-B, and HLA-DR are improving for both young patients (68%) and patients between the ages of 40 and 55 (67%). Progress has been made in the success of autologous stem cell transplants by purging the stem cell product of malignant cells and by enriching for normal stem cells that are CD34(+) and HLA-DR(-). Donor lymphocyte infusions (DLI) have produced durable complete remissions in >70% of patients who have relapsed following allogeneic transplantation with a sibling donor. The mechanism of activity in DLI appears to be T cells directed at CD34(+) CML progenitor cells.16

The future of CML therapy appears to be focusing on specific molecular targets that block signal transduction pathways altered by the BCR-ABL fusion protein. The first and most promising therapeutic intervention involves synthetic tyrosine kinase inhibitors. Since most, if not all, of the transforming capability of the fusion protein stems from its tyrosine kinase activity, selective inhibition of this activity has proven successful. STI-571, formerly called CGP57148, is a synthetic tyrosine kinase inhibitor designed to selectively inhibit the tyrosine kinase activity of the BCR-ABL fusion protein by binding the ATP binding cleft. Binding of STI-571 to the ATP binding cleft blocks the binding of ATP thus preventing the abnormal phosphorylation events caused by the BCR-ABL fusion protein.

Phase I/II clinical trials involving CML patients resistant to interferon have been underway since June 1998 and the results look very promising. When given as a 300+ mg oral daily dose, all CML patients achieved complete hematological remissions (defined as a normal white blood cell count) with some cytogenetic remissions in three weeks of therapy. In many cases, even patients who failed standard INF-α therapy have achieved complete hematologic remission. The drug must be taken daily because it has a short half-life of 12-14 hours. Some patients achieved cytogenetic responses suggesting that the therapy may be inducing apoptosis in the malignant clone. STI-571 would be considered effective if its only function was to inhibit the tyrosine kinase activity that causes the malignant phenotype, but if it also kills the malignant cells by inducing apoptosis, the effectiveness is dramatically amplified. The drug appears to have minimal side effects and no dose-limiting toxicities have been encountered.17 STI-571 is also called imatinib mesylate (imatinib) and is manufactured commercially by Novartis Pharmaceutical under the name Gleevec® or Gleevec™. Studies are underway to maximize imatinib dosing, to combine imatinib with other therapies, and to monitor the effectiveness of therapy using molecular monitoring techniques.

MOLECULAR MONITORING OF THERAPEUTIC RESPONSES

The most effective molecular monitoring approach to date involves the quantitation of BCR-ABL transcripts that remain in blood after therapy. Competitive or real-time quantitative reverse transcriptase PCR (Q-PCR) was first used to monitor patients receiving INF-α+ or allogeneic stem cell transplants (allo-SCT).18-21 Results demonstrated a correlation between the copy number of BCR-ABL transcripts in the blood and the Ph1+ metaphases in the bone marrow for patients on INF-α and was a predictor of cytogenetic and hematologic relapse in patients who received allo-SCT.21-23 As with INF-α, Q-PCR has also demonstrated close correlation between Ph1+ metaphases in bone marrow and BCR-ABL transcripts in peripheral blood from patients receiving imatinib therapy.24,25 One study reported that 28 patients who achieved CCR following imatinib therapy had <1% BCR-ABL transcripts with only one exception, whereas of the 48 patients not achieving CCR only two had <1% BCR-ABL transcripts in the peripheral blood.26 This work was corroborated by a second study that reported that 40 of 42 patients who achieved CCR demonstrated BCR-ABL transcripts in blood of <2%.27 Laboratories are beginning to report BCR-ABL copy number from peripheral blood in terms of the number of log reductions in copy number compared to the patient’s original, pre-treatment copy number (baseline). This is a more universal reporting method that partially corrects for differences in testing sensitivities between labs and in initial tumor burden between CML patients. Using this nomenclature, the IRIS study defined a major cytogenetic response as a 3-log reduction or greater in BCR-ABL transcripts detectable in peripheral blood. Maximum sensitivity for most assays is at least 4.5-logs below baseline so maximum measurable response was defined by the IRIS study as 4.5-logs below baseline. Although molecular monitoring of BCR-ABL appears to correlate with karyotyping analysis and is predic-
tive of therapeutic response, karyotype analysis is still valuable. Chromosomal abnormalities, other than Ph1, occur in some CML patients treated with imatinib as first-line therapy and are predictors of disease progression, thus warranting periodic karyotype analysis.28

EFFECTIVENESS OF IMATINIB THERAPY AS MEASURED BY MOLECULAR METHODS

Although hematological remissions are extremely high when imatinib is given as first-line therapy, major molecular responses (MMR), as defined as a >3-log reduction in BCR-ABL transcripts from baseline, are more difficult to achieve. The IRIS study group reported that 39% of newly diagnosed CML patients achieved MMR after 12 months of imatinib therapy. This is a more impressive result when compared to a MMR of 2% in the patients treated with a combination of INF-α and Ara-C. In addition, 20% of patients demonstrated a 2- to 3-log reduction in BCR-ABL transcripts from baseline and 19% achieved a 4-log or greater reduction following 12 months of imatinib therapy. Maximum molecular response of >4.5-log reduction was also observed in other smaller studies.27 It is expected that the MMR is likely to increase as patients on first-line imatinib therapy are followed beyond one year. Data suggest that CCR and MMR are good predictors of disease progression. Among all the patients in the IRIS study that achieved CCR on imatinib, 58% also achieved MMR and none of these patients showed disease progression in the subsequent 12-month follow-up period. In addition, there is only a 15% probability of disease progression among the imatinib-treated patients who did not achieve CCR, and a 3% among those who achieved CCR but not MMR.

The primary goal of imatinib therapy is to achieve undetectable levels of BCR-ABL transcripts in the blood that would produce ongoing remission and avoid disease progression. However, it is unclear if imatinib therapy alone can permanently disable the BCR-ABL leukemogenic pathway and/or eliminate BCR-ABL bearing CML cells through apoptosis or other mechanisms. Eventually, imatinib may need to be withdrawn from patients who have achieved long-term MMR (at undetectable BCR-ABL levels) and followed to determine if reemergence of BCR-ABL transcripts will occur. As for the present, it is of much greater concern that strategies be developed to address patients who have lost remission through the generation of additional cytogenetic abnormalities or resistance to imatinib therapy.

IMATINIB RESISTANCE AND DOSING

Primary resistance to imatinib has been defined as newly diagnosed CML patients who do not achieve complete hematologic remission (CHR) by three months, MCR by six months, or CCR by twelve months. In the IRIS study using newly diagnosed CML patients on 400 mg/day imatinib, 4% did not achieve CHR at three months, 23% did not reach MCR at six months and 31% failed to attain CCR at twelve months. These imatinib-resistant patients represented between 20% to 30% of the study group.29 However, in patients treated with 800 mg/day imatinib who had previously failed on INF-α, all 36 achieved CHR and only 11% did not reach CCR.30 Some experts predict that approximately 90% of newly diagnosed CML patients might achieve CCR given higher doses of imatinib. Nevertheless, the 10% of patients predicted of having primary resistance can’t be explained by the currently identified mutations and polymorphisms in BCR-ABL.31

Acquired resistance to imatinib is defined as a loss of a previously established response (CHR, MCR, CCR) or progression of disease and is a much greater problem. In the IRIS study, 8% of patients treated with imatinib developed resistance in 18 months while other studies report patients who began imatinib therapy both early and late in the chronic phase of CML who developed resistance at a rate of 15% and 25%, respectively, after 14 months.29,31 There are two dominant theories that explain acquired resistance to imatinib; expansion of CML cells with transforming mechanisms independent of the BCR-ABL protein or genetic lesions that have altered the BCR-ABL protein either quantitatively or qualitatively. An example of the former theory would involve CML cells that previously had or have since developed additional genetic mutations, whether detectable or not, that possess transforming capabilities independent of BCR-ABL. However, the latter explanation is the more common scenario. Over 23 distinct point mutations have been identified in the BCR-ABL kinase domain and associated with imatinib resistance. The incidence of point mutations increases with the duration of the disease.

This suggests that the CML clone is incurring sequence errors during DNA replication, which is consistent with most cancer models. These point mutations are presumably occurring in multiple loci within the genome but many have been identified in the BCR-ABL kinase domain. The mutations are not necessarily stimulated by imatinib because similar mutations have been identified in many patients who have had CML for months to years but have never been exposed to imatinib.
However, these mutations are affecting the binding affinity of imatinib, resulting in resistance. The mutant clones will either not bind imatinib or bind it at a lower affinity resulting in continued proliferation of CML cells in the presence of imatinib. This process will select for imatinib resistance clones and promote disease progression. It is unclear if the control of cell proliferation initially induced by imatinib when administered early in the course of the disease will reduce mutations that produce imatinib resistance.

A potential strategy to rescue CML patients who have developed imatinib resistance involves the identification of the particular BCR-ABL mutation present and the administration of specific tyrosine kinase inhibitors unaffected or less affected by that mutation. For those mutations that abrogate imatinib binding, imatinib should be discontinued or used in combination with another tyrosine kinase inhibitor less affected by the mutation. In cases where the binding affinity of imatinib is diminished, dose escalation may prove successful. Prognosis for imatinib resistant patients appears particularly poor if the mutation is in the P-loop of the ATP phosphate binding domain. However, a class of small molecule kinase inhibitors, called pyrido-pyrimidines, are currently being tested to determine kinase inhibition in both wild-type BCR-ABL proteins and in BCR-ABL mutants that have developed acquired imatinib resistance. These molecules are potent tyrosine kinase inhibitors and appear to bind the ATP binding site of BCR-ABL using different contact points as compared to imatinib. Von Bubnoff tested 13 different pyrido-pyrimidines in a murine system and found all to exhibit tyrosine kinase inhibition and reverse the CML phenotype. However, three of these molecules exhibited potent tyrosine kinase inhibition: SKI DV-2-43, PD166326, and SKI DV-M016. In preliminary studies using a mouse model of CML, PD166326 inhibited BCR-ABL tyrosine kinase activity between 4 and 100 times greater than imatinib. Further studies are underway to determine the effectiveness of PD166326 at treating CML without crippling normal tyrosine kinase pathways and without inducing drug related sequalae.

Three additional therapeutic approaches for patients who have developed imatinib resistance are to combine imatinib with drugs that inhibit other enzyme systems downstream of the tyrosine kinase activity of BCR-ABL, with chemotherapeutic drugs, or with CML vaccines. O’Hare found that a Src/Abl kinase inhibitor, AP23464, exhibited an 8-fold greater inhibition of BCR-ABL than imatinib using wild-type expressing Ba/F3 cells. In addition, it also inhibited several other cell lines expressing common imatinib resistant BCR-ABL mutations. Two farnesyl transferase inhibitors, Lonafarnib (SCH66336) and Tibifarnib (ZarnestraTM, R115777) have been shown to reverse the CML phenotype in imatinib resistant patients when administered with imatinib. Favorable results have also been achieved in imatinib resistant patients when combining chemotherapeutic agents like semi-synthetic homoharringtonine (Myelostat®) and 5-aza-2’-deoxycytidine (decitabine) with imatinib. A CML vaccine, CMLVAX100, has been shown to reduce the number of Ph1 positive cells in patients treated with imatinib who were exhibiting consistent residual disease. Over half of the patients achieved CCR and half of these CCR patients were negative for BCR-ABL transcripts by real-time quantitative PCR.

**STRATEGIES FOR THE MANAGEMENT OF CML PATIENTS**

Even in light of the promising results achieved with imatinib, the most appropriate therapeutic approach to patients newly diagnosed with CML is still under discussion. It is clear that imatinib therapy has proven superior to the previously established treatment regimen using INF-α and Ara-C. However, several questions concerning imatinib therapy still remain. First, what are the long-term outcomes for patients who respond to imatinib therapy? Are they cured or will some, many, or all eventually succumb to some form of drug intolerance or resistance resulting in relapse? How should imatinib non-responders be treated? What criteria should be applied to imatinib treated patients to distinguish responders from non-responders to determine the efficacy of continuing imatinib therapy? In addition, allo-SCT has proven successful and potentially curative in a subset of CML patients. However, in contrast to the safety of imatinib, allo-SCT incurs the risk of transplant-related mortality, morbidity, and chronic graft-versus-host disease, limiting the procedure to only low risk candidates. What criteria should be used to determine which patients are better served by allo-SCT versus imatinib therapy?

It seems clear that there is a subset of patients who hold the hope of cure if they receive an uncomplicated allo-SCT. The ideal candidate for allo-SCT is a CML patient who is less than 40 years old, in the chronic phase of the disease, and within one year of diagnosis, and has an HLA-identical donor. The decision is more complicated if the patient is slightly older, just outside the one-year diagnosis window, or the donor is not a perfect HLA match. Other variables that complicate the decision to proceed with allo-SCT include recipient/donor gender combinations and CMV serostatus.
the patient’s clinical features exceed these parameters, imatinib therapy is likely to be the better choice. Allo-SCT may also be a viable option for CML patients who are expected to show imatinib resistance or in patients who have failed imatinib therapy. Currently, there are no reliable markers to predict imatinib resistance but mutations in the ATP binding site of the BCR/ABL fusion protein will produce such a resistance. Another version of transplantation that has shown success is the reduced intensity conditioning transplantation (RIST). The procedure is designed to gradually ablate host hematopoietic tissues by donor T cells and can be administered as an original graft, given again in the post-transplant period or by additional lymphocyte infusions. One study reported an overall disease-free survival of 85% at five-year post RIST with all survivors being negative for BCR-ABL by RT-PCR.44 Some argue that a strategy that combines the effects of imatinib and allo-SCT may be a viable approach. Since imatinib produces a more durable and complete remission than INF-α, it is possible that allo-SCT outcomes may be improved if imatinib is used as the pre-transplant conditioning regimen instead of INF-α. Another combination approach may be to use imatinib following allo-SCT to reduce residual disease. This approach has not been studied as a primary strategy but has been successful when performed as a rescue procedure following the failure of allo-SCT therapy.45

Therapeutic approaches to patients who failed to respond to front-line imatinib therapy or who have lost remission is also under debate. Data suggest that there are at least five possible approaches to this problem: 1) increasing the dose of imatinib to 600 or 800mg/day46-47; 2) adding another agent to the imatinib regimen like INF-α, Ara-C, hydroxyurea, decitabine, or homoharringtonine48,49; 3) changing the treatment to one or more of these agents without imatinib50; 4) autologous transplant51; and 5) new tyrosine kinase inhibitors. Adoptive immunotherapy (vaccines) approaches are currently being investigated to include using the e14a2 fusion protein, proteinase 3, Wilm’s tumor protein, and heat shock protein 70 (Hsp-70) as the vaccine material but results are not as promising as hoped.52-55

In summary, it seems reasonable to start all newly diagnosed patients on imatinib and measure their hematologic, cytogenetic, and molecular response at 12 months. Those patients who are responding and meet the criteria for allo-SCT would be given that option. Those who are responding to imatinib but do not meet the allo-SCT criteria would continue on imatinib therapy. Those who demonstrate primary resistance to imatinib by not achieving hematologic remission at three months, MCR by six months, and CCR by twelve months would be dose escalated on imatinib, treated with a combination of drugs with or without imatinib, or potentially started on a next generation tyrosine kinase inhibitor. For any given patient with imatinib resistance, selection of the appropriate next generation tyrosine kinase inhibitor would be based on the identification of the mutation that produced the imatinib resistance. If remission is achieved through second-line therapy, patients would be evaluated as a transplant candidate. If patients are not eligible for transplant, second-line therapy would continue. This same strategy could be applied to patients who achieve remission but eventually develop an acquired resistance to imatinib. Autologous transplant could also be considered for those patients experiencing imatinib resistance.

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**Book Review**

*Cases in Human Parasitology*
by Judith S Heelan
ASM Press, Washington DC: 2004
ISBN 1-55581-296-1
Paperback: 243 pp, $59.95

Not since Reifsnyder’s *Parasitic Diseases Case Studies* (1980) has a book of parasitic disease case studies been published. A welcomed edition, Heelan’s *Cases in Human Parasitology* includes cases of emerging, as well as classical parasites, along with wonderfully colored photomicrographs of the organisms in question. As stated in the introduction, the purpose of the book is to “present cases solely involving parasites to supplement conventional textbooks in human parasitology and to provide an interesting and educational challenge to health care scientists.” The book contains 62 cases of patients who presented to an emergency department or to their physician with symptoms of a parasitic infection.

The book is divided into five sections: Intestinal Protozoa; Blood and Tissue Protozoa; Cestodes, Trematodes, and Intestinal Nematodes; Blood and Tissue Nematodes; and Challenging Cases. The latter section also includes some infections in patients with symptoms closely resembling parasitic infection. A glossary is also available at the end of the book.

Each section is preceded by a concisely written introduction of background information and ends with a reference list. Each case includes a brief presentation of pertinent patient history appropriate to the infection—travel history, symptoms, age of patient, season, and characteristics of the organism in question, accompanied by a photomicrograph. The history is followed by a list of questions suggesting topics discussed in a comprehensive parasitic textbook; such as, identification, epidemiology, treatment, life cycle, transmission, prevention, and control. The question section is followed by concise answers.

This would be an ideal book for use in a human/medical parasitology course whether for clinical laboratory science students, medical students, infectious disease residents, clinical pathology residents, or even biology undergraduates. It could easily be adapted because its sectional organization is similar to that of most parasitology courses. Since many health curricula include case-based approach, Heelan’s text would be an excellent tool for such. Individuals preparing for national examinations should also find *Cases in Human Parasitology* an excellent means for reviewing the topic. I highly recommend the book.

*John P Seabolt EdD, Department of Biology, University of Kentucky, 101 Morgan Bldg, Lexington KY 40506*
Continuing Education Questions

FOCUS: MYELOCYTIC LEUKEMIAS

CONTINUING EDUCATION QUESTIONS

WINTER 2005

To receive 5 contact hours of advanced level P.A.C.E.®, credit for the Myelocytic Leukemias questions, insert your answers in the appropriate spots on the immediately following page; then complete and mail the form as directed.

NOTE: There may be more answer spaces on the answer sheet than needed. If so, leave them blank. Make sure the number of the answer space you fill matches the number of the question you are answering.

LEARNING OBJECTIVES

1. Explain the cancer stem cell hierarchical model and how it applies to acute myelocytic leukemias (AML).
2. Correlate cytogenetic and molecular genetic findings in the diagnosis and prognosis of AML.
3. Compare and contrast Class I and Class II mutations in AML and give examples of each.
4. Explain the functions of the PML/RARA fusion protein in PML.
5. Propose what will occur at the molecular level when ATRA is given to patients with APL and correlate with clinical findings in the patients.
6. Assess how advances in our understanding of the biology and genetics of hematopoietic neoplasms have affected the classification of these disorders.
7. Compare and contrast hematologic remission, cytogenetic remission, and molecular remission.
8. Discuss the first scientific description of CML.
9. Discuss the history of the Philadelphia chromosome to include the discovery of the “minute” chromosome 22 and the t(9;22) reciprocal translocation.
10. Describe the clinical and laboratory features of CML.
11. Sketch the t(9;22) translocation that produces the Philadelphia chromosome.
12. Describe the molecular biology of the four primary BCR/ABL fusion genes to include the four discrete breakpoints and the resulting Gene arrangements.
13. Discuss the leukemogenic mechanisms in CML involving the BCR/ABL fusion protein.
14. Compare three different versions of the fusion protein and discuss disease associations.
15. Describe the first documented therapy for CML.
16. Discuss the chemotherapeutic approach to CML treatment.
17. Discuss one advantage and one disadvantage of alpha interferon and bone marrow/stem cell transplants in the treatment of CML.
18. Discuss the therapeutic approach to CML involving tyrosine kinase inhibitors to include:
   a. molecular target of tyrosine kinase inhibitors.
   b. function of the tyrosine kinase inhibitors.
   c. effectiveness of therapy.
   d. drug resistance and adverse events.
   e. alternative therapeutic approaches in patients with drug resistance.

ACUTE MYELOCYTIC LEUKEMIAS

1. The molecular genetic finding that correlates with t(15;17) is:
   a. BCR/ABL.
   b. PML/RARA.
   c. APA/RAR.
   d. MLL/RAR.
2. Tyrosine kinase receptors may be mutated and result in:
   a. constitutive activation.
   b. inhibition of transcription.
   c. increased apoptosis.
   d. increased adhesion.
3. The PML/RARA fusion gene affects the ability of the cell to:
   a. proliferate.
   b. adhere to bone marrow.
   c. differentiate.
   d. exit the bone marrow.
4. ATRA has the following effect on PML cells when given in pharmacologic doses:
   a. induces apoptosis.
   b. induces proliferation.
   c. decreases survival.
   d. restores ability to differentiate.
5. The most sensitive method to detect complete remission in acute leukemia is:
   a. bone marrow examination.
   b. immunophenotyping.
   c. karyotyping.
   d. molecular analysis.

**CHRONIC MYELOCYTIC LEUKEMIA: PART 1**

6. Who is credited with the first scientific description of CML?
   a. David Craigie
   b. Robert Virchow
   c. John Bennett
   d. Paul Donne’

7. The Philadelphia chromosome is best described as:
   a. a reciprocal translocation between chromosomes 9 and 22.
   b. the loss of genetic material from a deletion in chromosome 22.
   c. a point mutation involving chromosome 9.
   d. a duplication of chromosomal material involving chromosomes 9 and 22.

8. Chronic phase CML is characterized by all of the following laboratory findings EXCEPT a:
   a. high WBC count.
   b. high blast count in the peripheral blood.
   c. left shift on the differential.
   d. low LAP score.

9. At the molecular level, the Philadelphia chromosome results in:
   a. a single chimeric gene that is transcribed and translated into one fusion protein of 310 kilodaltons.
   b. a fusion protein that expresses an ABL moiety at the amino terminus and a BCR moiety at the carboxy terminus.
   c. three fusion proteins, p190, p210, and p230 that all result in classic CML.
   d. four dominant chimeric genes, all of which result in the translocation of the ABL gene from chromosome 9 to the BCR gene on chromosome 22 creating BCR/ABL.

11. Select the function that is NOT associated with leukemogenic mechanisms in CML.
   a. Activation of apoptotic mechanisms
   b. Reduced contact inhibition
   c. Activation of the RAS pathway
   d. Constitutive tyrosine kinase activity

**CHRONIC MYELOCYTIC LEUKEMIA: PART 2**

12. Which of the following was NOT used as a treatment for CML early in the evolution of therapy?
   a. Arsenic
   b. Radiotherapy
   c. Nitrogen mustards
   d. Mercury

13. Name the alkylating agent that was instrumental in ushering in the era of chemotherapy.
   a. Busulfan
   b. Arsenic
   c. Cyclosporine
   d. Cytarabine

14. The most widely used tyrosine kinase inhibitor to date has been called all the following EXCEPT:
   a. STI-571.
   b. CGP57148.
   c. INF-α.
   d. Gleevec.
   e. imatinib mesylate.

15. The principal function of imatinib is to:
   a. bind the substrate binding site of the BCR/ABL fusion protein.
   b. bind and neutralize ATP molecules in solution.
   c. bind BCR/ABL promoter regions to reduce fusion gene transcription.
   d. bind the ATP binding site of BCR/ABL.

16. Quantitative reverse transcriptase polymerase chain reaction is used to monitor CML patients by measuring:
   a. Ph1 metaphases in the bone marrow.
   b. BCR/ABL transcripts in the blood.
   c. the number of p210 molecules in the blood.
   d. the presence of BCR/ABL chimeric genes in bone marrow.

17. Acquired resistance to imatinib is primarily attributed to:
   a. point mutations in the ATP binding loop of BCR/ABL.
   b. additional translocations.
   c. multiple copies of the original BCR/ABL gene.
   d. excessive expression of p53.
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Answers
Circle correct answer (questions are on previous two pages).

1. a  b  c  d  e  8. a  b  c  d  e  15. a  b  c  d  e  22. a  b  c  d  e
2. a  b  c  d  e  9. a  b  c  d  e  16. a  b  c  d  e  23. a  b  c  d  e
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6. a  b  c  d  e  13. a  b  c  d  e  20. a  b  c  d  e  27. a  b  c  d  e
7. a  b  c  d  e  14. a  b  c  d  e  21. a  b  c  d  e  28. a  b  c  d  e

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Please circle the most appropriate answers.

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(a) state license (b) NCA (c) employment (d) other

2. Specialty: (a) biochemistry/urinalysis (b) microbiology (c) lab administration (d) hematology/hemostasis (e) education (f)immunology (g) immunohematology

3. Workplace: (a) hospital over 500 beds (b) hospital 200–499 beds (c) hospital 100–199 beds (d) hospital under 100 beds (e)private lab (f ) community blood bank (g) group practice (h) private physician (i) clinic (j) other

4. Salary range: (a) under $10,000 (b) $10,000 to $20,000 (c) $20,000 to $30,000 (d) $30,000 to $40,000 (e) over $40,000

5. Did these articles achieve their stated objectives?  
(a) yes  (b) no

6. How much of these articles can you apply in practice?  
(a) all (b) some (c) very little (d) none

7. Employment status: (a) full time (b) part time (c) student (d) not employed (e) retired

8. How long did it take you to complete both the reading and the quiz? _____________ minutes

9. What subjects would you like to see addressed in future Focus articles?
CONTINUING EDUCATION

Answers to 2003 FOCUS
Continuing Education Questions

16(1) Winter 2003
VIRAL INFECTIONS

Dengue Fever in the Western Hemisphere
1. Dengue fever virus belongs to which of the following viral families?
   b. Flaviviridae

2. What is the vector associated with the transmission of the dengue virus?
   a. Aedes sp.

3. Which of the following methods may not detect dengue virus antibodies in secondary infections?
   a. MAC-ELISA

4. Dengue virus is transmitted
   a. by bite from an infected female Aedes mosquito.

5. Dengue fever is also known as:
   c. Breakbone fever.

6. The diagnosis of dengue infections can be accomplished by:
   d. all of the above.

7. Dengue shock syndrome is staged as Grades III and IV because of:
   c. remarkable circulatory collapse.

8. Aedes aegypti is also the vector for:
   b. Yellow fever virus.

9. Factors that promote dengue virus infections in endemic areas include:
   d. all of the above.

10. A person may suffer from dengue hemorrhagic fever when he or she becomes reinfected with:
    d. two different dengue virus serotypes.

Hantavirus
11. HPS is primarily transmitted by:
    b. deer mice.

12. All of the following are prevention measures for HPS, EXCEPT:
    c. wash hands often

13. Treatment of HPS may include:
    a. ribavirin.

14. A commonly used clinical laboratory methodology for diagnosis of HPS is:
    a. ELISA

15. Two organs which demonstrate increased concentration of virus in HPS are:
    a. lung and heart or c. spleen and heart.

16. The geographical area of initial endemcity of HPV was:
    b. Four Corners region.

17. Characteristic manifestations seen in the cardiac phase of HPS is/are:
    a. shock and pulmonary edema.

18. Antigen detection of SNV in tissue is accomplished by:
    a. immunohistochemistry.

19. A hematological finding most common in HPS is:
    a. thrombocytopenia.

20. IgM capture-ELISA detects antibodies to SNV:
    a. during an acute infection.

West Nile Virus: An Emerging Virus in North America
21. The following is (are) known vector(s) for West Nile Virus?
    d. a and c

22. West Nile Virus belongs to the family:
    b. flaviviridae.

23. Which of the following methods is (are) recommended to confirm WNV infections?
    d. All of the above.

24. West Nile virus is closely related genetically to:
    a. St Louis Encephalitis virus or c. Dengue fever virus.

25. The following segments of the population are at highest risk of developing severe encephalitis from WNV infection.
    d. A and B

26. WNV infection can be transmitted from:
    c. natural reservoir bird host to mosquito.

27. WNV infections have been reported in:
    d. all of the above.

28. Which of the following WNV lineage has been associated with clinical human encephalitis infections?
    a. Lineage 1
29. The WNV recovered in the NYC outbreak was determined to be closely related to the strain causing infections in:
   b. Israel.
30. A probable WNV infection can be demonstrated by showing:
   d. a, b, and c.

16(2) Spring 2003
HEMORRHAGIC ABNORMALITIES
1. What is the most common acquired hemorrhagic disorder?
   c. Liver disease
2. Both liver disease and vitamin K deficiency cause a prolonged PT and PTT. How do factor V and factor VII assays help determine which condition is present?
   a. FV is decreased in liver disease but not in vitamin K deficiency
3. A patient experiences petechiae and purpura on arms and legs, repeated nosebleeds, and hematemesis. What type of hemorrhage is present?
   a. Systemic hemorrhage
4. What factor deficiency is the prothrombin time most sensitive to?
   b. VII
5. What condition causes a prolonged thrombin time test result?
   c. Presence of heparin
6. If a patient has an anatomic bleeding disorder and poor wound healing but the prothrombin time, APTT, thrombin time, platelet count, and platelet functional assay results are all normal, what factor deficiency could exist?
   d. Factor XIII
7. The typical DIC test profile uses what tests?
   a. D-dimer, platelet count, fibrinogen assay, PT, and PTT
8. What is the most likely interpretation of the following tests?

<table>
<thead>
<tr>
<th>Test</th>
<th>Patient</th>
<th>Reference range</th>
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<tbody>
<tr>
<td>PT</td>
<td>18 sec</td>
<td>11-14 sec</td>
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<td>PTT</td>
<td>48 sec</td>
<td>25-35 sec</td>
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<tr>
<td>TCT</td>
<td>31 sec</td>
<td>15-22 sec</td>
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<tr>
<td>Platelet count</td>
<td>56 x 10^3/µL</td>
<td>150-450 x 10^3/µL</td>
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<tr>
<td>Semi-quantitative</td>
<td>1:8</td>
<td>Negative</td>
</tr>
<tr>
<td>D-dimer</td>
<td>Pos</td>
<td>Negative</td>
</tr>
</tbody>
</table>

   d. Disseminated intravascular coagulation (DIC)
9. The activated partial thromboplastin time (APTT, PTT) will be prolonged in each of the following EXCEPT a:
   a. FVII deficiency
10. A patient’s PTT was 62 seconds. After mixing the patient’s plasma with normal plasma and repeating the test, the PTT was 58 seconds. This indicates:
    c. The presence of inhibitor
11. The test for von Willebrand factor (vWF) activity is:
    b. Ristocetin cofactor
12. What is the plasma half-life of von Willebrand factor?
    b. 12 hours
13. What is the most commonly used treatment for acquired multiple factor deficiencies such as in liver disease?
    d. Fresh frozen plasma
14. What therapy might be used for a hemophiliac who is bleeding but who has a low titer factor VIII inhibitor?
    d. High dosage factor VIII concentrate
15. A patient with von Willebrand disease needs replacement therapy. Which product would be appropriate to use?
    a. Intermediate purity factor VIII concentrate (Humate P)
16. How many bags of cryoprecipitate should be given to obtain a hemostatic level of fibrinogen in a 64 kg patient with hypo-fibrinogenemia?
    c. Nine
17. A bleeding patient with a platelet count of 7.0 x 10^9/L received one unit of ‘Platelets, Pheresis’ containing 4.2 x 10^11 platelets. The one-hour post-transfusion platelet count was 20 x 10^9/L. Is this an adequate response to transfusion? (The patient’s body surface area is 1.8 m^2.)
    d. No, with a CCI of 5600 the patient is likely refractory to platelet transfusion.
18. Fresh frozen plasma is indicated for transfusion in which of the following situations?
    c. Coagulopathy due to massive transfusion
19. Of the following, which would be the safest choice to treat a bleeding patient who has hemophilia B?
    b. BeneFIX
20. Which of the following prothrombin complex concentrates is appropriate for treatment of a patient with factor VII deficiency?
    a. Proplex
21. Calculate the dosage of FVIII needed to raise the FVIII activity of a 175 lb (80 kg) man from 2% to 80%. His hematocrit is 35%.
d. 2840 units

16(3) Summer 2003
RELIABILITY AND VALIDITY MEASURES
1. Reliability may be defined as:
c. how well an instrument measures the same input at varying times and under varying conditions.
2. Reliability is a ratio or fraction of the combined score variance to observed variance.
a. True
b. False
c. False
d. True
3. Reliability varies between:
a. 1 and 0
b. 0 and 1
c. 0 and 0.80
d. 0 and 1.0
4. If a measure is perfectly reliable there is no error in measurement.
a. True
b. False
c. False
d. True
5. Which is NOT a measure for assessing reliability?
b. Gher-Wong.
c. Inter-rater reliability
6. Researchers agree that the reliability of an instrument should not be below:
d. 0.80.
7. Validity may be defined as:
d. how accurately an instrument measures what one believes is being measured.
8. The process by which a theoretical concept is measured is called the:
a. operationalization of the concept.
9. A type of validity that is common is:
d. all of the above.
10. Measuring how well the operationalization of the concept compares to the relevant content domain is which type of validity?
a. Content related
b. Face validity
11. Which validity method is applicable to concepts measured by multiple items?
a. Content related
b. Criterion-related
12. Which validity method is not assessed statistically?
a. Content related
b. Criterion-related
c. Face validity
d. Construct validity
13. Assessments of validity do not involve theory.
a. True
b. False
c. Partially True
14. Which validity method involves computing a correlation coefficient between the measure of the target concept and the measure of the criterion concept?
b. Criterion-related
c. Construct validity
d. Face validity
15. The theory that any measurement has two components; the true value and the observed value is referred to as:
d. both b and c.
16. The two types of error are:
ea. a and c.
b. Type I and Type II

16(3) Summer 2003
CARDIAC PROTOCOLS
1. Which of the following statements best describes the BNP molecule?
c. BNP is a 32-amino acid peptide with a loop held in place by a disulfide bond.
2. Which combination of renal responses do elevated levels of BNP promote?
c. BNP promotes the renal excretion of both Na⁺ and H₂O.
3. Binding of BNP to the cell surface leads to an increase in which ‘second messenger’ system in the target cell?
b. BNP binding to the cell surface receptor leads to an increase in cGMP.
4. Identify the correct series of events involved with development of arterial plaque and leading to rupture and thrombosis.
b. Macrophages engulf oxidized LDLs, become foam cells, form fatty streaks, plaques rupture, and cause thrombosis.
5. Which of the following combinations of physiological conditions leads to ventricular release of BNP?
d. Hypervolemia and hypertension
6. Accelerated cardiac protocols for MI diagnosis and rule-out include the following cardiac markers and frequency of sequential testing:
c. myoglobin, troponin, CK-MB drawn at 30 minute to 2-hour intervals.
7. The reported diagnostic sensitivity and specificity of BNP assays (with a cutoff of 80 pg/mL) for diagnosis of CHF are approximately:
b. sensitivity of 98% and specificity of 92%.
8. A 53-year old male patient with dyspnea and chest pain has the following values for cardiac markers two hours after admission:
troponin 0.3 ng/mL (reference <0.1 ng/mL), BNP 248 pg/mL (reference <80 pg/mL), myoglobin 77 (reference 20 to 90 ng/mL), and CK-MB 2.5 (reference 0.3 to 4.0 ng/mL).
c. He is experiencing an episode of congestive heart failure.
9. A 48-year old female patient with dyspnea and chest pain has the following values for cardiac markers at admission: troponin 0.5 ng/mL (reference <0.1 ng/mL), BNP 321 pg/mL (reference <80 pg/mL), myoglobin 457 (reference 20 to 90 ng/mL), and CK-MB 5.6 ng/mL (reference 0.3 to 4.0 ng/mL).
   a. She is having an acute myocardial infarction.

10. A 35-year old male patient with elevated baseline levels of CRP has which of the following:
   c. increased risk of development of ACS.

16(4) Fall 2003
TRANSFUSION RISKS
1. NAT testing on donor units may soon be required to detect:
   c. West Nile virus.

2. The blood supply in the U.S. is currently tested for which of the following viruses?
   a. HTLV I

3. NAT testing of donor units for HAV has been proposed because:
   b. transfusion-transmission of HAV is postulated to be possible, or
   c. it has been shown that some cases of HAV are transfusion-transmitted.

4. The implementation of NAT testing has reduced the incidence of transfusion-transmitted HCV to approximately:
   b. 1 in 1,600,000.

5. S-D treatment of FFP will NOT inactivate:
   d. Parvovirus B19.

6. Psoralens will intercalate with nucleic acids when activated by:
   a. ultraviolet A.

7. The detergent used in S-D treatment of FFP:
   a. disrupts lipid envelopes.

8. A 14-day-old unit of donor red blood cells appears hemolyzed when selected from the blood bank refrigerator for crossmatching. If hemolysis is due to bacterial contamination, what organism is most likely involved?
   d. Yersinia enterocolitica

9. The most common group of organisms associated with platelet contamination is:
   a. normal skin flora.

10. The most sensitive detection method available for bacterial contamination in platelets is:
    b. automated blood culture system.

11. The risk of bacterial contamination is greatest with which of the following components?
    c. Pooled platelets

12. The underlying cause of death in patients who receive red cells contaminated with *Yersinia enterocolitica* or *Serratia liquefaciens* is:
    a. septic shock due to endotoxin.

13. The major factor that encourages growth of bacteria in platelet concentrates is:
    d. storage temperature.

14. The minimum level of bacterial contamination detected by microscopy with Gram’s stain is:
    b. $10^4 – 10^5$ CU/mL.

15. The major method of bacterial inactivation used for platelets is:
    b. psoralen compounds.

16. The transfusion-transmitted parasite that is endemic in the U.S. is:
    c. Babesia microti.

17. Which of the following organisms is of concern in donors from Central and South America?
   c. *Trypanosoma cruzi*

18. Prolonged asymptomatic parasitemia is responsible for the transfusion transmission of all of the following EXCEPT:
   d. ehrlichiosis.

19. The combination of gentian violet, ascorbic acid, and exposure to light will inactive which of the following organisms?
   a. *Trypanosoma cruzi*

20. Donor screening questions address risks for all of the following diseases EXCEPT:
    b. ehrlichiosis.

21. The greatest percentage of transfusion-transmitted cases of malaria in the U.S. is due to which organism?
    a. *P. falciparum*
TRENDS AND TECHNOLOGY

Trends and Technology: Winter 2005

MARY JANE GORE

ONLINE
For this issue, I found a listing of graduate schools in the clinical laboratory sciences (CLS), part of a more general web site called gradschools.com. The link to graduate CLS programs is programs.gradschools.com/usaclinical_labscience.html. The site is not comprehensive, nor is it precisely about CLS—a couple of the listings include grad school degrees in biotechnology or in exercise physiology. For the most part, however, it is a list of graduate schools in the clinical laboratory sciences (36 to date). This might be a good site to use in a clinical laboratory career day setting for college or community college students. Likewise, if your institution does not have its graduate school on this listing, it might be worth exploring a listing. You can also explore the site further by going to www.gradschools.com and searching the school programs by subject; you have to go to “C” to find “Clinical Laboratory Science”.

NEW PRODUCTS
The VITROS 5,1 FS Chemistry System features Ortho-Clinical Diagnostics MicroSlide Technology for the highest reportable result efficiency plus Intellilecheck Technology for system monitoring and advanced clot and bubble detection. In addition, the VITROS 5,1 FS Chemistry System is the first system to support the new VITROS MicroTip reagents, which will provide an extended menu of proteins, therapeutic drugs, drugs of abuse, and other critical assays. MicroTip Technology is unique in eliminating the maintenance and cost associated with traditional chemistry systems, such as plumbing, drains, fixed probes, mixing assemblies, and water supplies. MicroTip Technology will eliminate sample and reagent carryover that may occur with sample probes and reusable cuvettes. Contact Darcie Schwarz at (585) 248-2300 or darcie.schwarz@pulleyn.com.

Leica Microsystems introduces a high-performance microtome, the Leica RM2265, designed primarily for users in biomedical research and industry. The introduction of the Leica RM2265 replaces its predecessor, the RM2165. This latest microtome is a fully motorized and programmable rotary microtome with an emphasis on safety, ergonomics, and performance reliability. Contact Molly Lundberg at (847) 405-0123.

American Diagnostica has announced the availability of the new ActiScreen™ XL-FDP assay, an innovative and rapid immunoagglutination procedure for the detection of XL-FDPs in human plasma. Convenient enough to be used in a variety of diagnostic testing facilities, including physician office, hospital, and reference laboratories, the assay is intended for use as a clinical aid in diagnosing several thrombotic disorders. By quantitating XL-FDPs, laboratories also can monitor thrombolytic therapy with tissue plasminogen activator and streptokinase. Contact Robert Trinka at (203) 602-7777, ext 31.

Bayer Diagnostics has launched its newest chemistry analyzer, the ADVIA® 1200 Chemistry System. Designed to improve productivity and optimize laboratory efficiency, this fully automated system meets the on-demand testing needs of small- and medium-sized laboratories. The system complements the suites of other ADVIA analyzers and shares the same level of standardization of reagents, software, and results across all ADVIA.

Pickering Laboratories, a leader in the design, manufacture, and supply of post-column derivitization instruments and consumables, has introduced a more sensitive method for identifying hard-to-detect chemical
compounds. At the heart of Pickering’s industry-advancing development is the pulse-free syringe pump, designed for the new Pinnacle PCX post-column derivitization system, which is now available for shipping. Contact Michael Gottschalk at (800) 654-3330 or (650) 694-6700 or mgottschalk@pickeringlabs.com.

ESA Inc announces an advance in HPLC technology: the Corona Charged Aerosol Detector. Charged Aerosol Detection (CAD) offers all-in-one performance benefits that refractive index (RI), low wavelength (UV), evaporative light scattering (ELS), and chemiluminescence nitrogen (CLN) detector methods lack. Corona™ CAD™ represents a new HPLC detection technology that typically provides ten times the sensitivity of ELS. The technology provides consistently high sensitivity and low limits of detection independent of molecular structure, detecting compounds in low nanogram quantities. Contact John Christensen, (978) 250-7175 or johnc@esainc.com.

The Model C85-12 laboratory freezer from So-Low Enviromental Equipment can be used for preserving tissue, plasma, blood cells, microbiology specimens, and other research and storage purposes. The cabinet is an all-steel construction with a counter-balanced lid. The temperature control allows a range from −40 °C to −85 °C. No liquid is needed for the air-cooled condenser for this unit, which has a 12-cubic-foot capacity. Contact Jim Schrum at (513) 772-9410 or visit www.so-low.com.

So-Low environmental freezer

Dade Behring is introducing its benchtop Viva-E™ Drug Testing System for its Syva® product line. The Viva-E is designed to run Dade Behring’s Syva Emit®-branded therapeutic drug monitoring, drugs of abuse, and serum toxicology assays as well as its tests for determining the validity of drug test samples. The product was scheduled at press time to become available in the fourth quarter of 2004. Contact Melissa Ziriakus at (847) 236-7038.

AGREEMENTS

Dade Behring, the University of Frankfurt, and Innovectis announced the signing of an exclusive license agreement which provides Dade Behring with worldwide rights for the inventions of Professor Zeiher’s group at the University of Frankfurt that relate to placental growth factor (PIGF) for cardiovascular diseases (CVD), soluble CD40 ligand (sCD40L) for the prognosis of the course of disease in acute coronary syndrome (ACS), and additional markers for cardiovascular diseases. Recent studies show that these markers add substantial information for the diagnosis of acute coronary syndrome (ACS(1-3)). Contact Melissa Ziriakus at (847) 236-7038.

APPROVALS

Diagnostic Chemicals Limited (DCL) announced the launch of Ammonia L3K, the latest addition to DCL’s L3K line of clinical chemistry products. Ammonia L3K is a single vial, stable liquid reagent which incorporates a patented and proprietary co-enzyme analog technology. The use of liquid reagents eliminates the need for reagent preparation, thereby saving time and labor, and reducing potential for error. The U.S. Food and Drug Administration has granted 510(k) clearance for this product. Contact Robert Janetschek at (203) 881-2020, ext 304.

Streck Laboratories, announced that the company has perfected a technology for preserving patient blood samples for up to seven days. The technology, called Cyto-Chex BCT is a vacuum blood collection tube that contains a preservative reagent. Cyto-Chex BCT preserves the integrity of white blood cells at room temperature three times longer than traditional methods. Streck Laboratories has received FDA clearance to manufacture and sell Cyto-Chex BCT. The product allows for more cost-effective, reliable, and safer testing for immune system diseases. This new technology gives the health community the ability to ensure that fragile blood samples arrive at the laboratory intact, even in less developed countries, where within a day’s access to clinical laboratories is not always possible. Previously, samples could only be preserved for a short period of time. Contact Streck Laboratories Inc at (800) 843-0912 or visit www.streck.com.
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