UNIVERSITY OF ILLINOIS
COLLEGE OF MEDICINE
AT
URBANA-CHAMPAIGN

CLINICAL LABORATORY SCIENCES

2014-2015
INTRODUCTION

The Clinical Laboratory Sciences (CLS) course deals with laboratory medicine involving lab testing.

It is that branch of Pathology that applies scientific laboratory methods relevant to patient care, health promotion, and disease prevention.

*The three pillars of evidence-based medicine are:*

A. What are the results?
B. Are the results valid?
C. How are the results applicable to patient care?

The CLS course will attempt to answer the above utilizing a clinical “case format”. The significance and implications of lab testing, frequency of tests, turn-around time (TAT), limitations of lab data, quality control and cost effectiveness will be discussed. The overall approach is to emphasize lab testing and how it relates to CLINICAL DIAGNOSIS AND MANAGEMENT.

RECOMMENDED TEXTBOOKS  (need not purchase)


EXAM

There will be about 4 questions per teaching/learning session. The questions will be incorporated into the overall pathology/integrated exams to be held during the academic year.
LAB TESTING: IMPLICATIONS AND SIGNIFICANCE

Steve Nandkumar, MD
CONCEPTS IN LAB MEDICINE

I. LAB ORGANIZATION AND FUNCTIONS

A. Anatomic Pathology
   1. Surgical Pathology
   2. Cytopathology
   3. Autopsy Pathology

B. Clinical Pathology (Lab Medicine)
   1. Microbiology/Serology/Immunology
   2. Chemistry
   3. Hematology
   4. Blood Bank
   5. Others
      a. Radioisotope Pathology
      b. Cytogenetics

B. Role of Pathologist
   1. Research
   2. Administration
   3. Teaching
   4. Service

We are Lab Rats!!

II. WHY ORDER LAB TESTS?

A. Lab Tests are Helpful in the Following Ways:
   1. Establishing a diagnosis
   2. Monitoring the progress of a disease
   3. Guiding therapy and patient management
   4. Understanding the prognosis of a disease
   5. Screening/identification of risk factors in health promotion and disease prevention
   6. Research

III. WHAT ARE THE DIFFERENT AVAILABLE LAB TESTS?

A. There are many lab tests:
   1. Common ones are:
      a. BMP = Basic Metabolic Profile
      b. CMP = Comprehensive Metabolic Profile (see attached sheet on test menu)
      c. CBC = Complete Blood Count
      d. UA = Urinalysis
   2. Disease organ panels (see attached sheet)
   3. Others (specialized tests, esoteric tests, etc.)

Each lab decides the “test menu” with approval of the medical staff members. The test menu is reviewed/revised on a yearly basis.

IV. HOW DOES ONE SELECT APPROPRIATE LAB TESTS?

1. Ordering of lab tests depends on the clinical diagnosis and differential diagnoses of a case. Symptoms/signs of a disease plus physical exam findings result in a dx/diff dx. Tests are ordered to rule in or rule out a diagnosis. Know your DRGs (see attached sheet).
2. Clinical pathways/algorithms are helpful.
3. Training and experience help in diagnostic accuracy and hence selection of appropriate tests.
REMEMBER:
– Common manifestations and tests of common diseases
– Rare manifestations and tests of common diseases
– Common manifestations and tests of rare diseases
– Rare manifestations and tests of rare diseases

V. WHERE ARE “LAB TESTS” PERFORMED?

Most Lab Tests are Performed:
1. On-Site:
   a. Hospital labs (main lab).
   b. POCT (point of care testing)
   c. POLs (physician office labs).
2. Send Outs:
   a. Reference labs, e.g., Mayo Clinic, Quest, Lab Corp., etc.

VI. HOW LONG DOES IT TAKE TO RECEIVE TEST RESULTS?

TAT (Turn Around-Time) for Lab Tests:
1. Routine: Time taken 6-8 hours or overnight
2. ASAP: 2-4 hours
3. STAT: Within 1 hour

VII. HOW TO INTERPRET TEST RESULTS?

A. WHAT IS THE SIGNIFICANCE/IMPLICATION, ETC.?

B. WHAT SHOULD ONE DO, AFTER RECEIVING TEST RESULTS?

Test results may be:
   a. Quantitative – numerical value (within expected range, high or low)
   b. Qualitative – positive/negative
   c. Descriptive/diagnostic, etc.

REMEMBER REFERENCE INTERVALS
(Expected range)/critical or panic values

\[ \text{SIGNIFICANCE:} \]
\[ \text{Diagnostic} \quad \text{Monitoring disease, prognosis, etc.} \]

Useful in managing/treating cases.

INTERPRETATION

NORMAL – Disease ruled out
ABNORMAL – Disease usually verified
Repeat tests if unsure or need to confirm

Reflex testing: Additional relevant tests are automatically performed by the lab once a test is “positive”, e.g., abnormal pap smear cytology. Do HPV (viral) testing to categorize low risk from high risk cases.

TOP TEN RULES IN UTILIZING / INTERPRETING LAB TESTS
10. Reference ranges of laboratory tests vary due to age, gender, race, body mass and physiologic status.
9. Effect of drugs on laboratory values must not be overlooked.
8. Pre-analytic errors are more common than post-analytic errors. Analytic (technical) errors are the least common.
7. More tests may yield more abnormal test results. This may cause “Ulysses Syndrome”.
6. Multiple test abnormalities are more likely to be significant than single test abnormality. The greater the degree of abnormality of test results, the more likely the clinical significance.
5. Osler’s Rule attribute all abnormal lab findings to a single disease (especially if the patient is < 60 years). Multiple diseases must be considered as a last resort. ALWAYS CORRELATE CLINICALLY.
4. Never rely on a single (out-of-reference range) value to make a diagnosis. Look for a trend in particular values.
3. No test is perfect, (e.g., 100% sensitivity, specificity, predictive value).
2. Order tests that are relevant to patients’ diagnosis, prognosis, treatment, and management.
1. REMEMBER ALL THE OTHER RULES!

VIII. WHAT “PROBLEMS” OCCUR IN LAB TESTING?

A. PHASES OF LAB ANALYSIS (LAB TESTING)

<table>
<thead>
<tr>
<th>Pre-Analytic</th>
<th>Analytic</th>
<th>Post-Analytic</th>
</tr>
</thead>
<tbody>
<tr>
<td>From the time a test is ordered to the time a specimen reaches the lab.</td>
<td>From the time the specimen is received in the lab, prepared, and analyzed, (by instrument) till result is obtained</td>
<td>Result is reported</td>
</tr>
</tbody>
</table>

B. PROBLEMS IN “LAB ANALYSIS”

Pre-Analytic Errors
- Sample from wrong patient
- Inappropriate patient preparation
- Interfering drugs/medications
- Collection of specimen at wrong time, in wrong tubes, or in insufficient amounts
- Transportation/storage problems

Analytic Errors
- Instrument malfunction
- Use of outdated reagents

Postanalytic Errors
- Clerical errors

NOTE: Pre-analytic followed by post-analytic errors are the usual COMMON CAUSES of problems in lab analysis.
IX. WHAT VARIABLES AFFECT TEST RESULTS?

1. Age
2. Gender
3. Race
4. Body mass
5. Patient preparation
6. Posture
7. Blood samples (venous, arterial, capillary)

What is the difference between PLASMA and SERUM?

8. Drugs/Food (Important)
9. Hemolysis, lipemia, hyperbilirubinemia etc. affect blood test results yielding false positive or false negative results.

These cause color changes in plasma and hence interfere with spectrophotometric analysis (which measures changes in the color of plasma/serum after a chemical reaction).

X. REFERENCE RANGE

This is established by analyzing blood samples from normal healthy random people (minimum 20, ideal 100).

For results with a non-Gaussian distribution, the central 95% of the results will determine the reference range.

For results with a Gaussian distribution (bell-shaped curve), the mean plus or minus 2 SD (standard deviation) determines the reference range.

NOTE: Five percent of individuals can have a test result outside the reference range established by the central 95% of healthy individuals. Thus if an individual without disease has 20 different tests, there is a statistical chance that there will be 1 abnormal test (5% = 1/20) value.

Thus there is a problem when too many tests are ordered. Always order tests that are relevant to the clinical features and diagnosis.

XI. QUALITY CONTROL/QUALITY ACCOUNTABILITY – QC/QA

A. ANATOMIC PATHOLOGY
   1. Internal
   2. External (consultations)

B. CLINICAL PATHOLOGY
   1. Internal
   2. External e.g., CAP (College of American Pathologists), check samples, etc.
XII. DEFINITIONS

A. SENSITIVITY
“Diagnostic sensitivity” is the ability of the test to be positive in diseased individuals.

\[
\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive + False negative}} \times 100
\]

Sensitivity focuses on those WITH DISEASE.

Analytic sensitivity: The ability of a test is to detect “small quantities” of the substance of interest.

B. SPECIFICITY
Diagnostic specificity is the ability of the test to be negative in healthy individuals.

\[
\text{Specificity} = \frac{\text{True negative}}{\text{True negative + False positive}} \times 100
\]

Specificity focuses on those WITHOUT DISEASE.

Analytic specificity: It is the ability of a test to react only to the substance of interest.

C. PREVALENCE
The prevalence of a disease reflects the number of EXISTING CASES in a (percentage of) certain population.

D. INCIDENCE
Incidence refers to the number of NEW CASES occurring within a period of time (usually one year).

E. ANALYTIC PRECISION (REPRODUCIBILITY)
It is defined as the closeness with which repetitive test results agree with one another.

F. ACCURACY (CORRECTNESS)

Analytic accuracy. It is defined as the closeness with which the test results approach (hypothetically) correct results.

Diagnostic accuracy. It is the ability of the test to classify patients correctly as healthy or diseased.

Lab error rate is 0.2 to 3.5%.

NOTE: Technical validity of a test is determined by Precision and Accuracy.
G. **PREDICTIVE VALUE**

It would be helpful if the lab could give a precise probability statement that the patient has disease with each diagnostic lab test reported (i.e., probability of disease). One way to determine this is to note the exact distribution of results in healthy and diseased populations and the number of individuals involved at each concentration level. A simple method is to calculate the predictive value. It depends on prevalence, sensitivity and specificity.

1. **Predictive value of a positive test** = \[
\frac{\text{True positives}}{\text{True positives} + \text{False positives}} \times 100
\]

   It indicates the likelihood of a positive test result identifying someone **WITH** disease.

2. **Predictive value of a negative test** = \[
\frac{\text{True negatives}}{\text{True negatives} + \text{False negatives}} \times 100
\]

   It indicates the likelihood of a negative test result identifying someone **WITHOUT** disease.

**AN IDEAL LAB TEST SHOULD BE BOTH SENSITIVE AND SPECIFIC. ACCURACY DEPENDS ON PRECISION AND SPECIFICITY.**

XIII. **COST OF TESTING**

**REFERENCES:**

1. Lab Medicine Clinical Pathology in the Practice of Medicine by M. Laposata, M.D., Ph.D., (ASCP Press).

2. Interpretation in Clinical Chemistry by Myrton Beeler, M.D., (ASCP Press)
## TEST MENU

<table>
<thead>
<tr>
<th>Basic Metabolic Profile</th>
<th>Comprehensive Metabolic Profile</th>
<th>CBC</th>
<th>UA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>Glucose</td>
<td>WBC</td>
<td>Micro</td>
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<tr>
<td>BUN</td>
<td>BUN</td>
<td>RBC</td>
<td>Macro</td>
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<td>Sodium</td>
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<td>Potassium</td>
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<td>MCV</td>
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<td>Chloride</td>
<td>Chloride</td>
<td>MCH</td>
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<tr>
<td>CO2</td>
<td>CO2</td>
<td>Platelets</td>
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<tr>
<td>Anion</td>
<td>Anion Gap</td>
<td><strong>DIFF. COUNT</strong></td>
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<tr>
<td>SGOT (AST)</td>
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<td>Neutrophils</td>
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<td>SGPT (ALT)</td>
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<td>Basophils</td>
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<td>Alk. Phos.</td>
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<td>Eosinophils</td>
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<td>T. Bilirubin</td>
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<td>Lymphocytes</td>
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<td>T. Protein</td>
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<td>Monocytes</td>
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<td>Albumin</td>
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<td>Others</td>
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<td>Globulin</td>
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<td>A/G Ratio</td>
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</table>

Other tests can be ordered on an as-needed basis.
# DISEASE/ORGAN PANELS

<table>
<thead>
<tr>
<th>Anemia</th>
<th>Hypertension</th>
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<tbody>
<tr>
<td>CBC with indices, reticulocyte count and microscop examination</td>
<td>Basic metabolic panel</td>
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<tr>
<td>Microcytic: Iron, ESR</td>
<td>Urinary free cortisol</td>
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<tr>
<td>Normocytic: ESR, hemolysis profile</td>
<td>Renin</td>
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<tr>
<td>S=mic; folate, TSH</td>
<td>Thyroid Screening</td>
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<tr>
<td>Arterial Blood Gas</td>
<td>Urinary metanephrines</td>
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<tr>
<td>pH</td>
<td>Urinalysis</td>
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<tr>
<td>PCO₂</td>
<td>Iron/Hemochromatosis</td>
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<tr>
<td>O₂ saturation</td>
<td>Serum iron</td>
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<tr>
<td>P₅O₂</td>
<td>TIBC (total iron-binding capacity)</td>
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<tr>
<td>CO₂ content</td>
<td>% Saturation</td>
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<tr>
<td>PaCO₂</td>
<td>Ferritin</td>
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<tr>
<td>Arthritis</td>
<td>Alanine Amino Transferase (ALT)</td>
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<td>ESR (sedimentation rate)</td>
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<td>Uric acid</td>
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<td>ANA</td>
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<td>C-reactive protein</td>
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<td>Rheumatoid factor</td>
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<td>Cyclic citrullinated peptide antibody</td>
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<td>Bone/Joint</td>
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<td>Albumin</td>
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<td>Calcium</td>
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<td>Phosphorus</td>
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<td>Osteocalcin</td>
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<td>Protein, total</td>
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<td>Uric acid</td>
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<td>Alkaline phosphatase</td>
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<td>Cardiac Injury*</td>
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<td>Creatine kinase (CK)</td>
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<td>CK-MB</td>
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<td>Myoglobin</td>
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<td>Troponin-I</td>
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<td>Coagulation Screening</td>
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<td>Prothrombin time</td>
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<td>Thrombin time</td>
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<tr>
<td>Partial thromboplastin time</td>
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<td>Platelet count</td>
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<td>Bleeding time</td>
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<td>Collagen Disease/SLE</td>
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<td>ESR</td>
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<td>C-reactive protein</td>
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<td>C₃</td>
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<td>C₄</td>
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<td>ANCA (antibody neutrophil cytoplasmic antigen)</td>
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<td>ANA</td>
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<td>Anti-DNA</td>
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<td>Coma</td>
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<td>Basic Metabolic Panel</td>
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<td>Toxicology screen</td>
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<td>Salicylate</td>
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<td>Ammonia</td>
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<td>Anion gap</td>
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<td>Arterial blood gas profile</td>
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<td>Alcohol</td>
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<td>Lactic acid</td>
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<td>Calcium (total and ionized)</td>
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<td>Serum osmolality</td>
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<td>DIC</td>
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<td>Platelet count</td>
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<td>Thrombin time</td>
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<td>Fibrogenogen</td>
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<td>Fibrin split products</td>
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<td>Prothrombin time</td>
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<td>Partial thromboplastin time</td>
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<td>CBC with examination of blood film</td>
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<td>Diabetes Mellitus</td>
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<td>Management</td>
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<td>Basic Metabolic Panel</td>
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<td>Hemoglobin A₁</td>
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<td>Anion gap</td>
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<td>Lipid profile</td>
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<td>Electrolyte/Fluid Management</td>
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<td>Basic Metabolic Panel</td>
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<td>Plasma and urine osmolality</td>
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<td>Creatinine clearance</td>
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<td>Free water clearance</td>
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<td>Anion gap</td>
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<td>Enteral/Parenteral Nutrition</td>
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<td>Basic Metabolic Panel</td>
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<td>Magnesium</td>
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<td>Albumin</td>
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<td>Total protein</td>
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<td>Alkaline phosphatase</td>
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<td>Phosphorus</td>
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<td>Triglyceride</td>
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<td>Prealbumin</td>
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<td>CBC</td>
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<td>General Health</td>
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<td>CBC</td>
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<td>Comprehensive Metabolic Panel</td>
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<td>Lipid Panel</td>
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<td>LD</td>
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<td>Hemolysis</td>
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<td>Bilirubin</td>
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<td>Haptoglobin</td>
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<td>Free hemoglobin (serum and urine)</td>
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<tr>
<td>Lactate dehydrogenase (LD)</td>
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<tr>
<td>Antiglobulin (direct and indirect)</td>
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<tr>
<td>Reticulocyte count</td>
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<tr>
<td>Hepatitis Serology, Chronic Carrier</td>
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<td>Hepatitis Be Ab</td>
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<td>Hepatitis B surface Ag</td>
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<td>Hepatitis Be Ag</td>
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<td>Hepatitis C Ab</td>
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<td>HIV</td>
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<td>HIV 1 &amp; 2 Ab (EIA) with Western blot confirmation</td>
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<tr>
<td>HIV viral load</td>
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<td>HIV genotype</td>
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<tr>
<td>CBC w/CD4 &amp; CD8 lymphocyte subsets</td>
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</tbody>
</table>

*Troponin-I is replacing CK-MB in Cardiac Injury panel. CK-MB determination on initial patient serum may clarify same initial specimen elevation of cTn and classify appropriately a patient with a myocardial injury in the previous week.
Pancreatic
Amylase
Calcium (total and ionized)
Triglycerides
Lipase
Glucose

Parathyroid
Albumin
Alkaline phosphatase
Magnesium
Creatinine
PTH (whole molecule, amino terminal)
Protein, total
Calcium (total and ionized)
Phosphorus
Urinary calcium

Prenatal Screening
CBC
BUN
Uric Acid

A1C and Rh typing
Urinalysis
Toxoplasmosis Ab
CMV Ab
Hepatitis B surface Ag
HIV antibody
Cervical Pap smear
Cervical culture/amplification for GC,
Chlamydia, group B Streptococci
Glucose
Creatinine
Pree T4
Erythrocyte Antibody screen
Urine culture
Rubella titer
VDRL
Herpes simplex I & II Ab

Renal
Basic Metabolic Panel
Magnesium
Albumin
24-hr urine protein
Creatinine clearance
Phosphorus
Protein, total
24-hr creatinine
CBC

Thyroid Screening
Thyroxine (free T4)
TSH (third or fourth generation)

Toxicology Screening (Urine)
Amphetamines
Benzodiazepines
Marijuana metabolites
Methaqualone
Phencyclidine
Barbiturates
Cocaine metabolites
Methadone
Opiate metabolites
Propoxyphene

AMA DESIGNATED DISEASE/ORGAN PANELS*

80048 Basic Metabolic Panel
Calcium
Carbon Dioxide
Chloride
Creatinine
Glucose
Potassium
Sodium
Urea Nitrogen (BUN)

80076 Hepatic Function Panel
Albumin
Alkaline Phosphatase
Alanine Amino Transferase (ALT)
Aspartate Amino Transferase (AST)
Direct Bilirubin
Total Bilirubin
Total Protein

80069 Renal Function Panel
Albumin
Calcium

Carbon Dioxide
Chloride
Creatinine
Glucose
Potassium
Sodium

Total Bilirubin
Total Protein
Urea Nitrogen (BUN)

80074 Acute Hepatitis Panel
Hepatitis A Antibody, IgM
Hepatitis B Core Antibody, IgM
Hepatitis B Surface Antigen
Hepatitis C Antibody

80051 Electrolyte Panel
Carbon Dioxide
Chloride
Potassium
Sodium

80061 Lipid Panel
Triglycerides
HDL-cholesterol, Direct
Total cholesterol
LDL-cholesterol, Calculated


References
TOP DRGs

(DIAGNOSIS RELATED GROUPINGS)s

The following list is based on commonly seen diseases in a community hospital. The disease incidence may, however, vary depending on the community and the type of hospital (e.g., rural, urban, VA, tertiary care academic center, specialty hospitals).

1. Chest pain
2. Myocardial infarction/complications
3. Congestive heart failure/shock
4. Pneumonia/pleurisy
5. COPD (Chronic Obstructive Pulmonary Disease)
6. Respiratory failure
7. Abdominal pain (appendix, gallbladder, stomach, pancreas)
8. GERD (Gastroesophageal reflux disease)
9. GI hemorrhage
10. Diarrhea
11. CVA (Cerebrovascular Accident – Stroke)
12. Hypertension
13. Diabetes Mellitus
14. Sepsis
15. Osteoarthritis/Fractures
16. Pelvic pain/abnormal vaginal bleeding
17. Cancers (lung, breast, GI tract, prostate, leukemias, lymphomas)
*18. Gestation/c. section/newborn

**NOTE**: URTI( upper resp. tract infections) /UTI ( urinary tract infections) are usually seen in an outpatient setting

**"Gestation – altered physiology” (not a disease)**
CRITICAL VALUES FOR THERAPEUTIC DRUG LEVELS

The concept of critical values for drug levels was originally developed by the late Daniel M. Baer, MD, and first published in the April 1982 issue of MLO. This table is an expanded version of that publication and newly revised for 2014-2015 by Yashpal Agrawal, MD, PhD: Associate Professor of Clinical Pathology and Laboratory Medicine; Director, Central Laboratory; Director Point-of-Care Testing Services; Department of Pathology and Laboratory Medicine, New York Presbyterian Hospital-Cornell Campus, New York, NY.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Therapeutic range</th>
<th>Critical/Value</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Analgesic</td>
<td>5-20 mg/L</td>
<td>&gt;500 mg/L</td>
<td>obtained 4 hours after ingestion</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>Anticoagulant</td>
<td>Peak: 7-17 mg/dL</td>
<td>&gt;500 mg/L</td>
<td>Peak 30 min after injection</td>
</tr>
<tr>
<td>Amiodarone</td>
<td>Antiarrhythmic</td>
<td>Base: 10-200 ng/mL</td>
<td>&gt;250 ng/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Amelitine</td>
<td>Antidepressant</td>
<td>150-350 ng/mL</td>
<td>&gt;500 ng/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Anesthetic agents</td>
<td>Anticonvulsant</td>
<td>0.5-2.5 mg/L</td>
<td>&gt;2.5 mg/L</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>Anticonvulsant</td>
<td>10-12 µg/mL</td>
<td>&gt;250 µg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Immunomodulator</td>
<td>10-40 mg/mL</td>
<td>&gt;500 mg/mL</td>
<td>Specific concentration dependent on clinical presentation.</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>Steroid</td>
<td>1-10 µg/mL</td>
<td>&gt;10 µg/mL</td>
<td>Concentrations should be drawn 1-2 hours after dose.</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>Antiinflammatory</td>
<td>1-10 mg/mL</td>
<td>&gt;100 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Etoricoxib</td>
<td>Antiinflammatory</td>
<td>1-10 mg/mL</td>
<td>&gt;100 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Flurazepam</td>
<td>Anticonvulsant</td>
<td>0.1-0.5 µg/mL</td>
<td>&gt;1 µg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Fluoxetine</td>
<td>Antidepressant</td>
<td>5-50 µg/mL</td>
<td>&gt;500 µg/mL</td>
<td>Trough concentration should be a peak 2-3 hours after dose.</td>
</tr>
<tr>
<td>Garelnine</td>
<td>Antidepressant</td>
<td>Peak: 15-25 mg/mL</td>
<td>&gt;250 mg/mL</td>
<td>Monitoring of serum levels is suggested in patients with height and weight that are much different than normal.</td>
</tr>
<tr>
<td>Imipramine</td>
<td>Antidepressant</td>
<td>&gt;100-100 mg/mL</td>
<td>&gt;500 mg/mL</td>
<td>Concentrations should be drawn 2 hours after last dose.</td>
</tr>
<tr>
<td>Lamotrigine</td>
<td>Anticonvulsant</td>
<td>1-4 µg/mL</td>
<td>&gt;4 µg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Antiinfective</td>
<td>1.5-5.5 µg/mL</td>
<td>&gt;500 mg/mL</td>
<td>High concentrations generally associated with increased vomiting/nausea.</td>
</tr>
<tr>
<td>Lithium</td>
<td>Mood stabilizer</td>
<td>Acute: 1-14 mg/L Chronic: 0.6-1.2 mg/L</td>
<td>&gt;20 mg/L</td>
<td>Serum concentrations may increase in presence of hyperactivity.</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>Antiinfective</td>
<td>50-150 mg/mL</td>
<td>&gt;500 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Pharmacological</td>
<td>Anticonvulsant</td>
<td>15-40 µg/mL</td>
<td>&gt;40 µg/mL</td>
<td>Trough concentrations</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Antiinfective</td>
<td>10-20 µg/mL</td>
<td>&gt;20 µg/mL</td>
<td>Trough concentrations</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Antiinfective</td>
<td>5-12 µg/mL</td>
<td>&gt;25 µg/mL</td>
<td>Metabolized by Phenobarbital.</td>
</tr>
<tr>
<td>Procainamide</td>
<td>Antiinfective</td>
<td>PA: 1-4 mg/mL</td>
<td>&gt;10 mg/mL</td>
<td>Serum concentrations used in conjunction with clinical presentation.</td>
</tr>
<tr>
<td>Prazosin</td>
<td>Antiinfective</td>
<td>5-20 mg/mL</td>
<td>&gt;50 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Quinidine</td>
<td>Antiinfective</td>
<td>2-6 µg/mL</td>
<td>&gt;4 µg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Sulfonylurea</td>
<td>Antidiabetic</td>
<td>100-300 mg/mL</td>
<td>&gt;100 mg/mL</td>
<td>Serum concentration used in conjunction with clinical presentation.</td>
</tr>
<tr>
<td>Sotalol</td>
<td>Antiinfective</td>
<td>40-80 mg/mL</td>
<td>&gt;80 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Immunomodulator</td>
<td>5-20 mg/mL</td>
<td>&gt;25 mg/mL</td>
<td>Trough 12 hours after dose.</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Antiinfective</td>
<td>5-20 mg/mL</td>
<td>&gt;25 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Tolazoline</td>
<td>Antiinfective</td>
<td>4-8 mg/L</td>
<td>&gt;8 mg/L</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>Antiinfective</td>
<td>5-12 mg/mL</td>
<td>&gt;10 mg/mL</td>
<td>Trough concentration</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Antiinfective</td>
<td>Peak: 15-25 mg/mL</td>
<td>&gt;40 mg/mL</td>
<td>Trough, not recommended.</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>Antiinfective</td>
<td>Peak: 15-25 mg/mL</td>
<td>&gt;40 mg/mL</td>
<td>Trough concentration</td>
</tr>
</tbody>
</table>

*Ranges are approximate and may vary with laboratory and/or assay. Proper interpretation of therapeutic drug concentrations requires that the specimen be drawn at an appropriate time in relation to drug administration.*
# TABLE OF CRITICAL LIMITS

## Critical Limits

Critical limits define boundaries of life-threatening values of laboratory test results. Critical results or values are those that fall outside high and low critical limits. Urgent clinician notification of critical limits is the lab's responsibility. The system of critical value reporting was first implemented in a hospital by George D. Landberg, MD, and first published in *MLE* in 1972. These tables are based on three national surveys by Gerald J. Kost, MD, FAPBMS, FACCBO, of the University of California-Davis Health System. Adapted with permission from his articles, "the tables summarize critical limits used by 92 responding US medical centers, including 20 trauma centers, and 39 children's hospitals. Mean and standard deviation (SD) data are presented. The frequency with which critical limits were listed can be found in the original articles. As a rule of thumb, the "mean low" and "mean high" figures may be considered the critical limits for each test listed. Each institution should establish its own list of critical limits and clinician notification policy.

Dr. Kost conducted an independent national survey of U.S. medical centers and children's hospitals to determine estimated calcium critical limits. His extensive overview of critical limits and patient outcomes appeared in the March 1993 issue of *MLE*. Readers are also encouraged to review general practice guidelines.

The Joint Commission (JC) identifies critical values in current National Patient Safety Goals (NPSGs). One goal is to report critical results of tests and diagnostic procedures on a timely basis (Rationale for NPSG 02.03.01).

It is worth noting that the JC still lists critical values reporting in the January 1, 2014, set of NPSGs for hospitals. Inspectors check for compliance on this topic.

Critical results of tests and diagnostic procedures fail significantly outside the normal range and may indicate a life-threatening situation. The objective is to provide the responsible licensed caregiver these results within an established time frame so that the patient can be promptly treated.

## Elements of Performance for NPSG 02.03.01:

1. Develop written procedures for managing the critical results of tests and diagnostic procedures that address the following: the definition of critical results of tests and diagnostic procedures; by whom and to whom critical results of tests and diagnostic procedures are reported; the acceptable time period of time between availability and reporting of critical results of tests and diagnostic procedures.

2. Implement the procedures for managing the critical results of tests and diagnostic procedures.

3. Evaluate the timeliness of reporting the critical results of tests and diagnostic procedures.

In "Global trends in critical values practices and their harmonization," Dr. Kost and Kristin N. N. contribute.

## Clinical Chemistry

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>2.6 (0.4)</td>
<td>1.7-3.9</td>
<td>24.8 (6.0)</td>
<td>6.1-55.5</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>2.8 (0.5)</td>
<td>2.5-3.5</td>
<td>5.7 (0.4)</td>
<td>5.0-6.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>mmol/L</td>
<td>1.65 (0.17)</td>
<td>1.25-2.15</td>
<td>3.52 (0.23)</td>
<td>2.02-3.48</td>
</tr>
<tr>
<td>Sodium</td>
<td>mmol/L</td>
<td>129 (15)</td>
<td>110-137</td>
<td>156 (8)</td>
<td>145-170</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mmol/L</td>
<td>1.0 (0.1)</td>
<td>0.8-1.8</td>
<td>2.9 (0.8)</td>
<td>2.6-3.4</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mmol/L</td>
<td>0.29 (0.09)</td>
<td>0.26-0.35</td>
<td>2.97 (0.43)</td>
<td>2.26-3.23</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>mmol/L</td>
<td>257 (86)</td>
<td>236-280</td>
<td>326 (18)</td>
<td>295-375</td>
</tr>
<tr>
<td>Chloride</td>
<td>mmol/L</td>
<td>75 (0)</td>
<td>60-80</td>
<td>126 (12)</td>
<td>115-156</td>
</tr>
<tr>
<td>Calcium</td>
<td>mmol/L</td>
<td>250 (13)</td>
<td>230-280</td>
<td>326 (18)</td>
<td>295-375</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mmol/L</td>
<td>2.1 (0.01)</td>
<td>2.0-2.2</td>
<td>24.3 (1.4)</td>
<td>23.0-25.0</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mmol/L</td>
<td>37 (10)</td>
<td>30-50</td>
<td>438 (206)</td>
<td>275-700</td>
</tr>
<tr>
<td>Bicarbonate</td>
<td>mmol/L</td>
<td>0.92 (0.14)</td>
<td>0.50-1.17</td>
<td>1.55 (0.13)</td>
<td>1.26-2.08</td>
</tr>
<tr>
<td>Lactate</td>
<td>mmol/L</td>
<td>3.29 (0.50)</td>
<td>2.60-4.26</td>
<td>6.21 (0.78)</td>
<td>5.21-8.62</td>
</tr>
</tbody>
</table>

## Hematology

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>L/L</td>
<td>0.38 (0.05)</td>
<td>0.28-0.48</td>
<td>0.61 (0.08)</td>
<td>0.50-0.83</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/dL</td>
<td>66 (17)</td>
<td>49-120</td>
<td>109 (27)</td>
<td>70-140</td>
</tr>
<tr>
<td>Platelets</td>
<td>x10^9/L</td>
<td>378 (13)</td>
<td>150-1000</td>
<td>519 (147)</td>
<td>555-1000</td>
</tr>
<tr>
<td>WBC count</td>
<td>x10^9/L</td>
<td>2.0 (0.7)</td>
<td>1.0-4.0</td>
<td>373 (0.77)</td>
<td>100-1000</td>
</tr>
<tr>
<td>PT</td>
<td>s</td>
<td>27 (6)</td>
<td>20-30</td>
<td>52 (12)</td>
<td>40-70</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>g/L</td>
<td>0.88 (0.17)</td>
<td>0.50-1.00</td>
<td>7.75 (2.63)</td>
<td>5.00-10.00</td>
</tr>
</tbody>
</table>

## BLOOD GASES AND pH

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO₂</td>
<td>mm Hg</td>
<td>19 (3)</td>
<td>15-25</td>
<td>27 (6)</td>
<td>50-90</td>
</tr>
<tr>
<td>pH</td>
<td>mm Hg</td>
<td>7.21 (0.08)</td>
<td>7.00-7.5</td>
<td>7.59 (0.03)</td>
<td>7.50-7.65</td>
</tr>
<tr>
<td>pO₂</td>
<td>mm Hg</td>
<td>43 (0)</td>
<td>30-55</td>
<td>57 (0.8)</td>
<td>40-73</td>
</tr>
</tbody>
</table>
### CLS-M-2-Lab Testing Implications and Significance

#### CLINICAL CHEMISTRY

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>mmol/L</td>
<td>2.6 (0.5)</td>
<td>1.7-3.3</td>
<td>24.7 (8.8)</td>
<td>13.0-55.5</td>
</tr>
<tr>
<td>Potassium</td>
<td>mmol/L</td>
<td>2.8 (0.2)</td>
<td>2.0-3.5</td>
<td>6.0 (0.5)</td>
<td>5.0-8.0</td>
</tr>
<tr>
<td>Calcium</td>
<td>mmol/L</td>
<td>1.62 (0.17)</td>
<td>1.25-1.87</td>
<td>3.17 (0.22)</td>
<td>2.74-3.74</td>
</tr>
<tr>
<td>Sodium</td>
<td>mmol/L</td>
<td>121 (5)</td>
<td>110-130</td>
<td>156 (5)</td>
<td>150-170</td>
</tr>
<tr>
<td>CO₂ content</td>
<td>mmol/L</td>
<td>11 (2)</td>
<td>6-18</td>
<td>29 (3)</td>
<td>33-45</td>
</tr>
<tr>
<td>Magnesium</td>
<td>mmol/L</td>
<td>0.45 (0.04)</td>
<td>0.41-0.49</td>
<td>1.77 (0.45)</td>
<td>1.23-3.00</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>mmol/L</td>
<td>0.42 (0.16)</td>
<td>0.16-0.65</td>
<td>2.67 (0.39)</td>
<td>2.26-3.23</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>µmol/L</td>
<td>—</td>
<td>—</td>
<td>257 (60)</td>
<td>86-342</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>mmol/L</td>
<td>77 (9)</td>
<td>70-90</td>
<td>121 (5)</td>
<td>115-120</td>
</tr>
<tr>
<td>Urea</td>
<td>mmol/L</td>
<td>252 (12)</td>
<td>240-270</td>
<td>318 (10)</td>
<td>302-320</td>
</tr>
<tr>
<td>Creatinine</td>
<td>µmol/L</td>
<td>—</td>
<td>—</td>
<td>195 (114)</td>
<td>5.5-5.5</td>
</tr>
<tr>
<td>Ionized calcium°</td>
<td>mmol/L</td>
<td>0.65 (0.13)</td>
<td>0.50-1.08</td>
<td>1.53 (0.11)</td>
<td>1.35-1.75</td>
</tr>
<tr>
<td>Lactate</td>
<td>mmol/L</td>
<td>—</td>
<td>—</td>
<td>4.1 (1.2)</td>
<td>2.4-5.5</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>17 (5)</td>
<td>10-25</td>
<td>69 (10)</td>
<td>60-90</td>
</tr>
<tr>
<td>Ammonia</td>
<td>µmol/L</td>
<td>—</td>
<td>—</td>
<td>109 (9)</td>
<td>35-200</td>
</tr>
<tr>
<td>Protein</td>
<td>g/L</td>
<td>34 (3)</td>
<td>30-49</td>
<td>95 (6)</td>
<td>90-100</td>
</tr>
<tr>
<td>CSF protein</td>
<td>mg/L</td>
<td>—</td>
<td>—</td>
<td>1875 (54)</td>
<td>1000-3000</td>
</tr>
</tbody>
</table>

#### HEMATOLOGY

<table>
<thead>
<tr>
<th>Test</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit</td>
<td>L/L</td>
<td>0.20 (0.06)</td>
<td>0.10-0.30</td>
<td>0.62 (0.05)</td>
<td>0.54-0.70</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>g/L</td>
<td>68 (13)</td>
<td>50-100</td>
<td>268 (25)</td>
<td>170-250</td>
</tr>
<tr>
<td>Platelets</td>
<td>x10⁹/L</td>
<td>53 (25)</td>
<td>20-100</td>
<td>915 (23)</td>
<td>630-1500</td>
</tr>
<tr>
<td>WBC count</td>
<td>x10⁹/L</td>
<td>2.1 (0.8)</td>
<td>0.5-3.5</td>
<td>42.9 (22.1)</td>
<td>15.6-90.0</td>
</tr>
<tr>
<td>PT</td>
<td>s</td>
<td>21 (6)</td>
<td>15-35</td>
<td>21 (6)</td>
<td>15-35</td>
</tr>
<tr>
<td>PTT</td>
<td>s</td>
<td>62 (21)</td>
<td>40-100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>g/L</td>
<td>0.77 (0.30)</td>
<td>0.20-12.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bleeding time</td>
<td>min</td>
<td>14.0 (9.0)</td>
<td>0.5-20.0</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

#### BLOOD CASES AND pH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCO₂°</td>
<td>mm Hg</td>
<td>21 (9)</td>
<td>15-40</td>
<td>66 (22)</td>
<td>50-150</td>
</tr>
<tr>
<td>pH</td>
<td>—</td>
<td>7.21 (0.05)</td>
<td>7.16-7.30</td>
<td>7.59 (0.04)</td>
<td>7.50-7.70</td>
</tr>
<tr>
<td>pO₂°</td>
<td>mm Hg</td>
<td>45 (7)</td>
<td>30-55</td>
<td>124 (29)</td>
<td>100-150</td>
</tr>
</tbody>
</table>

#### NEWBORN°

<table>
<thead>
<tr>
<th>Test</th>
<th>Facility</th>
<th>Units</th>
<th>Mean (SD)</th>
<th>Range</th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>CH</td>
<td>mmol/L</td>
<td>1.6 (0.4)</td>
<td>1.1-2.8</td>
<td>18.2 (3.8)</td>
<td>16.5-27.8</td>
</tr>
<tr>
<td>Potassium</td>
<td>CH</td>
<td>mmol/L</td>
<td>2.8 (0.4)</td>
<td>2.5-3.7</td>
<td>7.9 (0.5)</td>
<td>6.5-8.0</td>
</tr>
<tr>
<td>Modified potassium</td>
<td>CH</td>
<td>mmol/L</td>
<td>2.8 (0.4)</td>
<td>2.5-3.7</td>
<td>6.5 (5.0)</td>
<td>(See Ref. 3)</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>CH</td>
<td>µmol/L</td>
<td>—</td>
<td>—</td>
<td>222 (88)</td>
<td>86-306</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>USMC</td>
<td>g/L</td>
<td>65 (25)</td>
<td>50-150</td>
<td>223 (83)</td>
<td>210-250</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>USMC</td>
<td>L/L</td>
<td>0.23 (0.08)</td>
<td>0.24-0.45</td>
<td>0.71 (0.29)</td>
<td>0.65-0.75</td>
</tr>
<tr>
<td>pO₂°</td>
<td>USMC</td>
<td>mm Hg</td>
<td>37 (7)</td>
<td>30-50</td>
<td>92 (12)</td>
<td>70-100</td>
</tr>
</tbody>
</table>

References

# CUT-OFF AND TOXICITY LEVELS FOR DRUGS-OF-ABUSE TESTING

This table summarizes information useful in the interpretation of drugs-of-abuse assays. It was originally developed by the late Daniel M. Baer, MD, and updated by Richard A. Paulson, MT(ASCP), supervisor of Chemistry and Toxicology, VA Medical Center, Portland, OR. The table has been updated annually and it was reviewed this year by O.M. Haverstick, PhD, DABCC, FACB, Associate Professor of Pathology, University of Virginia, Charlottesville, VA.

<table>
<thead>
<tr>
<th>Drug (and trade name)</th>
<th>Some common street names</th>
<th>Detectable duration in urine after</th>
<th>Urine cutoff points for reporting positive last dose</th>
<th>Toxic blood level by screening assay*</th>
<th>Blood reference (therapeutic) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heroin (Diacetylmorphine)</td>
<td>Horse, Stuff, Smack, Junk</td>
<td>1-2 days (total opiate)</td>
<td>2000 ng/mL (as morphine) 150 ng/mL (as 6-monacetylmorphine)</td>
<td>&gt;200 ng/mL (as morphine)</td>
<td>None detected</td>
</tr>
<tr>
<td>Morphine (Diamorphine)</td>
<td>M, Junk, Morphi, White stuff</td>
<td>2 days</td>
<td>2000 ng/mL 300 ng/mL</td>
<td>&gt;200 ng/mL 10-50 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Methadone (Dolophine, Amidone)</td>
<td>Methadose</td>
<td>3 days</td>
<td>2000 ng/mL 150 ng/mL</td>
<td>&gt;2000 ng/mL For narcotic stabilization: 300-1000 ng/mL For pain: 100-400 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Meperidine (Demerol, Peridone)</td>
<td>Fortis, Damies</td>
<td>2-3 days</td>
<td>200 ng/mL</td>
<td>&gt;1000 ng/mL 70-500 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Codeine (Analgexics with codeine)</td>
<td>Rajo, School boy</td>
<td>2 days</td>
<td>2000 ng/mL 300 ng/mL</td>
<td>&gt;1000 ng/mL 10-100 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Tramadol (Ultram, Tramal, Ultrascan)</td>
<td>Ultra T</td>
<td>8-12 hours</td>
<td>not established</td>
<td>not established</td>
<td>Variable by patient</td>
</tr>
<tr>
<td>Opiates</td>
<td>Dey, DC, Daycotton, Killer</td>
<td>1-3 days</td>
<td>100 ng/mL 300 ng/mL</td>
<td>&gt;200 ng/mL 10-100 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Hydrocodone (Lorcet, Vicodin, Lortab, Hydrocon)</td>
<td>Vikes, Predo, Norce</td>
<td>1-2 days</td>
<td>200 ng/mL 100 ng/mL</td>
<td>&gt;100 ng/mL 10-50 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Hydromorphone (Dilaudid)</td>
<td>Dust, Julie, Smack, V, Foulbacks</td>
<td>1-2 days</td>
<td>2000 ng/mL 300 ng/mL</td>
<td>&gt;1000 ng/mL 10-50 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Fentanyl (Sublimaze, Duragesic, Actiq, Pentrace)</td>
<td>Percoco, Apoace, China girl, China white, Dance fever, Friend, Goodfellas, Jadopi, Mujor B, TNT, Tango and Cash</td>
<td>1-2 days</td>
<td>5 ng/mL</td>
<td>&gt;3 ng/mL &lt;3 ng/mL (naive patients)</td>
<td>1-3 ng/mL (highly variable depends on dose and route of administration)</td>
</tr>
<tr>
<td>Lysergic acid, diethylamide (LSD)</td>
<td>Acid, Microdot, White lightning</td>
<td>1-5 days</td>
<td>0.5 ng/mL 100 ng/mL</td>
<td>&gt;2 ng/mL</td>
<td>None detected</td>
</tr>
<tr>
<td>Marijuana and cannabinoids</td>
<td>Mary Jane, Hashish, Blimp, Blends, Sensibles</td>
<td>Single use: 2-7 days (as 6b-THC-CODH) Prolonged use: 1-2 months (as 6b-THC-CODH)</td>
<td>100, 50, 25, or 20 ng/mL 15 ng/mL</td>
<td>50-200 ng/mL</td>
<td>None detected</td>
</tr>
<tr>
<td>Phencyclidine</td>
<td>PDP, Angel dust, Killer weed, Hug</td>
<td>Single use: 1 week Prolonged use: 2-4 weeks</td>
<td>25 ng/mL</td>
<td>100 ng/mL</td>
<td>None detected</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Coke, Crack, Rake, Snow</td>
<td>Single use: 3 days</td>
<td>200 ng/mL 150 ng/mL (as metabolite benzoylecgonine)</td>
<td>&gt;1000 ng/mL 100-500 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Amphetamine (Benzedrine, Dextroamphetamine)</td>
<td>Speed, Bunnies, Loppers, Glasses</td>
<td>Single use: 48 hours</td>
<td>100 ng/mL 500 ng/mL</td>
<td>&gt;200 ng/mL 20-30 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Methylene-3,4-dioxyl-Methamphetamine (MDMA)</td>
<td>Ecstasy, Adam, XTC, Love drug, Hug drug</td>
<td>Single use: 24 hours</td>
<td>3000-5000 ng/mL 500 ng/mL</td>
<td>100-1000 ng/mL 20-30 ng/mL</td>
<td></td>
</tr>
<tr>
<td>Methamphetamine (Deseret, Methedrine)</td>
<td>Speed, Meth, Crystal ice, Crank</td>
<td>Single use: 48 hours</td>
<td>100 ng/mL 200 ng/mL</td>
<td>&gt;500 ng/mL 10-50 ng/mL</td>
<td></td>
</tr>
</tbody>
</table>

*Based on common screening assays currently in use (2014) and CAP Proficiency Testing reporting (2006-2014) unless otherwise indicated. Confirmation results by Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) vary by lab.

Confirmation results by Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) vary by lab.

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**Note:** The cutoff and toxicity levels provided are based on common screening assays currently in use and CAP Proficiency Testing reporting (2006-2014) unless otherwise indicated. Confirmation results by Gas Chromatography-Mass Spectrometry (GC-MS) or Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) vary by lab. The table has been updated annually and it was reviewed this year by O.M. Haverstick, PhD, DABCC, FACB, Associate Professor of Pathology, University of Virginia, Charlottesville, VA.
### BARBITURATES

<table>
<thead>
<tr>
<th>Drug (and trade name)</th>
<th>Some common street names</th>
<th>Detectable duration in urine after last dose</th>
<th>Urine cutoff points for reporting positive by screening assay*</th>
<th>Toxic blood level</th>
<th>Blood reference (therapeutic) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentobarbital (Nembutal)</td>
<td>Goofballs, Downers, Nemos, Yellow jackets, Yellow submarine</td>
<td>2 days</td>
<td>200 ng/mL, 200 ng/mL</td>
<td>&gt;10 μg/mL</td>
<td>1-5 μg/mL</td>
</tr>
<tr>
<td>Secobarbital (Seconal)</td>
<td>Bulls, Pink ladies, Reda</td>
<td>2 days</td>
<td>200 ng/mL, 200 ng/mL</td>
<td>&gt;1 μg/mL</td>
<td>1-2 μg/mL</td>
</tr>
<tr>
<td>Butobarbital (Butisol)</td>
<td>Goofballs, Goofball, Peanuts, Stopoars</td>
<td>2 days</td>
<td>200 ng/mL, 200 ng/mL</td>
<td>2 μg/mL</td>
<td>3-25 μg/mL</td>
</tr>
<tr>
<td>Butalbital (Flinoral)</td>
<td>Goofballs, Sleeper, Stopoars, Peanuts</td>
<td>2 days</td>
<td>200 ng/mL, 200 ng/mL</td>
<td>2 μg/mL</td>
<td>5-15 μg/mL</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Barbs, Downers</td>
<td>1-3 weeks</td>
<td>300 ng/mL, 300 ng/mL</td>
<td>&gt;40 μg/mL</td>
<td>10-40 μg/mL</td>
</tr>
</tbody>
</table>

### ALCOHOLIC TOXICS & ANESTHETICS

<table>
<thead>
<tr>
<th>Drug (and trade name)</th>
<th>Some common street names</th>
<th>Detectable duration in urine after last dose</th>
<th>Urine cutoff points for reporting positive by screening assay*</th>
<th>Toxic blood level</th>
<th>Blood reference (therapeutic) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>Booze, Hoosh</td>
<td>&lt;1 day</td>
<td>10 mg/dL</td>
<td>80-400 mg/dL</td>
<td>100-1000 mg/dL (for treatment of toxic alcohols)</td>
</tr>
<tr>
<td>Methanol</td>
<td>Wood alcohol</td>
<td>&lt;1 day</td>
<td>5 mg/dL (GC)</td>
<td>&gt;10 mg/dL</td>
<td>&lt;0.15 mg/dL</td>
</tr>
<tr>
<td>Isopropyl</td>
<td>Rubbing alcohol</td>
<td>&lt;1 day</td>
<td>5 mg/dL (GC)</td>
<td>&gt;10 mg/dL</td>
<td>None detected</td>
</tr>
<tr>
<td>Acetone</td>
<td></td>
<td>&lt;1 day</td>
<td>5 mg/dL (GC)</td>
<td>&gt;33 mg/dL</td>
<td>&lt;1.0 mg/dL</td>
</tr>
<tr>
<td>Ethylene Glycol</td>
<td>Antifreeze</td>
<td>&lt;1 day</td>
<td>5 mg/dL (GC)</td>
<td>&gt;50 mg/dL</td>
<td>None detected</td>
</tr>
</tbody>
</table>

### SEDATIVES/HYPNOTICS/ANESTHETICS

<table>
<thead>
<tr>
<th>Drug (and trade name)</th>
<th>Some common street names</th>
<th>Detectable duration in urine after last dose</th>
<th>Urine cutoff points for reporting positive by screening assay*</th>
<th>Toxic blood level</th>
<th>Blood reference (therapeutic) range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diazepam (Valium)</td>
<td>Tranics, Downers, Blues, Yellows, Blue Magoo, V</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>Drug plus Metabolite: &gt;0 μg/mL</td>
<td>Drug plus Metabolite: &gt;0.1-0.5 μg/mL</td>
</tr>
<tr>
<td>Oxazepam (Serax)</td>
<td>Tranics, Downers, Blues, Yellows</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>&gt;0.8 μg/mL</td>
<td>0.2-1.4 μg/mL</td>
</tr>
<tr>
<td>Alprazolam (Xanax)</td>
<td>Tranics, Downers, Blues, Yellows</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>&gt;0.6 μg/mL</td>
<td>0.2-1.0 μg/mL</td>
</tr>
<tr>
<td>Clonazepam (Klonopin)</td>
<td>Tranics, Downers, Blues, Yellows</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>&gt;0.4 μg/mL</td>
<td>0.1-0.5 μg/mL</td>
</tr>
<tr>
<td>Chlordiazepoxide (Librium)</td>
<td>Tranics, Downers, Blues, Yellows</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>&gt;0 μg/mL</td>
<td>0.2-0.5 μg/mL</td>
</tr>
<tr>
<td>Lorazepam (Ativan, Loraz)</td>
<td>Tranics, Downers, Blues, Yellows</td>
<td>Single use: Not detected</td>
<td>300 ng/mL, 200 ng/mL, 150 ng/mL</td>
<td>0.3-0.6 μg/mL</td>
<td>0.2-0.5 μg/mL</td>
</tr>
<tr>
<td>Flunitrazepam (Rohypnol)</td>
<td>Roofies, Rib, Ropic, Roach-2, R n 2</td>
<td>72 hours</td>
<td>0.1-0.5 ng/mL (immunoassay)</td>
<td>50 ng/mL</td>
<td>15-15 ng/mL</td>
</tr>
<tr>
<td>Gamma-Hydroxybutyrate (Sonatomax)</td>
<td>SHB, G-Caps, Eetlers, Fantasy, Liquid Ectasy</td>
<td>12 hours</td>
<td>1-10 mg/L (GC, GC-MS)</td>
<td>&gt;150 mg/L</td>
<td>48-125 mg/L (for narcolepsy)</td>
</tr>
<tr>
<td>Ketamine Hydrochloride (Ketalar)</td>
<td>Special K, Lady Kaye, Vitamin K, Jet, Cat Vilium</td>
<td>&lt;72 hours</td>
<td>5-10 ng/mL (GC-MS)</td>
<td>&gt;1.27 μg/mL (highly variable)</td>
<td>0.5-5.0 μg/mL</td>
</tr>
</tbody>
</table>
## TABLE OF REFERENCE INTERVALS

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Test</th>
<th>Reference interval (conventional units)</th>
<th>Conversion factor (multiply by)</th>
<th>Reference interval (SI units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>Albumin*</td>
<td>3.5-5.2 g/dL</td>
<td>10</td>
<td>35.5-2 L</td>
</tr>
<tr>
<td>B</td>
<td>Base excess (men)</td>
<td>-3.3 to +1.2 mmol/L</td>
<td>1</td>
<td>-3.3 to +1.2 mmol/L</td>
</tr>
<tr>
<td>B</td>
<td>Base excess (women)</td>
<td>-2.4 to +2.3 mmol/L</td>
<td>1</td>
<td>-2.4 to +2.3 mmol/L</td>
</tr>
<tr>
<td>P</td>
<td>Bicarbonate</td>
<td>21-29 mmol/L</td>
<td>1</td>
<td>21-29 mmol/L</td>
</tr>
<tr>
<td>S/P</td>
<td>Bilirubin, conjugated*</td>
<td>0.0-0.2 mg/dL</td>
<td>20</td>
<td>0.0-0.2 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Bilirubin, total*</td>
<td>0.0-0.2 mg/dL</td>
<td>20</td>
<td>0.0-0.2 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Calcium, total</td>
<td>8.5-10.3 mg/dL</td>
<td>0.25</td>
<td>8.5-10.3 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>CO₂ content, venous</td>
<td>23-26 mmol/L</td>
<td>1</td>
<td>23-26 mmol/L</td>
</tr>
<tr>
<td>P</td>
<td>Chloride*</td>
<td>98-107 mM/L</td>
<td>1</td>
<td>98-107 mM/L</td>
</tr>
<tr>
<td>S/P</td>
<td>Cholesterol (NCEP recommendation)</td>
<td>140-200 mg/dL</td>
<td>0.055</td>
<td>140-200 mg/dL</td>
</tr>
<tr>
<td>P</td>
<td>Cortisol (24 h)*</td>
<td>5.2-9.2 mg/dL</td>
<td>20</td>
<td>5.2-9.2 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Creatinine (Jaffe, men)*</td>
<td>0.9-1.3 mg/dL</td>
<td>60</td>
<td>0.9-1.3 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Creatinine (Jaffe, women)*</td>
<td>0.6-1.1 mg/dL</td>
<td>60</td>
<td>0.6-1.1 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Ferritin (men)*</td>
<td>20-250 ng/mL</td>
<td>10</td>
<td>20-250 ng/mL</td>
</tr>
<tr>
<td>S/P</td>
<td>Ferritin (women)*</td>
<td>10-120 ng/mL</td>
<td>10</td>
<td>10-120 ng/mL</td>
</tr>
<tr>
<td>P</td>
<td>Fibrinogen</td>
<td>200-400 mg/dL</td>
<td>0.01</td>
<td>2-4 g/L</td>
</tr>
<tr>
<td>S/P</td>
<td>Folate</td>
<td>25-122 mg/L</td>
<td>2.265</td>
<td>60-260 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Glucose, fasting*</td>
<td>100-74 mg/dL</td>
<td>0.055</td>
<td>100-74 mg/dL</td>
</tr>
<tr>
<td>S</td>
<td>Hepatitis C virus (liver)</td>
<td>30-200 mg/dL</td>
<td>0.01</td>
<td>3-20 g/L</td>
</tr>
<tr>
<td>B</td>
<td>Hemoglobin (men)*</td>
<td>140-150 g/dL</td>
<td>0.055</td>
<td>140-150 g/dL</td>
</tr>
<tr>
<td>B</td>
<td>Hemoglobin (women)*</td>
<td>120-140 g/dL</td>
<td>0.055</td>
<td>120-140 g/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Iron, total</td>
<td>60-150 μg/dL</td>
<td>0.175</td>
<td>60-150 μg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Iron binding capacity</td>
<td>250-400 μg/dL</td>
<td>0.175</td>
<td>45-71 μg/dL</td>
</tr>
<tr>
<td>B</td>
<td>Lactate (venous)</td>
<td>2-7 mg/dL</td>
<td>0.111</td>
<td>0.36-0.75 mM/L</td>
</tr>
<tr>
<td>B</td>
<td>Lead</td>
<td>&lt;5 μg/dL</td>
<td>0.048</td>
<td>&lt;0.2 μg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Lithium, therapeutic</td>
<td>0.5-1.2 mM/L</td>
<td>1</td>
<td>0.5-1.2 mM/L</td>
</tr>
<tr>
<td>S/P</td>
<td>Magnesium*</td>
<td>1.7-2.4 mM/L</td>
<td>0.4114</td>
<td>1.7-2.4 mM/L</td>
</tr>
<tr>
<td>B</td>
<td>MCHC (RBC index)</td>
<td>26.3-32.0 g/dL</td>
<td>1</td>
<td>26.3-32.0 g/dL</td>
</tr>
<tr>
<td>B</td>
<td>MCV (RBC index)</td>
<td>80.0-95.0 fL</td>
<td>1</td>
<td>80.0-95.0 fL</td>
</tr>
<tr>
<td>B/P</td>
<td>Osmolality</td>
<td>270-295 mOsm/kg</td>
<td>1</td>
<td>270-295 mOsm/kg</td>
</tr>
<tr>
<td>B</td>
<td>pCO₂ arterial</td>
<td>35-45 mmH</td>
<td>0.133</td>
<td>35-45 mmH</td>
</tr>
<tr>
<td>B</td>
<td>pH arterial*</td>
<td>7.31-7.42</td>
<td>1</td>
<td>7.31-7.42</td>
</tr>
<tr>
<td>B/P</td>
<td>Phosphatase (as P)</td>
<td>7.5-4.5 mg/dL</td>
<td>0.23</td>
<td>0.81-1.45 mM/L</td>
</tr>
<tr>
<td>B</td>
<td>pO₂ arterial**</td>
<td>5.7-30.5 mmH</td>
<td>0.133</td>
<td>5.7-30.5 mmH</td>
</tr>
<tr>
<td>B/P</td>
<td>Platelet count</td>
<td>150-450 ×10⁹/mm³</td>
<td>1</td>
<td>150-450 ×10⁹/mm³</td>
</tr>
<tr>
<td>B</td>
<td>Potassium (men)*</td>
<td>3.5-4.5 mM/L</td>
<td>1</td>
<td>3.5-4.5 mM/L</td>
</tr>
<tr>
<td>B</td>
<td>Potassium (women)*</td>
<td>3.4-4.4 mM/L</td>
<td>1</td>
<td>3.4-4.4 mM/L</td>
</tr>
<tr>
<td>S</td>
<td>Protein, total (femoral)</td>
<td>6.0-7.8 g/dL</td>
<td>10</td>
<td>60-78 g/L</td>
</tr>
<tr>
<td>B</td>
<td>RBC count (men)</td>
<td>4.6-8.2 ×10⁹/mm³</td>
<td>1</td>
<td>4.6-8.2 ×10⁹/mm³</td>
</tr>
<tr>
<td>B</td>
<td>RBC count (women)</td>
<td>4.2-5.2 ×10⁹/mm³</td>
<td>1</td>
<td>4.2-5.2 ×10⁹/mm³</td>
</tr>
<tr>
<td>P</td>
<td>Sodium</td>
<td>136-145 mM/L</td>
<td>1</td>
<td>136-145 mM/L</td>
</tr>
<tr>
<td>S</td>
<td>Thyroxine, free*</td>
<td>0.8-2.7 ng/dL</td>
<td>12.9</td>
<td>0.3-3.47 ng/dL</td>
</tr>
<tr>
<td>S</td>
<td>Thyroxine (T₄), total (men)*</td>
<td>4.6-10.5 ng/dL</td>
<td>12.9</td>
<td>59-135 ng/dL</td>
</tr>
<tr>
<td>S</td>
<td>Thyroxine (T₄), total (women)*</td>
<td>5.5-11 ng/dL</td>
<td>12.9</td>
<td>55-138 ng/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Triglyceride (NCEP recommendation)</td>
<td>10-150 mg/dL</td>
<td>0.0113</td>
<td>0.11-1.7 mg/dL</td>
</tr>
<tr>
<td>S/P</td>
<td>Urea nitrogen (BUN)*</td>
<td>5-20 mg/dL</td>
<td>0.357</td>
<td>2-7.1 mg/dL</td>
</tr>
<tr>
<td>S</td>
<td>Uric acid (men)*</td>
<td>4.4-7.6 mg/dL</td>
<td>0.059</td>
<td>0.26-4.5 mg/dL</td>
</tr>
<tr>
<td>S</td>
<td>Uric acid (women)*</td>
<td>2.3-8.6 mg/dL</td>
<td>0.059</td>
<td>0.13-3.9 mg/dL</td>
</tr>
<tr>
<td>B</td>
<td>Vitamin B₁</td>
<td>206-678 pg/mL</td>
<td>0.733</td>
<td>151-493 pg/mL</td>
</tr>
<tr>
<td>S</td>
<td>WBC count</td>
<td>4.1-11 ×10⁹/mm³</td>
<td>1</td>
<td>4.1-11 ×10⁹/mm³</td>
</tr>
<tr>
<td>B</td>
<td>Zinc</td>
<td>89-120 μg/dL</td>
<td>0.152</td>
<td>12-18 μg/dL</td>
</tr>
</tbody>
</table>

*Specimens: B, whole blood; P, plasma; S, serum. Reference intervals depend on test method and the demographics of the normal population used.

*Adult intervals (18-60y). Age-specific ranges apply for pediatric and geriatric populations.

Various Factors Affecting Laboratory Test Interpretation

Interpretation of laboratory test results is much more complicated than simply comparing the test result against a so-called normal range, labeling the test values normal or abnormal according to the normal range limits, and then fitting the result into patterns that indicate certain diseases. Certain basic considerations underlie interpretation of any test result and often are crucial when one decides whether a diagnosis can be made with reasonable certainty or whether a laboratory value should alter therapy.

SENSITIVITY AND SPECIFICITY
All laboratory tests have certain attributes. Sensitivity refers to the ability of the test to detect patients with some specific disease (i.e., how often false negative results are encountered). A test sensitivity of 90% for disease Z indicates that in 10% of patients with disease Z, the test will not detect the disease. Specificity describes how well test abnormality is restricted to those persons who have the disease in question (i.e., how often false positive results are produced). A specificity of 90% for disease Z indicates that 10% of test results suggestive of disease Z will, in fact, not be due to disease Z.

PREDICTIVE VALUE
In recent years, Galen and Gambino have popularized the concept of predictive value, formulas based on Bayes’ theorem that help demonstrate the impact of disease prevalence on interpretation of laboratory test results (Table 1-1). Prevalence is the incidence of the disease (or the number of persons with the disease) in the population being tested. Briefly, predictive value helps dramatize the fact that the smaller the number of persons with a certain disease in the population being tested, the lower will be the proportion of persons with an abnormal test result who will be abnormal because they have the disease in question (i.e., the higher will be the proportion of false positive results). For example, if test Y has a sensitivity of 95% and a specificity of 95% for disease Z (both of which would usually be considered quite good), and if the prevalence of disease Z in the general population is 0.1% (1 in 1,000 persons), the predictive value of a positive (abnormal) result will be 1.9%. This means that of 100 persons with abnormal test results, only 2 will have disease Z, and 49 of 50 abnormal test results will be false positive. On the other hand, if the prevalence of disease Z were 10% (as might happen in a group of persons referred to a physician’s office with symptoms suggesting disease Z), the predictive value would rise to 68%, meaning that 2 out of 3 persons with abnormal test results would have disease Z.

Predictive value may be applied to any laboratory test to evaluate the reliability either of a positive (abnormal) or a negative (normal) result. Predictive value is most often employed to evaluate a positive result; in that case the major determinants are the incidence of the disease in question for Table 1-1 Influence of disease prevalence on predictive value of a positive test result*

<table>
<thead>
<tr>
<th>Prevalence of disease in population tested (%)</th>
<th>Predictive value (%) for test with 95% sensitivity and 95% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>68</td>
</tr>
<tr>
<td>20</td>
<td>83</td>
</tr>
<tr>
<td>50</td>
<td>95</td>
</tr>
</tbody>
</table>

*Percentage of patients with a positive test result who actually have the disease for which they are being tested.
the population being tested and the specificity of the test. However, predictive value is not the only criterion of laboratory test usefulness and may at times be misleading if used too rigidly. For example, a test may have excellent characteristics as a screening procedure in terms of sensitivity, low cost, and ease of technical performance and may also have a low positive predictive value. Whether or not the test is useful would depend on other factors, such as the type and cost of follow-up tests necessary in case of an abnormal result and the implications of missing a certain number of persons with the disease if some less sensitive test were employed.

There may be circumstances in which predictive value is misleading or difficult to establish. If one is calculating the predictive value of a test, one must first know the sensitivity and specificity of that test. This information requires that some accurate reference method for diagnosis must be available other than the test being evaluated; that is, a standard against which the test in question can be compared (a “gold standard”). This may not be possible. There may not be a more sensitive or specific test or test combination available; or the test being evaluated may itself be the major criterion by which the diagnosis is made. In other words, if it is not possible to detect all or nearly all patients with a certain disease, it will not be possible to provide a truly accurate calculation of sensitivity, specificity, or predictive value for tests used in the diagnosis of that disease. The best one could obtain are estimates, which vary in their reliability.

**REPRODUCIBILITY AND ACCURACY**

Reliability of laboratory tests is quite obviously affected by technical performance within the laboratory. The effect of these technical factors is reflected by test reproducibility and accuracy. **Reproducibility** (precision or inherent error) is a measure of how closely the laboratory can approach the same answer when the test is performed repeatedly on the same specimen. Theoretically, exactly the same answer should be obtained each time, but in actual practice this does not happen due to equipment and human imperfection. These deviations from the same answer are usually random and thereby form a random or gaussian distribution (Fig. 1-1). Variation from the average (mean) value is expressed in terms of standard deviation (SD). The laboratory frequently converts the standard deviation figure to a percentage of the mean value and calls this the coefficient of variation (CV). The majority of tests in a good laboratory can be shown to have reproducibility—expressed as CV—in the neighborhood of 4% (some may be a little better and some a little worse). This means that two thirds of the values obtained are actually somewhere between 4% above and 4% below the true value. Since ±2 SD (which includes 95% of the values) is customarily

![Gaussian distribution](image)

**Fig. 1-1** Gaussian (random) value distribution with a visual display of the area included within increments of standard deviation (SD) above and below the mean: ±1 SD, 68% of total values; ±2 SD, 95% of total values; ±3 SD, 99.7% of total values.
used to define acceptable limits (just as in determination of normal ranges), plus or minus twice the CV similarly forms the boundaries of permissible technical error. Returning to the 4% CV example, a deviation up to ±8% would therefore be considered technically acceptable. In some assays, especially if they are very complicated and automated equipment cannot be used, variations greater than ±8% must be permitted. The experience and integrity of the technical personnel, the reagents involved, and the equipment used all affect the final result and influence reproducibility expressed as CV. In general, one can say that the worse the reproducibility (as reflected in higher CVs), the less chance for accuracy (the correct result), although good reproducibility by itself does not guarantee accuracy.

These considerations imply that a small change in a test value may be difficult to evaluate since it could be due to laboratory artifact rather than to disease or therapy. Larger alterations or a continued sequence of change are much more helpful.

Accuracy is defined as the correct answer (the result or value the assay should produce). Besides inherent error, there is the possibility of unexpected error of various kinds, such as human mistake when obtaining the specimen, performing the test, or transcribing the result. Investigators have reported erroneous results in 0.2%–3.5% of reports from one or more areas of the laboratory. The laboratory analyzes so-called control specimens (which have known assay values of the material to be tested) with each group of patient specimens. The assumption is that any technical factor that would produce erroneous patient results would also produce control specimen results different from the expected values. Unfortunately, random inaccuracies may not affect all of the specimens and thus may not alter the control specimens. Examples of such problems are a specimen from the wrong patient, the effect of specimen hemolysis or lipemia, inaccurate pipetting, and insufficient mixing when the assay method uses a whole blood specimen. In addition, clerical errors occasionally occur. In my experience, the majority of clerical difficulties are associated with the patients who have the same last name, patients who have moved from one room to another, decimal point mistakes, transcription of results onto the wrong person's report sheet, and placement of one person's report sheet into the chart of someone else. These considerations imply that unexpected laboratory abnormalities (or sometimes even the degree of abnormality) should be interpreted in the context of the clinical picture. This does not imply that unexpected test values should be ignored; but if there is doubt, or if the

result would call for extensive workup or therapeutic action, it may be advisable to have the test repeated. If possible, the repeat should be performed on the original specimen or, if that is no longer available, on a new specimen obtained without delay. The greater the time lapse between the original and the new specimen, the more problems will be encountered in differentiating an error in the original specimen from true change that occurred before the next specimen. One of the more frustrating duties of a laboratory director is to receive a question or complaint about a laboratory test result several days or even weeks after the test was performed, when it is usually too late for a proper investigation.

NORMAL (REFERENCE) RANGES
The most important single influence on laboratory test interpretation is the concept of a normal range, within which test values are considered normal and outside of which they are considered abnormal. The criteria and assumptions used in differentiating normal from abnormal in a report, therefore, assume great importance. The first step usually employed to establish normal ranges is to assume that all persons who do not demonstrate clinical symptoms or signs of any disease are normal. For some tests, normal is defined as no clinical evidence of one particular disease or group of diseases. A second assumption commonly made is that test results from those persons considered normal will have a random distribution; in other words, no factors that would bias a significant group of these values toward either the low or the high side are present. If the second assumption is correct, a gaussian (random) distribution would result, and a mean value located in the center (median) of the value distribution would be obtained. Next, the average deviation of the different values from the mean (SD) can be calculated. In a truly random or gaussian value distribution, 68% of the values will fall within ±1 SD above and below the mean, 95% within ±2 SD, and 99.7% within ±3 SD (see Fig. 1–1). The standard procedure is to select ±2SD from the mean value as the limits of the normal range.

Accepting ±2 SD from the mean value as normal will place 95% of clinically normal persons within the normal range limits. Conversely, it also means that 2.5% of clinically normal persons will have values above and 2.5% will have values below this range. Normal ranges created in this way represent a deliberate compromise. A wider normal range (e.g., ±3 SD) would ensure that almost all normal persons would be included within normal range limits and thus would increase the specificity of abnormal results. However, this would place addi-
tional diseased persons with relatively small test abnormality into the expanded normal range and thereby decrease test sensitivity for detection of disease.

**Nonparametric calculation of the normal range.** The current standard method for determining normal ranges assumes that the data have a gaussian (homogeneous symmetric) value distribution. In fact, many population sample results are not gaussian. In a gaussian value distribution, the mean value (average sample value) and the median value (value in the center of the range) coincide. In nongaussian distributions, the mean value and the median value are not the same, thus indicating skewness (asymmetric distribution). In these cases, statisticians recommend some type of nonparametric statistical method. Nonparametric formulas do not make any assumption regarding data symmetry. Unfortunately, nonparametric methods are much more cumbersome to use and require a larger value sample (e.g., \( \geq 120 \) values) than do gaussian distributions (e.g., \( \geq 20 \) values). One such nonparametric approach is to rank the values obtained in ascending order and then apply the nonparametric percentile estimate formula.

**Problems derived from use of normal ranges**

1. A small but definite group of clinically normal persons may have subclinical or undetected disease and may be inadvertently included in the supposedly normal group used to establish normal values. This has two consequences. There will be abnormal persons whose laboratory value will now be falsely considered normal; and the normal limits may be influenced by the values from persons with unsuspected disease, thereby extending the normal limits and accentuating overlap between normal and abnormal persons. For example, we tested serum specimens from 40 clinically normal blood donors to obtain the normal range for a new serum iron kit. The range was found to be 35-171 \( \mu g/dl \), very close to the values listed in the kit package insert. We then performed a serum ferritin assay (the current gold standard for iron deficiency, see chapter 3) on the 10 serum samples with the lowest serum iron values. Five had low ferritin levels suggestive of iron deficiency. After excluding these values, the recalculated serum iron normal range was 60-160, very significantly different from the original range. The kit manufacturer conceded that its results had not been verified by serum ferritin or bone marrow.

2. Normal ranges are sometimes calculated from a number of values too small to be statistically reliable.

3. Various factors may affect results in nondiseased persons. The population from which specimens are secured for normal range determination may not be representative of the population to be tested. There may be differences due to age (see pages 650, 660), sex, locality, race, diet, upright versus recumbent posture (Table 1-2), specimen storage time, and so forth. An example is the erythrocyte sedimentation rate (ESR) in which the normal values by the Westergren method for persons under age 60 years, corrected for anemia, are 0-15 mm/hour for men and 0-20 mm/hour for women, whereas in persons over age 60, normal values are 0-25 mm/hour for men and 0-30 mm/hour for women. There may even be significant within-day or between-day variation in some substances in the same person.

4. Normal values obtained by one analytical method may be inappropriately used with another method. For example, there are several well-accepted techniques for assay of serum albumin. The assay values differ somewhat because the techniques do not measure the same thing. Dye-binding methods measure dye-binding capacity of the albumin molecule, buter procedures react with nitrogen atoms, immunologic methods depend on antibodies against antigenic components, and electrophoresis is influenced primarily by the electric charge of certain chemical groups in the molecule. In fact, different versions of the same method may not yield identical results, and even the same version of the same method, when performed on different equipment, may display variance.

5. As pointed out previously, normal values supplied by the manufacturers of test kits rather frequently do not correspond to the

<p>| Table 1-2 Decrease in test values after change from upright to supine position |
|---------------------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th>Test</th>
<th>% Decrease*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>4 (0-17)</td>
<td></td>
</tr>
<tr>
<td>Hematocrit</td>
<td>6 (4-9)</td>
<td></td>
</tr>
<tr>
<td>Potassium</td>
<td>1 (0-3)</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>4 (2-6.8)</td>
<td></td>
</tr>
<tr>
<td>Total protein</td>
<td>9 (7-10)</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>9 (6.2-14)</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>9 (5-15)</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>10 (3-20)</td>
<td></td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>9 (5-11)</td>
<td></td>
</tr>
<tr>
<td>Aspartate transaminase (SGPT)</td>
<td>7 (4-14)</td>
<td></td>
</tr>
</tbody>
</table>

*Average percent change with range of values found in the literature.
results obtained on a local population by a local laboratory, sometimes without any demonstrable reason. The same problem is encountered with normal values obtained from the medical literature. In some assays, such as fasting serum glucose using so-called true glucose methods, there is relatively little difference in normal ranges established by laboratories using the same method. In other assays there may be a significant difference. For example, one reference book suggests a normal range for serum sodium by flame photometry of 136-142 mEq/L, whereas another suggests 135-155 mEq/L. A related problem is the fact that normal ranges given in the literature may be derived from a laboratory or group of laboratories using one equipment and reagent system, whereas results may be considerably different when other equipment and reagents are used. The only way to compensate for this would be for each laboratory to establish its own normal ranges. Since this is time-consuming, expensive, and a considerable amount of trouble, it is most often not done; and even laboratories that do establish their own normal ranges are not able to do so for every test.

6. Population values may not be randomly distributed and may be skewed toward one end or the other of the range. This would affect the calculation of standard deviation and distort the normal range width. In such instances, some other way of establishing normal limits, such as a nonparametric method, would be better, but this is rarely done in most laboratories.

One can draw certain conclusions about problems derived from the use of the traditional concept and construction of normal ranges:

1. Some normal persons may have abnormal laboratory test values. This may be due to ordinary technical variables. An example is a person with a true value just below the upper limit of normal that is lifted just outside of the range by laboratory method imprecision. Another difficulty is the 2.5% of normal persons arbitrarily placed both above and below normal limits by using ±2 SD as the limit criterion. It can be mathematically demonstrated that the greater the number of tests employed, the greater the chance that at least one will yield a falsely abnormal result. In fact, if a physician uses one of the popular 12-test biochemical profiles, there is a 46% chance that at least one test result will be falsely abnormal. Once the result falls outside normal limits, without other information there is nothing to differentiate a truly abnormal from a falsely abnormal value, no matter how small the distance from the upper normal limit. Of course, the farther the values are from the normal limits, the greater the likelihood of a true abnormality. Also, if two or more tests that are diagnosis related in some way are simultaneously abnormal, it reinforces the probability that true abnormality exists. Examples could be elevation of aspartate aminotransferase (SGOT) and alkaline phosphatase levels in an adult nonpregnant woman, a combination that suggests liver disease; or elevation of both blood urea nitrogen (BUN) and creatinine levels, which occurring together strongly suggest a considerable degree of renal function impairment.

2. Persons with disease may have normal test values. Depending on the width of the normal range, considerable pathologic change in the assay value of any individual person may occur without exceeding normal limits of the population. For example, if the person's test value is normally in the lower half of the population limits, his or her test value might double or undergo even more change without exceeding population limits. (Fig. 1-2). Comparison with previous baseline values would be the only way to demonstrate that substantial change had occurred.

Because of the various considerations outlined previously, there is a definite trend toward avoiding the term "normal range." The most frequently used replacement term is reference range (or reference limits). Therefore, the term "reference range" will be used throughout this book instead of "normal range."

PROBLEMS WITH LABORATORY SPECIMENS

Specimen collection and preservation may create laboratory problems (see page 680). Probably the most frequent offender is contamination of urine from female patients by vaginal or labial secretions. Using more than 10 squamous epithelial cells per low-power field in a centrifuged urine sediment as the index of probable contamination, my surveys have found this present in 20%-30% of female random voided or midstream ("clean catch") specimens. These secretions may add red blood cells, white blood cells, protein, and bacteria to the urine. Nonfasting blood specimens may occasionally be troublesome, due to increased blood glucose and the effect of lipemia. This is most frequent in patients who are admitted in the afternoon and in outpatients. We have had some success in alleviating this problem by requesting
that physicians ask elective presurgical patients either to have admission laboratory tests drawn fasting before admission or to come to the hospital for admission after fasting at least 3 hours. Certain tests, such as blood gas analysis, biochemical acid phosphatase assay, and plasma renin assay, necessitate special preservation techniques to be reliable.

One of the most well-known specimen collection problems is that of ensuring completeness of 24-hour urine specimens. Some patients are not informed that the 24-hour collection begins only after a urine specimen has been voided and discarded. It is frequently helpful to give the patient written instructions as to how a clean-voided specimen may be obtained and how the 24-hour specimen is collected. The two standard criteria used to evaluate adequacy of collection are the specimen volume and the urine creatinine content. Specimen volume is helpful only when the volume is abnormally low (e.g., <400 ml/24 hours in adults). A small volume that does not have maximal concentration (as evidenced by a high specific gravity or osmolality) suggests incomplete collection. However, renal disease, medications such as diuretics, and other conditions may prevent concentration, so this criterion is difficult to apply unless the patient is known to have good renal function. The second criterion is a normal quantity of urine creatinine. Creatinine is derived from muscle metabolism and has a reasonably constant daily excretion. However, creatinine production and excretion are dependent on body muscle mass. It has also been shown by several investigators that even in the same individual, daily creatinine excretion may vary 5%-25%, with an average variation of about 10%. Meat, especially when cooked for a long time, may increase creatinine excretion up to 40% for short periods of time and possibly 10%-20% over a 24-hour period.

Since creatinine excretion correlates with muscle mass, it might be helpful to compare measured creatinine excretion with calculated ideal excretion based on body height and ideal body weight (see Table 37-11). This would be only a rough benchmark, but it might be more helpful than the population reference range, which is rather wide.

**EFFECTS OF PHYSIOLOGIC VARIABLES**

Physiologic differences between groups of persons may affect test results. These deviations may be attributable to normal metabolic alterations in certain circumstances. Some examples are age (e.g., an increase in alkaline phosphatase levels in children compared with adult values) (see page 650), sex (e.g., higher values for serum uric acid in males than in females), race (e.g., higher values for creatine phosphokinase in African American men than European men); time of day (e.g., higher values for serum cortisol in the morning than in the evening), meals (e.g., effect on blood glucose), and body position (e.g., change in values shown in Table 1-2 due to change in posture, resulting in possible decrease in many serum test values when an ambulatory outpatient becomes a hospital inpatient).

**EFFECTS OF MEDICATIONS**

The effect of medications is a major problem since a patient may be taking several drugs or may be taking over-the-counter pharmaceuticals without reporting them to the physician. Medication effects (see pages 652-659) may be manifest in several ways: drug-induced injury to tissues or organs (e.g., isoniazid-induced hepatitis), drug-induced alterations in organ function (e.g., increase in γ-glutamyltransferase produced by phenytoin microsomal induction in liver cells), drug competition effect (e.g., displacement of thyroxine from thyroxine-binding proteins by phenytoin), and interference by one drug with the analysis method of
another (e.g., decrease in serum glucose using glucose oxidase when large doses of vitamin C are ingested).

**EFFECTS OF HOSPITAL WORKING PROCEDURES**

Several common hospital conditions may affect laboratory results without such alteration being recognized by the physician. These include intravenous fluids running at the time the test specimen is drawn, the effect of dehydration, the effect of heparin flushes on some tests, the effects of various medications, and in certain cases the administration of medication at a time different from that expected or recorded. The last item refers to the common situation in which several patients are scheduled to receive medication at the same time (e.g., 8 A.M.). Although administration to each may be charted as being the same time, the actual time that any individual receives the medication may vary significantly.

Another frequent problem is defective communication between the physician and the laboratory. In some cases this takes the form of incorrectly worded, ambiguous, or illegible orders. Nursing or secretarial personnel can easily misinterpret such orders and relay them incorrectly to the laboratory. Nonstandard test abbreviations or acronyms created from the names of new tests not familiar to nursing personnel also cause difficulties. In some cases the physician should supply at least a minimal amount of pertinent clinical information to obtain better service. This information is most vitally needed in the microbiology department. The microbiology technologist must know from what area the specimen was obtained, exactly what type of culture is desired, and especially, whether any particular organism is suspected so that special growth media or special handling may be employed if necessary. Basic clinical information is even more essential to the surgical pathologist and the radiologist. The surgical pathologist must at least know where the tissue specimen originated, and both the pathologist and radiologist can do a much better job providing an answer to the clinician if they could only know what the clinician’s question is (i.e., for what reason is he or she requesting the study).

A word must be said about stat orders. **Stat** means emergency to the laboratory. Someone must stop whatever he or she is doing and perform the stat analysis immediately, possibly having to obtain the specimen first. After analysis the report must be delivered immediately. During this time that laboratory person may not do any other work. Stat tests result in great decrease of laboratory efficiency and cost effectiveness. The most efficient and least expensive way to perform tests is to analyze several patient specimens at the same time, so that the initial setup and quality control portions of the test need be performed only once and all specimens can be incubated simultaneously. Extra speed is obtained when a test is ordered stat, but results for everyone else are delayed. Unfortunately, many stat requests, sometimes even the majority, are ordered for reasons other than a true emergency need for the result. In some cases the order originates from nursing service because someone neglected to send a requisition for a routine test to the laboratory. In other cases the order is made stat because of convenience to the physician or the patient. Stat orders for these purposes at best are inconsiderate, wasteful, and disruptive. The physician should consider whether some other action-producing order category could be substituted, such as “as soon as possible.” If the actual problem is that of unacceptable turnaround time for routine tests, this is a matter to be discussed with the laboratory director rather than evaded by stat orders.

**LABORATORY TESTS AND THE MEDICAL LITERATURE**

One of the more interesting phenomena in medicine is the scenario under which new tests or new uses for old tests are introduced. In most cases the initial reports are highly enthusiastic. Also in most cases there is eventual follow-up by other investigators who either cannot reproduce the initial good results or who uncover substantial drawbacks to the test. In some cases the problem lies in the fact that there may not be any way to provide an unequivocal standard against which test accuracy can be measured. An example is acute myocardial infarction, because there is no conclusive method to definitively separate severe myocardial ischemia from early infarction (i.e., severe reversible change from irreversible change). Another example is acute pancreatitis. In other cases the initial investigators may use analytical methods (e.g., “homemade” reagents) that are not identical to those of subsequent users. Other possible variances include different populations tested, different conditions under which testing is carried out, and effects of medication. Historical perspective thus suggests that initial highly enthusiastic claims about laboratory tests should be received with caution.

Many readers of medical articles do not pay much attention to the technical sections where the materials and methods are outlined, how the subjects or patient specimens are selected and acquired, and how the actual data from the experiments are presented. Unfortunately, rather
frequently the conclusions (both in the article and in the abstract) may not be proven or, at times, even may not be compatible with the actual data (due to insufficient numbers of subjects, conflicting results, or most often magnifying the significance of relatively small differences or trends). This often makes a test appear to give clear-cut differentiation, whereas in reality there is substantial overlap between two groups and the test cannot reliably differentiate individual patients in either group. Another pitfall in medical reports is obtaining test sensitivity by comparing the test being evaluated with some other procedure or test. While there usually is no other way to obtain this information, the reader must be aware that the gold standard against which the new test is being compared may itself not be 100% sensitive. It is rare for the report to state the actual sensitivity of the gold standard being used; even if it is, one may find that several evaluations of the gold standard test had been done without all evaluations being equally favorable. Therefore, one may find that a new test claimed to be 95% sensitive is really only 76% sensitive because the gold standard test against which the new test is being compared is itself only 80% sensitive. One should be especially wary when the gold standard is identified only as “a standard test” or “another (same method) test.” In addition, even if the gold standard were claimed to be 100% sensitive, this is unlikely because some patients would not be tested by the gold standard test due to subclinical or atypical illness; or patients could be missed because of interferences by medications, various technical reasons, or how the gold standard reference range was established (discussed previously).

BIBLIOGRAPHY


PATHOLOGY LAB.

Diana Biggs
(Former) Laboratory Manager
Carle Foundation Hospital

FOR SELF-STUDY
Introduction to the Clinical Lab

Diana Biggs, MT(ASCP)SM
Clinical Systems Manager
Laboratory

Lab Management
- Medical Director
  - Pathologist
- Director of Operations
  - May or may not be MT
- Manager
  - MT
- Technical Specialist
  - MT

Where the process starts
- Outpatient draws done at the South Clinic drawing station
- Hospital rounds done by phlebotomy staff
- Branch blood specimens collected and spun (if appropriate), then sent in to main lab via couriers. All non-blood specimens also come to main lab.
- On-site specimens sent to main lab via pneumatic tube system
- Specimens for Microbiology often collected in the office. Some collected in South Clinic area, some at home.

After specimens get to the main testing lab......
Specimens are checked against orders to make sure patient information is correct and that the right test information is in the computer. Tests requiring whole blood are delivered to respective departments.
- Chemistry specimens centrifuged and serum separated for testing before going to the department.
- Microbiology and blood bank specimens always go directly to those departments for processing

Chemistry
- Largest department
- Mostly automated
  - Beckman LX 20 analyzers for Chem panels
  - Biorad Variant II for Hgb A1C
  - Beckman Access for Thyroid function, hormones, therapeutic drugs
  - Beckman Immage for Proteins
  - Tosoh for CEA, PSA
  - Rapid BNP for congestive heart failure
**Chemistry / Immunology**
- Infectious Disease Testing - most testing is part of Chemistry department. Some tests done in Microbiology
- Mostly EIA procedures
- New Vidas to automate some of the current manual kit testing
- Abbott equipment does HIV, Hepatitis testing
- Autoimmune disease testing

**Hematology**
- Sysmex line for CBC’s
- Sysmex analyzers for Coagulation studies
- Sysmex and Bayer Atlas for urinalysis
- Beckman Counter for Flow Cytometry - leukemia phenotyping, CD4 on HIV patients

**Microbiology**
- Cultures - Bacterial, Anaerobic, Acid Fast, Fungal, Viral
- Bactec automated analyzer for Blood Cultures
- Vitek system for automated Identification and Susceptibility
- Amplified probe technology for GC and Chlamydia
- EIA screening for Parasitology. Microscopic available.
- Smears for blood parasites
Blood Bank

- Gel semi-automated system for ABO and Rh typing
- Blood comes from Community Blood Services of Illinois
- Cross-match for transfusion
- Platelets

Cytology

- Pap Smears
- Fine Needle Aspirations
- Fluid Analysis for malignant cells, inflammatory cells, etc

Histology

- Tissues, removed organs sent here
- Sections made and stained for review by pathologist

Quality Assurance Issues

- Controls and calibrators must be run for automated instrumentation
- Controls with known values must be run with non-automated testing
- Controls on large analyzers run multiple times per day
- Microbiology has rules built into computer system to catch susceptibility patterns that do not match the organism identification.
**Alert Values**
- Significant, potentially life-threatening results. Must be called and documented in lab computer system.
- Reviewed yearly and modified as appropriate.

**Lab Manual**
- Available on-line
- Policies and procedures
- Specimen collection information
- Test information
- Important lab phone numbers
- Printable forms and instructions

**Obtaining Results - What to do and what NOT to do**
- Check the computer for results before calling
- Have the clinic number ready and know exactly what test results you are looking for
- Be patient - trying to obtain information on tests takes time
- Don't ask for STAT results if URGENT will do.

**Who to call if you have problems**
- First, ask to speak to the department technical specialist
- Next, ask to talk with the department manager
CHEST PAIN/CORONARY ARTERY DISEASE /CHF

CARDIAC BIOMARKERS

S. Nandkumar, MD
CLS CASE STUDY #1

PRESENT COMPLAINT:
A 56-year-old Caucasian male is brought to the Emergency Room with complaints of upper abdominal discomfort and squeezing type of chest pain with radiation to the upper arm, back, and neck. The patient indicates that he has had such pains/discomfort for the past two days with the symptoms being present at rest and worsening during physical exertion. The pain is accompanied by nausea, vomiting, weakness, and fever. There is no history of dizziness, syncope, shortness of breath or loss of consciousness.

FAMILY HISTORY:
The family history is noncontributory.

The patient admits to smoking 2 pack per day of cigarettes for several years. He is a “social drinker”.

PHYSICAL EXAMINATION:
The patient is alert and oriented.

VITAL SIGNS:
- Blood Pressure: 110/70 mm Hg
- Pulse: 88/min., regular
- Respiratory Rate: 20/minute
- Temperature: 101ºF

EXAMINATION OF SYSTEMS:
- CVS: Non-contributory
- RS: Non-contributory
- AS: Non-contributory
- CNS: Non-contributory

QUESTIONS:
1. What is the diagnosis?
2. What are the differential diagnoses?
3. What laboratory tests would be helpful?

The patient is admitted to the intensive care unit (ICU) and treated. Two days later he develops S4 and S3 (heart sounds), jugular venous distension, and occasional basal rales in the lung fields.

1. What is the diagnosis?
2. Would any laboratory tests help?

The patient is treated, but develops a “friction” rub, hypotension, and dies on the fourth day of admission.

Images will be shown.
CLS CASE STUDY #2

PRESENT COMPLAINT:
A 58-year-old male patient is admitted to the Emergency Room with complaints of severe crushing precordial pain, nausea, vomiting, and difficulty in breathing all of a few hours duration.

The past history is significant in that the patient is known to suffer from and treated for CAD for the past two years.

PHYSICAL EXAMINATION:

VITAL SIGNS:
Blood Pressure 90/50 mm Hg
Pulse 112/min., regular
Respiratory Rate 24/minute
Temperature 99ºF

The patient “collapses” during physical exam and dies.

QUESTIONS:
1. What is the diagnosis?
2. What are the differential diagnoses?
3. What laboratory tests would be helpful?
CHEST PAIN/CORONARY ARTERY DISEASE/CHF
CARDIAC BIOMARKERS

I. CREATINE KINASE (CK)
Enzyme creatine kinase is involved in energy storage in tissues (muscles).

**In Active Contracting Muscle**

<table>
<thead>
<tr>
<th>Creatine phosphate</th>
<th>Mg</th>
<th>ATP</th>
<th>Creatine + ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Energy use

**In Resting Muscle**

<table>
<thead>
<tr>
<th>Creatine + ATP</th>
<th>Mg</th>
<th>Creatine phosphate (energy stores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

There are Two SUBUNITS
1. M for muscle
2. B for brain

**These Form 3 ISOENZYMES**

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>Half-Life in Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK₁ (BB)</td>
<td>1–2</td>
</tr>
<tr>
<td>CK₂ (MB)</td>
<td>10–12</td>
</tr>
<tr>
<td>CK₃ (MM)</td>
<td>20–24</td>
</tr>
</tbody>
</table>

Relative Percent of CK Isoenzymes in Various Tissues

<table>
<thead>
<tr>
<th>Tissue</th>
<th>CK₁ (BB)</th>
<th>CK₂ (MB)</th>
<th>CK₃ (MM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle</td>
<td>0</td>
<td>2–3</td>
<td>97–98</td>
</tr>
<tr>
<td>Cardiac muscle, normal</td>
<td>0</td>
<td>2–3</td>
<td>97–98</td>
</tr>
<tr>
<td>Cardiac muscle, abnormal</td>
<td>0</td>
<td>10–15</td>
<td>85–90</td>
</tr>
<tr>
<td>Lung</td>
<td>20–50</td>
<td>0–5</td>
<td>30–60</td>
</tr>
<tr>
<td>Brain</td>
<td>97–98</td>
<td>2–3</td>
<td>0</td>
</tr>
<tr>
<td>Intestines</td>
<td>90–95</td>
<td>0</td>
<td>3–10</td>
</tr>
<tr>
<td>Prostate</td>
<td>95–100</td>
<td>0–2</td>
<td>0–5</td>
</tr>
<tr>
<td>Placenta</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Factors Affecting CK:
1. *AGE:* There is increased proportion of CK-MB in fetal life
2. *GENDER:* More muscle mass in men than women
3. *MUSCLE MASS:
4. *RACE:* CK levels are 30% higher in African Americans
5. *EXERCISE:* Strength exercises cause increased CK. Aerobic exercises cause low CK levels.

Intramuscular injections, muscle trauma, seizures, severe COPD, pregnancy etc., can all cause elevated levels of CK.
CK-MB
Normal Values  < 5 ng/ml  (mass immunoassay) – best test, directly measures the quantity of protein
< 14 U/L  (enzymatic assay)
< 5% of total CK

Isoforms of CK Isoenzymes

<table>
<thead>
<tr>
<th>Isoenzyme</th>
<th>MM-3</th>
<th>MM-2</th>
<th>MM-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>CK-MM</td>
<td>Carboxy peptidase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK-MB</td>
<td>Carboxy peptidase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MM-3/MM-1 isoform ratio is normally 1 to 1.3.
MB-2/MM-1 isoform ratio is normally 1.

Macro-CK  (Atypical CK)
1. Macro CK-1 is an abnormal isoenzyme in serum and is due to a complex formation between CK (usually CK-BB) and IgG.
2. Macro CK-2 is a mitochondrial CK.

Macro CK-1 occurs in elderly women, HIV infections, autoimmune diseases, etc. Macro CK-2 occurs in some malignancies. Both macro CK enzymes can be separated by electrophoresis and do not have any significance other than to cause interference in assay for CK.

Causes of Abnormal Results
Elevated total CK occurs due to cardiac or skeletal muscle damage. Other diseases of the brain, intestines, lung, prostate, etc., can cause increase in total CK. Isoenzymes CK-MB, CK-MM, and CK-BB can usually help identify these diseases.

Cardiac Damage
Acute coronary syndrome (silent ischemia, unstable angina, non Q-wave infarction, typical myocardial infarction).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Isoform Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TOTAL CK</td>
<td>elevated</td>
</tr>
<tr>
<td>2. CK-MB</td>
<td>elevated</td>
</tr>
<tr>
<td>3. CK-MB relative index</td>
<td>elevated</td>
</tr>
</tbody>
</table>

CK-MB R.I. = \( \frac{\text{CK-MB} \times 100}{\text{Total CK}} \)

Normal R.I. is < 2.5. It helps differentiate skeletal muscle injury from cardiac injury (CK-MB, R.I. is > 2.5 in cardiac injury).

NOTE: CARDIAC ENZYMES RISE AND FALL. IT IS HENCE IMPORTANT TO ORDER SERIAL CK/CK-MB MEASUREMENTS USUALLY 4 HOURS APART, TO DETERMINE THE TIMING OF MI (OR REINFARCTION).
II. MYOGLOBIN
Myoglobin is a heme containing protein that binds O₂ within cardiac and skeletal muscle. Following muscle damage, myoglobin released in plasma is cleared by the kidneys (half-life is about four hours). Muscle mass, gender and race effects on myoglobin are similar to those of CK. Myoglobin increases with increasing age due to decreased glomerular filtration rate.

**Myoglobin**
1. is the earliest marker in M.I.
2. increases within 1–3 hours, peaks in 8–12 hours, and becomes normal in 24–36 hours.
3. may be released in multiple short bursts and so 2–3 blood samples must be drawn at one hour intervals.
4. is NONSPECIFIC. It is increased in renal failure, shock, muscle injury, muscle dystrophy, cardiac surgery, etc.
5. is 95–100% sensitive (with serial sampling); has a high negative predictive value (96%).
6. is a strong predictor of adverse outcomes (i.e., 30 day mortality)

III. TROPONINS
Troponins are proteins that bind to tropomyosin and govern excitation-contraction coupling in muscle.

**NORMAL VALUES**
- Troponin I < 1.6 ng/ml
- Troponin T < 0.1 ng/ml

**Based on Function. There are Three Subunits of Troponin.**
1. Troponin T (tropomyosin-binding subunit)
2. Troponin I (inhibitory subunit)
3. Troponin C (calcium binding subunit)

Troponin C isoforms found in cardiac and skeletal muscle are identical and hence NOT used as markers for cardiac injury. Troponins T and I also have isoforms found in cardiac and skeletal muscles. These are DIFFERENT and immunoassays specific for the cardiac isoforms, cTnT and cTnI, are used as markers for cardiac injury.

Troponins T and I exist in a free form (in cytoplasm) and a bound form in cardiac muscle. Following injury, there is a slow release over 1–2 weeks and plasma levels fall slowly.

**NOTE:** NORMAL HEALTHY INDIVIDUALS USUALLY HAVE NO OR VERY LOW LEVELS OF TROPONIN. ELEVATED LEVELS ARE HIGHLY INDICATIVE OF CARDIAC INJURY.

**FALSE POSITIVE LEVELS** occur in
1. chronic renal failure
2. skeletal muscle injury/diseases/regeneration (cTnT)
3. Rheumatoid Factor/Heterophile antibodies
4. Fibrin in incompletely clotted samples

New 2nd generation assays avoid mass reactivity with skeletal muscle forms, and are hence highly specific for myocardial injury.
USE AND INTERPRETATION

1. Troponins T and I are equally diagnostic for myocardial injury. They may be considered as the “gold standard” for diagnosing M.I.
2. Troponins are usually detected 3–6 hours after injury, peak at about 24 hours and remain detectable for 7–10 days. They are not considered as “early biomarkers.”
3. Sensitivity of the test increases with time (after injury); specificity is about 100%.
4. Increased Troponins signify myocardial injury, even without EKG changes. It is helpful in diagnosing “unstable angina” induced injury.
5. Serial assays are useful in cardiac allografts rejection, to assess perfusion following thrombolytic therapy and diagnosis of perioperative acute myocardial infarction.

IV. ISCHEMIA MODIFIED ALBUMIN

(ACB – Albumin Cobalt Binding test)  

VALUE = 85-100 ku/L or more

Acute ischemic events lead to generation of reactive O₂ species/toxic free radicals. These reduce the ability of the N-terminus of serum albumin to bind to metal ions such as cobalt or copper.

Normal Person  
(No Ischemia)  

1. Normal albumin binds to Cobalt  
2. So less Cobalt is available to bind to “chromogen” added  
3. So less coloured compound forms

Patient with Ischemia  

1. Damaged albumin doesn’t bind to cobalt  
2. So more Cobalt is free to bind to chromogen added  
3. So coloured compound formed is MORE (inference: more damaged albumin in serum)

IMA is positive within 6-10 minutes of ischemia. Returns to baseline 6 hours after cessation of ischemia

CAUSES OF ELEVATION

Cardiac  

Non-cardiac  

e.g., brain ischemia, liver disease, kidney failure, cancers, infections, heavy exercise

Positive Predictive Value  33%  
Negative Predictive Value  96%

USE  

1. Rule out ACS in low to moderate pretest probability conditions when EKG and other markers are negative.
C-REACTIVE PROTEIN

Standard Test (in milligram / litre)

Serum (from patients with pneumococcal infections) + c-polysaccharide of pneumococcus = visible flocculates $\rightarrow$ c-reactive protein.

It is a general scavenger molecule the gene for which is located on chromosome 1. An acute phase reactant it is often associated with and elevated in infections, inflammations and tissue necrosis.

Injury/infection $\rightarrow$ tissue damage $\rightarrow$ Cytokine Release $\rightarrow$ CRP Synthesis in liver

CRP
1. binds to phosphocholine of microorganisms, activates complement mediated phagocytosis of organisms.
2. binds and detoxifies endogenous toxic substances produced by tissue damage.
3. has proinflammatory and procoagulant functions.
4. Stimulates macrophages and “foam cell” formation $\rightarrow$ CRP mediated uptake of lipids AS.
5. Stimulates endothelial cell function $\rightarrow$ ICAM expression increases $\rightarrow$ AS.

USE:
1. Presumptive diagnosis of bacterial infection (high CRP) versus viral infection (low CRP).
2. Monitor progression or remission of autoimmune disease.
3. Inflammatory disorders, e.g., Crohn’s disease.
4. Any tissue injury or necrosis, e.g., M.I., malignancies, burns, trauma, kidney rejection.

In acute M.I., CRP appears within 24–48 hours, peaks in 72 hours and returns to normal after 7 days.

hs-CRP
High sensitivity CRP (determined by turbidimetric assays) NORMAL VALUE = < 1 mg/L

1. It is a cardiac biomarker with 90% sensitivity and 82% specificity for predicting subsequent events (e.g., cardiac death, MI, or need for revascularization)

<table>
<thead>
<tr>
<th>Levels</th>
<th>Risk for Cardiac Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 mg/L</td>
<td>Low</td>
</tr>
<tr>
<td>1-3 mg/L</td>
<td>Intermediate</td>
</tr>
<tr>
<td>&gt; 3 mg/L</td>
<td>High</td>
</tr>
</tbody>
</table>

2. May be useful as a measure of outcome in severe unstable angina. High CRP levels may be a predictor of subsequent cardiac events (as in #1 mentioned above).
3. Persons in the high normal CRP concentrations are at greater risk for strokes and MI than those with low normal values.
4. Adds to the predictive value of serum lipids for identifying individuals at risk for cardiovascular events.
5. CRP measurement has been useful in suggesting preventive treatment, e.g., aspirin and/or prevastatin use (coronary heart disease events dropped by 50% with statin use when CRP levels were high).
CONGESTIVE HEART FAILURE

NATRIURETIC PEPTIDES

These hormones are antagonists to the renin angiotensin-aldosterone (RAA) axis. They cause vasodilation and hence reduced blood pressure; and also cause Na and water loss. The natriuretic peptides are:

1. **ATRIAL NATRIURETIC PEPTIDE (ANP)** – produced in atria (myocardial cells)
   pro ANP → ANP

2. **B-TYPE NATRIURETIC PEPTIDE (BNP)** – produced in ventricles
   pro BNP → active BNP + N-terminal pro BNP (inactive)

These hormones are produced in response to volume and pressure overload.

---

**Table 2**

<table>
<thead>
<tr>
<th>THE MAJOR ROLES OF BNP AND NT-ProBNP IN PATIENTS WITH CONGESTIVE HEART FAILURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Screening for patients with defined risk factors (e.g., ischemic heart disease, valve disease, diabetes, etc).</td>
</tr>
<tr>
<td>• Diagnosis of CHF from other diseases with similar findings and symptoms (e.g., chronic obstructive pulmonary disease)</td>
</tr>
<tr>
<td>• Determination of disease severity (correlates with other indices of heart failure such as the NYHA classification)</td>
</tr>
<tr>
<td>• Risk stratification for future cardiovascular events (i.e., CHF disease progression and cardiac death)</td>
</tr>
<tr>
<td>• Monitoring the effectiveness of drug therapy (e.g., ACE inhibitors)</td>
</tr>
</tbody>
</table>

**Reference Range**

- ANP  < 80 pg/ml
- BNP  < 1,000 fmol/ml or
  < 100 pg/ml
OTHER CAUSES OF ELEVATION:

Pulmonary embolism. Cor-Pulmonale, cirrhosis, renal disease, Cushing’s syndrome, hyperaldosteronism, etc. Any edematous state with hemodynamic stress can cause increased BNP/NT-pro BNP.

SOURCES:
1. Interpretation of Diagnostic Tests by Jacques Wallach, M.D.

Revised 07/27/09.
Serum Markers of Risk for Coronary Artery Disease

By David Plaut

Coronary artery disease (CAD) is the leading cause of death in the United States. In fact, there are more cases of CAD than the next two causes of death combined. In most cases, CAD builds up over a period of years.

The most common consequences of CAD are:

1. Angina—pain when exercising that usually abates when resting.
2. Acute myocardial infarction (AMI).
3. Heart failure (shortness of breath and swelling of the ankles resulting when the heart cannot pump enough blood due to restriction in the coronary arteries).
4. Irregularities in the rhythm of the heart (arrhythmias).
5. Cardiomyopathies and
damage to any one of the four values that control the flow of blood within the heart.

Generally, CAD is a disease of affluence—it is rare in the tropics and in developing countries. Interestingly, one study shows that the United States is 14th in incidence of CAD (after Latvia, which is first; Hungary; and the Russian Federation are above the United States). Causes of CAD

The table presents a list of factors that contribute to CAD. Some are modifiable, some are not.

Health care professionals do not know what precipitates the narrowing (occlusion) of the coronary arteries. The precipitating event could be an infection (both Helicobacter pylori and Chlamydia pneumoniae have been implicated) or an injury, resulting in inflammation, that could be attributed to hypertension, increased blood levels of cholesterol or triglycerides, or smoking (or perhaps other factors that we do not yet know).

The atherosclerotic process begins when damage or inflammation to the endothelium cells of the coronary artery increases the permeability of the artery to plasma lipoproteins, monocytes, T-lymphocytes and calcium. All of this is the beginning of the atherosclerotic lesion (plaque). These plaques are not distributed randomly but occur (for reasons not clear at this time) at particular junctions and certain areas where the artery curves. At these places, two factors probably contribute to plaque formation—low shear forces and slow or variable flow rates. These two factors, accompanied by an increased concentration of low-density lipoprotein (LDL), stimulates the migration of the monocytes and T-lymphocytes as well as the LDL and lipoprotein (a) (Lp(a)) through the endothelial barrier. The LDL and Lp(a) are then immobilized in the intima of the connective tissue.

Oxygen radical (free radicals) are generated by the macrophages (the presence of which suggests inflammation) and oxidize lipoproteins making them more toxic. Iron and copper ions have been reported to play a role in the production of the free radicals. It is for this reason that radical scavengers such as vitamin E and C and β-carotene may inhibit the progress of coronary artery occlusion.

Serum Markers

A number of serum markers have been proposed as indicators of risk for CAD. In this brief review, some but not all will be discussed. While most of the emphasis is on the clinical applications and utility of these assays, there are aspects of the analysis mentioned when appropriate.

1. Cholesterol. This was probably the first marker suggested as a risk factor. It has been studied for more than 50 years, going back to the Framingham Study in the early 1950s. Cholesterol was implicated with CAD when early studies found cholesterol in the plaques of MI patients. While there is no question that cholesterol levels are a factor in CAD, two facts must be kept in mind: 1) 35 percent...
of MI patients have total cholesterol levels (TC) below 200 and 50 percent have TC below 220. These facts are important reasons why we must look beyond the TC levels to other markers.

There are now standards for cholesterol and quite specific and reproducible assays for measuring it. However, some data suggest that differences within a lab and between labs still do exist and may have implications for the NCEP goals still a challenge. 4

(2) High-density lipoprotein (HDL). HDL, or a-cholesterol has been measured for more than 40 years. This fraction of the TC correlates negatively with triglycerides (TGs) and has antiatherogenic effects as a result of its reverse cholesterol transport (HDL moves cholesterol to the liver) and its antiplatelet activity, activation factor acetylsalicylic acid, and platelet aggregation. Most studies have found correlation between the incidence of CAD and HDL than between CAD and TC. One study (36,200 patients with angina and angiographically confirmed CAD found that HDL levels were more important risk factors than LDL, fibrinogen, CRP or Lp(a). This has not always been the conclusion of studies comparing various serum markers of risk.

Measuring HDL is more difficult than measuring TC in that it involves either a separation of the HDL from the other lipoproteins (e.g., LDL, VLDL) and then measuring the cholesterol in the extract or a direct immun assay, which depends on the specificity of the antibody. In this study there may be variations among the standards (36,200) used by various manufacturers that may lead to difference between laboratories. The study referred to above found total errors (TE = 2CV + bias) in HDL assays between 12 percent and 19 percent. These data suggest that our goals for HDL have not yet been achieved.

(3) Low-density lipoprotein. LDL is composed of two types of particles. The more common is the larger, less dense particle (pre-A) and the small dense (B), which is more commonly associated with increased levels of TC and fibrinogen and reduced levels of HDL. These smaller particles have less antioxidant capabilities and are more atherogenic.

LDL levels can be reduced by weight reduction, diet, exercise and lipid-lowering drugs (the statins). Maximum therapeutic benefit is obtained with a decrease in LDL levels of 20 percent to 30 percent, irrespective of baseline levels or LDL levels on treatment and, until now, there have been no data to suggest that decreases in LDL of greater than 30 percent give any additional benefit to patients in terms of improving their long-term outcome. We may need more data to resolve all the issues concerning the optimum decrease and optimum level of LDL.

In the past, LDL levels were calculated from TC and TG levels (the Friedenwald formula). This was an acceptable approach when the TG level was normal and no methods other than electrophoresis were available, but it gave incorrect values when the TGs were high. There are direct methods, similar to those for HDL, that do not require separation. These are simpler and less expensive—only one assay (vs. two) needs to be performed. In the study referred to above, total errors in LDL assays ranged between 13 percent and 16 percent.

(4) Apolipoprotein A and Apo B assays are not yet common. In part this is due to the fact that standards for them are not agreed upon and since there is more than one Apo A and several Apo Bs, it is difficult to produce antibodies that will react with all forms. Since each person can have different levels of B-100 and B-48, an assay that does not identify these equally is less useful.

(5) Triglycerides (TG). A number of reports demonstrate the importance of serum TG assays in predicting the clinical course of vascular disease. However, adjustment for measurements highly correlated with TG levels, such as age, sex, body mass index and high-density lipoprotein cholesterol (HDL-C) may lessen (if not remove) the TG contribution to outcomes. Recently, improved analytic approaches have increased the number of patients with elevated TGs as independent risk factors for an onset of cardiovascular disease. Elevated TG levels are the consequence of larger TG-rich particles, including very low density lipoprotein and atherogenic intermediate particles, which are associated with dense LDL.

It has been observed that a reduction in TG concentrations often proceeds in parallel with improved clinical outcomes; however, direct correlation between the two has been elusive. This has been demonstrated in multiple pharmacological trials. However, an improvement in these relationships has been observed when TG-correlated measurements of intermediate LDL density and HDL have been made.

(6) Lipoprotein(e), a highly heterogeneous lipoprotein, due to variations in the size of apolipoprotein(e), and the density of the ApoB-100-containing particle to which Apo(e) is linked. Although high plasma levels of Lp(e) have been associated with an increased risk for atherosclerotic cardiovascular disease, the pathophysiology associated with this condition has not been well characterized. The role of Lp(e) in CAD may also be influenced by ethnic differences in environmental factors and events related to oxidative processes, and the action of some enzymes.

The study of Lp(e) is complicated by the fact that there are a variety of methods for its quantification and, due to its complex structure, the immunosassays may yield different results as a binding to various sites (epitude) and the lack of a standard.

In one study to assess the value of measuring Lp(e), 15, middle-aged men with hypercholesterolemia and a history of MI were selected consecutively from referrals to a lipid clinic and compared to a control group that had not sustained an MI. Individuals of similar age, sex, cigarette smoking and blood pressure characteristics were also selected from the same clinic. Serum cholesterol, triglyceride, LDL cholesterol, HDL apolipoprotein A1 and B and Lp(a) were measured in both groups. The serum concentration of Lp(e) was more than twice as high in patients with MI. There were no significant differences in other variables. Lp(a) was the only significant predictor of MI. This is in contrast to Ridker's work, where no significant differences were found in Lp(a) and homocysteine (HC) comparing those men and women who did not have an MI or stroke with those who did during the eight-year follow-up. On the other hand, Ridker did find significance in the levels of LDL.

HC is a naturally occurring amino acid that is present in the serum of humans. In 1982, Curtin and Neill first described children with severe mental retardation who had high levels of HC in their serum. They also noted that these children had a higher than average incidence of AMI. The relation between serum levels of HC and CAD may be related to the cytotoxic effects of HC on endothelial cells. Several recent studies have shown that even moderately elevated serum levels of HC (which occurs in 5 percent to 7 percent of the general population) are associated with increased risk for fatal and non-fatal AMI and stroke, independent of other risk factors such as elevated cholesterol.
to both genetic and lifestyle factors, such as smoking, alcohol consumption, diet and inadequate exercise. In a clinical study, Ngoyed found an odds ratio of over nearly 3 when the H/L was between 15 and 19.9 and an odds ratio of greater than 5.5 when the serum H/L was more than 20.11

Another way of looking at this is that a 5 mg/dL increase in H/L raises the risk of CAD by as much as a 20 mg/dL increase in cholesterol or each increase of 5 mg/dL in serum H/L above the normal range raises the risk of CAD by about 20 percent. In patients with increased cholesterol, the relative risk of an MI increased by a factor of 2.8 from the lowest one-fifth of patients to the upper one-fifth. Lowering H/L appears to improve cardiac function.

An immunoassay that does not require pretreatment of the sample has been developed and is commercially available. There are also rapid, simple HPLC methods that make it possible for the widespread use of H/L assays in assessing patients who have a history to cardiac disease, as well as detection of those who are at risk for a future event. H/L levels and, thus, risk, can be reduced by dietary intake of folate acid (approximately 200 mcg/day).

C-reactive protein (CRP) was first measured in acutely ill patients in 1930.13 It was recognized early on that CRP was a marker of inflammation. CRP was measured in atherosclerotic plaque, possibly the soft, lipid-rich plaques normally associated with myocardioc infarcts with thrombus. CRP and inflammation may be more involved in plaque stability and rupture than in plaque formation. (Animal studies suggest that IL-6, which regulates the production of CRP, increases the rate of early arterial plaque upbuilding when injected into mice.) Neuer, more sensitive assays have been developed to detect the association between H/L and CRP levels. These assays (sometimes referred to as high-sensitive or hs-CRP methods) have a reference range that begins at about 0.1 mg/L. Prospective studies show that chronic inflammation giving rise to low CRP. While still in the"normal range," these levels are indicative of long-term risk for cardiovascular disease. Differences in CRP levels have been found between men and women. A small increase in age has been observed. The intra-individual variation is in the order of 30 percent, indicating that rather large differences must be observed before considering the change pathological.

The European Concerted Action of Thrombosis Angina Pectoris Group found that CRP was about 50 percent higher (2.2 v 1.4) in a group of patients who had an event within two years. Those in the group of patients with the highest CRP were about twice as likely to have an event. In another study, Ridker found that hs-CRP assays added an independent predictor to lipid assays.14 Ridker's data from the Physicians Health Study found that among more than 22,000 men followed for eight years, those with the highest hs-CRP levels had a two-fold increase in risk of stroke and a three-fold increase of future AMI. In one of the TIMI studies, of more than 1,400 patients presenting with non-ST elevation but diagnosed with acute coronary syndrome, an elevated hs-CRP upon admission predicted a higher mortality in 14 days, even in patients with a negative TnT value.15

Not only has hs-CRP been helpful in detecting those patients at higher risk who have CAD, CRP measurement has been found useful in suggesting preventive treatment. In the PHS study, as GRP predicted those most likely to benefit from aspirin (325 mg twice daily). Additionally, it appears that CRP levels can predict risk reduction attributable to Prevasatin. Ridker found a 54 percent reduction in the occurrence of AMI when the CRP was high and Prevasatin given vs. only 25 percent with normal CRP levels.

Raffa has studied a number of methods for quantifying CRP and found good precision in them.18 There is a well accepted standard for CRP which should make interlaboratory comparisons meaningful. As a hemostatic marker, work with fibrinogen and some of the other coagulation factors has been studied as indicators of CAD risk. In one such study, the risk of coronary heart disease and all-cause mortality for patients in the highest one-third of patients fibrinogen were higher than those in the lowest third.19 An increase of about 1 SD of plasma fibrinogen level (75 mg/dL) was found to increase risk of CHD and all-cause mortality 29 percent and 31 percent, respectively (Benderly). The Framingham group concluded that since an elevated fibrinogen level is a predictor of cardiovascular disease these assays should be added to the cardiovascular risk factor profile (Kennel Framingham).

Tataro found that fibrinogen concentration was correlated to the severity of CHD and that patients with generalized arteriosclerosis had higher plasma fibrinogen concentrations than did those suffering from only CHD.20 Although there may be an association between circulating D-dimer values and CHD, further studies are needed to determine the extent to which this is causal.

Troponin I and T are well accepted markers of and have even been purposed as the gold standard for cardiac cell damage. Assays of troponin I and T also may be use in assessing the risk of future MI. For example, in a study that followed 91 patients for one year in the group (n = 22) who had an increase of TnI either on admission or at eight hours following admission, only 68 percent were free of cardiac events at one year compared to 90 percent of those with normal TnI on admission.21 Similarly, Anman found that in a group of nearly 600 patients with unstable angina or non-Q wave MI, a troponin of greater than the sensitivity of the method was associated with an increased mortality at six weeks. Even patients with non-ST segment elevation acute coronary syndrome are at risk for future events. These data suggest that perhaps in those patients with other risk factors (including the markers discussed above), a measurement of TnI or TnT might be of value in determining treatment.

Guidelines for Levels of Cholesterol and LDL

Like previous guidelines issued by NCEP, the ATP III focuses on lowering LDL cholesterol as a primary focus and using exercise, diet and drugs as important means of lowering the risk of CAD. The new guidelines recognize LDL levels of less than 100 mg/dL as optimal for all patients and increase our attention on high TG levels (above 200 mg/dL). ATP III places more emphasis on identifying patients at risk for CAD and LDL events. To apply the recommendations of ATP III in practice, clinicians should follow a six-step process:

1. assess the patient's lipid profile (full panel, not just total cholesterol);
2. assess and categorize the patient's CAD risk (using a point system reflecting the levels of risk inherent in certain factors);
3. establish treatment goals and approaches (the greater the risk, the more aggressive the management);
4. initiate therapeutic lifestyle changes (including new recommendations for low intake of saturated fats and dietary cholesterol);
5. consider LDL lowering drug therapy (often with combination therapy); and
6. consider other lipid factors (particularly hypertriglyceridemia and the metabolic syndrome).

Most patients who begin lipid-lowering therapy stop it within one year, and only about one-third of patients reach treatment goals. The release of the ATP III guidelines provides pharmacists a great opportunity to enhance pharmaceutical care services directed specifically at patients with hyperlipidemia.

Bush and Reidel from Johns Hopkins have suggested that "the relatively low sensitivity of total cholesterol as a screening tool should be the impetus for rethinking the screening guidelines. Specifically, the cost-benefit ratio of routine screening for lipoprotein, particularly HDL cholesterol, needs to be carefully considered."22

David Plant is a clinical chemist and statistician in Plano, TX.

For a complete list of references, contact David Plant at david_plant@dailyleafing.com.
Cardiac Damage Detection
A Review of Serum Markers

Over the past few years, several changes have been made in how patients with chest pain are triaged when they enter the emergency department (ED). These changes are due in large part to:

1. the treatments available today, including lytic agents such as tissue plasminogen activator (TPA) and streptokinase, emergency angioplasty and emergency bypass surgery. The rational goal for administering TPA to those who have had an acute myocardial infarction (AMI) is within 45 minutes if the patient presents to the ED within one to two hours of the onset of chest pain.

2. available space in the ED. Many EDs and coronary care units do not have enough space to hold patients for extended periods. This leads to a desire to release the non-myocardial infarction (MI) person as quickly as possible.

BY DAVID PLAUT
FIG. 1
Number of Patients with Chest Pain
Who Present to EDs in the United States

<table>
<thead>
<tr>
<th>Category</th>
<th>8 million</th>
<th>2.5</th>
<th>1.0</th>
<th>1.2</th>
<th>0.3M</th>
</tr>
</thead>
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<tr>
<td>DCM</td>
<td>non</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHF</td>
<td>AMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Cardiac</td>
<td>UA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td>sudden</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: table/surveys: David Plante

5. third party carriers. Their goal is to discharge the non-MI patients as quickly as possible and to administer TPA only to those who will benefit from this thrombolytic therapy.

For these reasons, the ED physician is under pressure to make correct decisions quickly.

Fig. 1 provides data on the number of patients with chest pain who present to EDs in the United States. It is important to try to place each patient in one of these categories as quickly as possible.

The Ideal Cardiac Marker

How can the most appropriate serum marker(s) for cardiac cell damage be chosen? The answer begins with the criteria for an ideal cardiac marker. Such a marker would:

* be specific for cardiac tissue;
* increase soon after the AMI;
* remain elevated long enough to be detectable in the serum of the late presenter;
* have a high concentration per gram of tissue; and
* be easy, rapid and inexpensive to measure.

The World Health Organization (WHO) criteria published in 1979 for making the diagnosis of MI are history of characteristic chest pain, diagnostic changes in the electrocardiogram (ECG) and changes in serum enzyme levels. Two of these are to be met in order to rule in an MI. Because a number of MI patients do not meet at least one of the first two criteria—only about 50 percent of MI patients present with a diagnostic EKG and only about 75 percent of MI patients present with chest pain, the laboratory often plays an important role in the early diagnosis of MI.

In September 2000, the American College of Cardiology and the

| TABLE 1 |
| NACB Guidelines for a Laboratory Cardiac Profile |
| Marker | Admission | 2-4 hrs | 6-9 hrs | 12-24 hrs |
| Early (+6 h) | x | x | x | (x) |
| Late (>6 h) | x | x | x | (x) |

x = recommended, (x) = optional.

European Society of Cardiology published a joint paper in which they proposed that the WHO criteria be supplemented by new guidelines for the diagnosis of AMI. The National Academy of Clinical Biochemistry (NACB) has proposed a protocol for the "detection of AMI by enzyme or protein markers, in the absence of definitive ECGs" (Table 1).

An early marker might be myoglobin or a second-generation troponin; a late marker might be total creatine kinase (TCK), the MB isoenzyme of CK or either troponin I or T. In this article, several case histories illustrate the use of this type of early rule-in or rule-out protocol. High-sensitive C-reactive protein (hs-CRP) is also discussed, as it might help triage the patient who has been diagnosed...
with coronary artery disease (CAD)—either to assist in following the patient with AMI or treating the patient with unstable angina (UA).

Because more patients will be ruled out for M1 than ruled in, a marker with high negative predictive value would be of value for those patients presenting early in their episode of chest pain.

Tucker et al showed that myoglobin has both early rule-out (Case 1) and early rule-in (Case 2) features that make it attractive. In case 2 only myoglobin changes during the period from To-T6.

Case No. 1 is the situation of some 50 percent to 60 percent of AMls and, of course, most non-Mls. Here, there are no changes in any of the markers over the first six hours. Since myoglobin appears in the serum 0.5–3.0 hours after an M1 and reaches a peak at six to nine hours after an event, we would expect to see some change in myoglobin during this period.

It is often argued, however, that myoglobin is not cardiac specific and should not be used to triage patients in the ED. The arguments for including myoglobin are a) the ED physician can choose not to use it when there is clear evidence of skeletal muscle damage and b) since the majority of patients presenting with chest pain will be ruled out for M1, a marker with high negative predictive value would be useful.

Gornall and Roth reported on a series of 100 patients presenting to the ED with chest pain. In this study, myoglobin was more specific for cardiac damage than the ECG (98 percent vs. 53 percent). When used properly, i.e., excluding known skeletal trauma patients and the late presenting AMls, myoglobin can be an extremely useful test.

Case No. 2 illustrates how an early rise in myoglobin can assist in the correct classification of the patient with chest pain. In these situations where there were no abnormal values on the first draw and where a diagnostic EKG is absent, it is necessary to collect a second sample. Here, the second sample shows an acute rise in the myoglobin. While such a rise in the myoglobin may not rule in cardiac damage, it certainly aids the clinician's decision not to discharge the patient until more is known about the cause of the rise in myoglobin.

A New Era

This is the era of the troponins. Troponin I, T and C form a complex with actin and troponyisin to create a large series of interconnected molecules that are responsible for muscle contraction. In contrast to MB and myoglobin, troponin T (TnnT) and troponin I (TnnI) are not the same in skeletal and cardiac muscle. There are several TnTs and three different Tnls; two of the Tnls are specific to skeletal muscle and one is specific to cardiac muscle. Because both TnnT and the Tnl found in cardiac tissue differ enough from the troponin T and I in skeletal muscle, assays for both TnnT and TnnI have been developed.

Assays for TnnT are available from Roche Diagnostics (Indianapolis) and include a point-of-care test and the Elecsys. Tosoh Medica (South San Francisco) offers the AIA-600 II for TnnI, CK-MB and myoglobin. TnnI is measured on instruments such as the:

- Dade Behring (Deerfield, IL) Rxl, Stratus CS and the Opus;
- Bayer (Tarrytown, NY) ADVIA Centaur and Immuno I;
- Beckman Coulter (Fullerton, CA) Access;
- First Medical (Mountain View, CA) Alpha Dx Point-of-Need System;
- DPC (Los Angeles) Immulite; and

Abbott (Abbott Park, IL) AxSYM.

Many of these commercial methods use sandwich assays (Ab-Ag-Ab) to quantify cTnl but employ different pairs of antibodies. Since different antibodies are used in some assays and there is no current internationally recognized standard for cTnl, results may differ among instruments.

Keep in mind that TnnI is not a simple compound like glucose. It exists in three different forms: two from skeletal and one from cardiac tissue. The differences in assay values are certainly related to what is actually being measured. Within the cell, the troponins are found in a complex with one another as well as with troponyisin and actin. Once released from the damaged cell, the molecule of TnnI may still be associated with TnnT or C. Also, a fact that appears in some of the serum, "free" TnnI can recomplex with TnnT or C. The fact that the different antibodies commercially used bind to different epitopes (sites) on the TnnI may be another reason different answers are obtained, whether the antibody measures only free TnnI or complexed and free TnnI.

The first sample in Case No. 2 yields normal values for TCK, MB and cTnl, and myoglobin, while the only myoglobin is increased at the second draw. As pointed out earlier, myoglobin is the first marker to rise after cardiac damage. At the 6th hour post presentation, the TCK, MB and cTnl are all elevated. It is generally true that these three markers increase at approximately the same time after cell damage. This case introduces the question of whether CK, MB and troponin (I or T) all need to be used.

In Case No. 3, the patient presented more than 30 hours after first experiencing chest pain; his EKG was non-diagnostic. The laboratory data, however, strongly suggest that an M1 had occurred. From the myoglobin and TnnI levels on the first draw, we infer that the event occurred more than 12 hours ago. Thus, the first sample answered two questions, 1) Did an MI occur? and 2) Approximately when? The next question to ask is whether more samples need to be drawn; the National Academy on Clinical Biochemistry does not recommend
that serum markers be used to size the infarct.

Case No. 4 illustrates the importance of looking at serial samples and the complementary nature of myoglobin and cardiac TnI (cTnI). Here, the myoglobin and troponin are both normal at T1 and both increase by T2. (Note that the MB levels are both within the reference range.) While the cTnI is not extremely high, together with the increase in myoglobin, recent acute cardiac damage is the most likely explanation. With current, more sensitive (second generation) assays for troponin I, earlier detection of an acute event is possible. This case illustrates the importance of looking at the significance of small changes in the level of the serum marker vs. using a single value as a definitive positive indicator of acute cell damage.

**CASE 1**

<table>
<thead>
<tr>
<th>Time* (h)</th>
<th>TCK (&lt;200)</th>
<th>CK-MB (&lt;5)</th>
<th>RI (&lt;2.5)</th>
<th>Myoglobin (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
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</tr>
<tr>
<td>2</td>
<td>131</td>
<td>2.7</td>
<td>2.0</td>
<td>33</td>
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</tr>
<tr>
<td>6</td>
<td>125</td>
<td>2.5</td>
<td>2.0</td>
<td>31</td>
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</table>

*Time after presentation to the ED

**MB X 100/TCK**

**CASE 2**

<table>
<thead>
<tr>
<th>Time* (h)</th>
<th>TCK (&lt;200)</th>
<th>CK-MB (&lt;5)</th>
<th>RI (&lt;2.5)</th>
<th>Myoglobin (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
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<tbody>
<tr>
<td>0</td>
<td>151</td>
<td>1.8</td>
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</tr>
<tr>
<td>1</td>
<td>126</td>
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</tr>
<tr>
<td>2</td>
<td>137</td>
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<td>222</td>
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</tr>
<tr>
<td>6</td>
<td>709</td>
<td>36.0</td>
<td>5.0</td>
<td>1989</td>
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</table>

**CASE 3**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>TCK (&lt;200)</th>
<th>MB (&lt;5)</th>
<th>RI (&lt;2.5)</th>
<th>Myoglobin (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
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<tbody>
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<td>12</td>
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<td>43</td>
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**CASE 4**

<table>
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<tr>
<th>Time (h)</th>
<th>MB (&lt;5)</th>
<th>Myoglobin (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.8</td>
<td>63</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>2.7</td>
<td>222</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**CASE 5**

A 52-year-old male with intermittent chest discomfort and inverted T-waves in inferior EKG

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>CK (&lt;200)</th>
<th>MB (&lt;5)</th>
<th>RI (&lt;2.5)</th>
<th>Myo (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
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<tbody>
<tr>
<td>0</td>
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<td>4</td>
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<td>5.5</td>
<td>2.2</td>
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<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>50</td>
<td>1.6</td>
<td>2.4</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**CASE 6**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>TCK (&lt;200)</th>
<th>MB (&lt;5)</th>
<th>RI (&lt;2.5)</th>
<th>Myoglobin (&lt;80)</th>
<th>cTnI (&lt;0.2)</th>
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<tr>
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<td>5.4</td>
<td>1.3</td>
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<tr>
<td>2</td>
<td>391</td>
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<td>1.2</td>
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<td>0.0</td>
</tr>
<tr>
<td>6</td>
<td>312</td>
<td>2.1</td>
<td>1.0</td>
<td>207</td>
<td>0.0</td>
</tr>
</tbody>
</table>

This is reiterated in Case No. 5. A cardiac catheterization revealed three-vessel CAD and a coronary artery bypass graft (CABG) was performed. The cTnI is increased on the first sample while the other tests are all negative. The two-hour sample shows a marked increase in myoglobin and CK; the cTnI increases slightly. The changes in cTnI and myoglobin, when combined, suggest that acute cardiac damage has occurred. Two interpretations can be made: first, this case is an extension of an MI that occurred some time before the patient was admitted (thus, the normal myoglobin and the increased cTnI); second, the patient has chronic cardiac damage that now presents itself as an MI (the cTnI increasing from a somewhat increased baseline and with the increasing myoglobin).

Case No. 6 depicts a patient with known renal failure (a condition that may elevate TCK, MB, and myoglobin). Therefore, it is important to obtain a second sample on this patient. The fact that none of the markers change over the period suggests no acute event.

While there has been little value in measuring myoglobin, TCK-MB, CK-MB in those patients with unstable angina, recent work indicates that quantifying troponin I or T may be of value in these patients. Levels of troponin in those patients with unstable angina would not be expected to change over the three- to 12-hour evaluation period.

In a series of more than 500 patients with unstable coronary artery disease, Lusher found that twice as many patients with increased levels of troponin I experienced an AMI or cardiac death within 30 days compared to those with low levels of TnI. Similarly, Anrman and Galvani in separate studies indicated that measuring cTnI in patients with acute coronary syndromes was of prognostic value. For example, in Anrman's work, there were more cardiac events at six weeks when the level of cTnI was greater than 0.4 than when it was less than 0.4 (3.7 percent events vs. 1.0 percent).

**Levels of CRP in AMI**

Work with an assay for CRP with greater sensitivity (hs-CRP) than the traditional method has indicated that these values may be of help in assessing the risk of a future event (e.g. MI, stroke) in apparently healthy individuals as well as in aiding with the prognosis of those with confirmed MIs. Patients whose CRP are increased for extended periods post MI are at greater risk of recurrent AMI, UA or even death. The degree of elevation is associated with the probability of an event within a year following an AMI (Table 2). The patients in the last group were also event free for a shorter period than those with lower levels of CRP. Levels of CRP, like those of creatine kinase, cTnI and cTnT, are associated with infarct size, usually considered a marker of long-term outcome.

David Plaut has been a clinical chemist for more than 30 years working in hospitals and most recently at Duke Behring. More references are available from the author at david_plaut@dukebehring.com

**References**

TRANSFUSION MEDICINE

Steve Nandkumar, MD
TRANSFUSION MEDICINE

DEFINITION
It is defined as a field of medicine associated with the transfusion of blood and blood components.

HISTORY
<table>
<thead>
<tr>
<th>Blood group system</th>
<th>Year</th>
<th>Discoverer</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABO</td>
<td>1900</td>
<td>Landsteiner</td>
</tr>
<tr>
<td>Rh (rhesus)</td>
<td>1939</td>
<td>Fisher-Race, Weiner</td>
</tr>
</tbody>
</table>

ABO SYSTEM
Antigens are complex carbohydrates carried on rbc glycoprotein and glycolipids.

Antibodies to ABO antigens are natural antibodies (IgM) occurring after the first year of life because of sensitization by similar antigens present in the environment (GI tract bacteria, etc.)

Maternal IgG antibodies may be present in newborn’s serum.

BASIC SUBSTRATE IS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product</th>
<th>Basic substrate</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>‘A transferase’</td>
<td>H antigen + N-acetyl galactosamine</td>
<td>A antigen</td>
</tr>
<tr>
<td>B</td>
<td>‘B transferase’</td>
<td>H antigen + galactose</td>
<td>B antigen</td>
</tr>
<tr>
<td>O</td>
<td>No transferase activity</td>
<td>H antigen</td>
<td>No A or B antigen</td>
</tr>
</tbody>
</table>

A and B genes are found on chromosome 9p. When both are present, A and B antigens co-exist.

NOTE:
Absence of ‘H gene’ results in a homozygous (h,h) state. **THERE IS NO ‘H’ ANTIGEN.** Such individuals have a **BOMBAY** phenotype (Oh), i.e., no H and hence no A or B antigens

The ABO group antigens are present on rbc membranes, sperms, squamous cells, and some tumour cells. They are also present in plasma and body fluids.
There are about 40 different antigens. The major ones are D, C, c, E, and e.

There are 3 adjoining gene loci on chromosome 1.

<table>
<thead>
<tr>
<th>Possible Genotype</th>
<th>Phenotype</th>
<th>Isohemagglutinins</th>
<th>Whites</th>
<th>African Americans</th>
<th>Asians</th>
<th>Native Americans</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0/0</td>
<td>0</td>
<td>Anti-A IgM</td>
<td>45%</td>
<td>49%</td>
<td>40%</td>
<td>79%</td>
<td>Universal donor (no antigens on the surface). Anti-AB IgG is responsible for ABO incompatibility. Can only receive O blood (antibodies would destroy A, B, or AB blood). Predisposition for peptic ulcer disease.</td>
</tr>
<tr>
<td>O/A, A/A</td>
<td>A</td>
<td>Anti-B IgM</td>
<td>40%</td>
<td>27%</td>
<td>28%</td>
<td>16%</td>
<td>Predisposition for gastric carcinoma.</td>
</tr>
<tr>
<td>O/B, B/B</td>
<td>B</td>
<td>Anti-A IgM</td>
<td>11%</td>
<td>20%</td>
<td>27%</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>A/B</td>
<td>AB</td>
<td>None present</td>
<td>4%</td>
<td>4%</td>
<td>5%</td>
<td>&lt;1%</td>
<td>Universal recipient (no antibodies).</td>
</tr>
</tbody>
</table>

**NOTE:** THERE IS NO d allele/antigen. ABSENCE OF D is usually referred to as d. (There is no D antigen production).

Two haplotypes can result in the phenotypic expression of 2 to 5 Rh antigens

e.g., CDE/cde
e/cDE, etc.

Rh positive (85% of population) → D antigen is present
Rh negative (15% of population) → D antigen is absent

Du variant is a weak variant of D antigen.

**IMPORTANT:** EXPOSURE OF Rh NEGATIVE INDIVIDUAL TO Rh POSITIVE RBCs BY TRANSFUSION/PREGNANCY CAN CAUSE ANTI-D ALLO ANTIBODY FORMATION.

**Kell (k) System**
- Present in 10% of population
- Kell antigen is formed from a precursor protein (gene located on Chromosome X)
- Kell negative patients may develop anti-Kell antibodies if transfused with Kell positive blood. This may cause hemolytic transfusion reaction on re-exposure to Kell positive blood.

**Duffy (Fy) System**
- Duffy antigens are codominant alleles Fya Fyb. These serve as receptors for malarial parasite (P. vivax).
- About 70% of people in malaria endemic areas are Duffy negative (selective protection).

**Kidd (Jk) System**
- The Kidd antigens are Jka Jkb. Formation of anti-Kidd antibodies may cause DELAYED hemolytic transfusion reaction.
Lewis (Le) System

- Lewis gene on chromosome 19 produces a fucosyl transferase (Lewis antigens). It is NOT an integral part of rbc membrane, but is ADSORBED onto the surface from plasma and body secretions.
- Anti-Lewis antibodies are IgM antibodies, do not cross the placenta, and are the most common cause of incompatibility during pretransfusion screening.

I, i System

- i antigen is an unbranched oligosaccharide. It is present in the newborns and is converted to I antigen (a branched chain carbohydrate) in adults by I gene product, a glycosyl transferase.
- Most adults lack i antigen. Antibodies directed against i or I antigen are IgM cold reacting antibodies (COLD AGGLUTININS).
  - anti i antibodies → seen in infectious mononucleosis
  - anti I antibodies → seen in Mycoplasma pneumonia and malignant lymphomas
- Cold agglutinins can cause cold autoimmune hemolytic anemia. Administration of “warm” blood prevents isoagglutination.

P System

- P antigens are carbohydrate antigens. They are also present on urothelial cells and act as receptors for E. coli leading to UTI (urinary tract infections).
- Autoantibodies to P antigen may bind to rbc in the cold, fix complements upon warming and cause PCH (paroxysmal cold hemoglobinuria) as seen in rare cases of syphilis and viral infections. These antibodies are “biphasic” (bind in cold and act in warm) and are called Donath-Landsteiner antibodies.

MNSsU System

- Regulated by genes on chromosome 4, M and N are determinants on glycophorin A (rbc membrane protein); S and s are on glycophorin B. All persons express U antigens.
- Antibodies to M, N, S, s and U can cause HDN/HTR.

Table 114-1. RBC Blood Group Systems and Alloantigens

<table>
<thead>
<tr>
<th>Blood Group System</th>
<th>Antigens</th>
<th>Alloantibody</th>
<th>Clinical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh (D, C/c, E/e)</td>
<td>RBC protein</td>
<td>IgG</td>
<td>HTR, HDN</td>
</tr>
<tr>
<td>Lewis (Le^a, Le^b)</td>
<td>Oligosaccharide</td>
<td>IgM/IgG</td>
<td>Rare HTR, HDN</td>
</tr>
<tr>
<td>Kell (K/k)</td>
<td>RBC protein</td>
<td>IgG</td>
<td>HTR, HDN</td>
</tr>
<tr>
<td>Duffy Fy^a/Fy^b</td>
<td>RBC protein</td>
<td>IgG</td>
<td>HTR (often delayed), HDN (mild)</td>
</tr>
<tr>
<td>Kidd Jk^a/Jk^b</td>
<td>RBC protein</td>
<td>IgG</td>
<td>None</td>
</tr>
<tr>
<td>I/I</td>
<td>Carbohydrate</td>
<td>IgM</td>
<td>Anti-M rare HDN, Anti-S, -s, and –U</td>
</tr>
<tr>
<td>MNSsU</td>
<td>RBC protein</td>
<td>IgM/IgG</td>
<td>HDN, HTR</td>
</tr>
</tbody>
</table>

NOTE: RBC, red blood cell; HDN, hemolytic disease of the newborn; HTR, hemolytic transfusion reaction.

HOME WORK What are the uses of blood groups?
1.
2.
3.
BLOOD DONATION

CRITERIA FOR BLOOD DONATION
Voluntary blood donors – paid donor
– unpaid donors
Autologous donation – donate blood for one’s own use
e.g., elective surgery
Directed donation – family members and friends donate blood designating it (directing it) for a specific patient.

BLOOD COLLECTION
About 450 ccs of blood are collected from the vein. CPDA-1 is used as a preservative
- Citrate → anticoagulant (about 63 ml.)
- Phosphate → maintains 2-3 DPG (diphosphoglycerate) in rbc’s
- Dextrose → provides nourishment for glycolysis
- Adenine (and phosphate) → ATP synthesis

BLOOD COMPONENT PREPARATION

BLOOD CENTRIFUGATION (SLOW)
Red blood cells (packed red blood cells)
Stored at 1-10ºC
Shelf-life 35 to 42 days
Platelet rich plasma
Secondary centrifugation (FAST)

NOTE: Glycerolized RBCs can be stored for a few years (above 10 yrs).
Leukopenic rbc - rbc unit with leukocytes removed
(10-20 ml of plasma) +
Cryoprecipitate contains factors VIII, vWF, I, XIII
precipitate forms
thaw at 1-6ºC
Cryoprecipitate poor plasma
Albumin, Immunoglobulin preparation

PHERESIS OR APHERESIS
Donated blood is used to separate the components of interest (either plasma or platelets) and the remaining blood elements are returned to patient.
Platelets obtained in this manner are called **SINGLE DONOR PLATELETS OR APHERESIS PLATELETS**.

<table>
<thead>
<tr>
<th>Apheresis Platelets</th>
<th>Platelet Concentrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of platelets</td>
<td>$3 \times 10^{11}$</td>
</tr>
<tr>
<td>Platelet ↑ foll. transfusion</td>
<td>30,000-50,000/µL</td>
</tr>
<tr>
<td>Expense</td>
<td>Expensive</td>
</tr>
<tr>
<td>Sensitization</td>
<td>less donor exposure to recipient so less alloimmunization</td>
</tr>
</tbody>
</table>

**Table 114-2 Characteristics of Selected Blood Components**

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume, mL</th>
<th>Content</th>
<th>Clinical Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRBC</td>
<td>180–200</td>
<td>RBCs with variable leukocyte content and small amount of plasma</td>
<td>Increase hemoglobin 10 g/dL and hematocrit 3%</td>
</tr>
<tr>
<td>Platelets</td>
<td>50–70</td>
<td>$5.5 \times 10^{10}$/RD unit</td>
<td>Increase platelet count 5000–10,000/µL</td>
</tr>
<tr>
<td></td>
<td>200–400</td>
<td>$\geq 3.0 \times 10^{11}$/SDAP product</td>
<td>CCI $\geq 10 \times 10^{9}$/L within 1 h and $\geq 7.5 \times 10^{9}$/L within 24 h post-transfusion</td>
</tr>
<tr>
<td>FFP</td>
<td>200–250</td>
<td>Plasma proteins—coagulation factors, proteins C and S, antithrombin</td>
<td>Increases coagulation factors about 2%</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>10–15</td>
<td>Cold-insoluble plasma proteins, fibrinogen, factor VIII, vWF</td>
<td>Topical fibrin glue, also 80 IU factor VIII</td>
</tr>
</tbody>
</table>

**NOTE:** PRBC, packed red blood cells; RBC, red blood cell; RD, random donor; SDAP, single-donor apheresis platelets; CCI, corrected count increment; FFP, fresh frozen plasma; vWF, von Willebrand factor.

**INDICATIONS FOR TRANSFUSION**

**Table 12.6 Indications for Transfusion**

**Packed RBCs**
- Hgb <7 g/dL or hematocrit <21% in a patient with uncompromised cardiovascular function
- Hgb <10 g/dL or hematocrit <30% in a patient with cardiovascular disease, sepsis, or hemoglobinopathy

**Platelets**
- Prophylactically for platelet count <10,000/µL (adults), <20,000/µL (children), <40,000/µL (neonate aged <1 week old) or <50,000/µL (neonate aged <72 hr old)
- <20,000 platelets/µL and bleeding or minor bedside procedure
- <20,000 platelets/µL and postoperative bleeding or in preparation for a major procedure
- <100,000 platelets/µL and bleeding post cardiopulmonary bypass
- Bleeding or invasive procedure in a patient with a history of platelet dysfunction

**Fresh Frozen Plasma**
- Deficiencies of Factors II, V, VII, X, XI, XIII, protein C, or protein S
- Thrombotic thrombocytopenic purpura (TTP) and hemolytic-uremic syndrome (HUS)
- Undefined factor deficiencies and PT or PTT >1.5 x upper limit of normal
- Emergency reversal of warfarin anticoagulation
- Disseminated intravascular coagulation (DIC) with bleeding

**Cryoprecipitate**
- Bleeding or preprocedure and 1 of the following:
  - Dysfibrinogenemia
  - Fibrinogen <100 mg/dL
- Uremia indication is controversial

Hgb, hemoglobin; PT, prothrombin time; PTT, partial thromboplastin time.
PRE-TRANSFUSION TESTING

I. ABO AND Rh GROUPING (TYPING)

A. FORWARD TYPING
   This determines (unknown) ABO and Rh phenotypes on rbcs by using KNOWN antisera against A, B, and D antigens.

B. BACK OR REVERSE TYPING
   This determines unknown isoagglutinins in serum by using KNOWN A, B, D antigens.

II. SCREENING OF PLASMA FOR UNEXPECTED ALLOANTIBODIES

If patient’s serum contains unexpected antibodies, they may react with antigens on donor rbcs and cause HTR. So make sure that donor rbcs LACK the target antigen.

Patients serum + group O rbcs (so that they are not agglutinated by anti A or anti-B isoagglutinins) containing many known antigens to which clinically significant alloantibodies are made).

LOOK FOR AGGLUTINATION

Positive agglutination = alloantibodies present, USE donor rbcs that LACK target antigen.
Negative agglutination = no alloantibodies

NOTE: Screening donor plasma for alloantibodies is less significant. Why?

III. TESTING FOR INFECTIOUS DISEASES (IN DONORS)

e.g., HIV-1, HIV-2
   HTLV-1, HTLV-2
   HBV, HCV, CMV
   SYPHILIS
   Others as necessary

IV. CROSSMATCH

Donor rbcs must be compatible with patient’s blood. This is determined by

A. INDIRECT ANTIGLOBULIN TEST (Indirect Coomb’s Test)
   This test detects the presence of alloantibodies in patient’s serum to donor rbcs (antigens).

   If patient’s serum + donor rbcs \( \xrightarrow{\text{antiglobulin reagent}} \) no agglutination

   This means that the crossmatch is compatible.

   If patient’s serum + donor rbcs \( \xrightarrow{\text{antiglobulin reagent}} \) agglutination

   This means patient’s serum has alloantibodies to donor rbs and hence the crossmatch is NOT COMPATIBLE. If so, use donor rbcs that LACK target antigens (to the alloantibodies).

NOTE: Alloantibodies are IgG type and do not readily react/agglutinate in vitro. Antiglobulin reagents (anti IgG and/or anticomplements) enhance this reaction.
B. IMMEDIATE SPIN CROSSMATCH

Patient’s serum + donor rbcs → centrifuge → observe immediately for agglutination.
If no agglutination, then the donor’s rbcs are compatible.

This test is useful in
1. emergencies when an abbreviated crossmatch saves time for providing blood rapidly.
2. patients who have a negative antibody screen.

This test may detect ABO incompatibility, but is not sensitive for other alloantibodies.

C. DIRECT ANTIGLOBULIN TEST (DAT) – (also known as direct Comb’s test)

This test detects IgG/complements BOUND TO PATIENT’S RBCs IN VIVO.

Patients’ rbcs (wash with saline) + antiglobulin reagent

Observe for incubate
Agglutination

If agglutination present positive DAT
If no agglutination negative DAT

A positive DAT is seen in HDN, HTR, AIHA (autoimmune hemolytic anemia) and drug-induced hemolytic anemia (DIHA).

NOTE: Antiglobulin reagent contains anti IgG/anti complements.

COMPATIBILITY TESTING FOR BLOOD COMPONENTS (OTHER THAN RBCs).

1. There is no need for cross matching.
2. Platelets and plasma must be ABO compatible with recipients’ blood. Isoagglutinins anti A and/or anti B can cause HTR in case of ABO mismatch.
3. Rh-ve recipients may receive plasma from Rh + ve or Rh-ve donors.
4. Rh-ve patients must receive platelets from Rh-ve donors.

COMPLICATIONS OF BLOOD TRANSFUSION

DEFINITION

Adverse reactions to transfusions (overall incidence is 3-3.5%) may be non-life threatening or can pose serious risk to patient’s health.

There are 3 types: Immunologic Non-immunologic Infectious

IMMUNOLOGIC REACTIONS

A. REACTIONS TO RBC (INCIDENCE < 0.1%).

1. Acute Hemolytic Transfusion Reaction (HTR)

Immune mediated hemolysis occurs when the recipient has preformed antibodies that lyse donor rbcs. ABO isoagglutinins (most common) and other alloantibodies of the Rh, Kell,
Duffy, and Kidd systems cause HTR. Such antibodies develop following prior transfusion with rbc or maternal-fetal hemorrhages. Acute HTR occurs usually within 24 hours of transfusion. There is Intravascular Hemolysis. The clinical features involve fever, chills, nausea, vomiting, tachycardia, tachypnea, low back pain, substernal pain/discomfort and hypotension.

- Hemoglobinemia (red plasma)
- Hemoglobinuria (red urine)
- D.I.C.
- Renal dysfunction due to tubular damage by free Hb and immune complexes

**Lab Tests for Hemolysis**
- LDH ↑
- Haptoglobin ↓
- Indirect bilirubin ↑

**Management**
**STOP TRANSFUSION AT ONCE**
- Report to blood bank
- Monitor for DIC
- Maintain renal function/diuresis

**NOTE:**
- Blood bank will
  - Check for clerical errors.
  - Test pre- and post-transfusion patient’s blood for hemolysis.
  - Repeat typing studies/cross matching.
  - Perform direct Coombs’ test (DAT) of post transfusion blood sample (to detect Ab or complement bound to rbc's in vivo).

**IMPORTANT:** THE MOST COMMON CAUSE OF HTR IS CLERICAL ERROR. (Mismatched transfusion due to labeling error, blood given to wrong patient, etc.)

2. **Delayed Hemolytic Transfusion Reaction** (Delayed serologic transfusion reaction)
- These reactions occur in patients previously sensitized to alloantigens on rbc's. The antibody screening is negative due to low Ab levels.
- Transfusions of an Ag positive blood trigger an ANAMNETIC RESPONSE leading to antibody formation and rbc binding with subsequent HTR.
- The reaction occurs usually within 3-21 days (usually 5-7 days) after transfusion. There is evidence of EXTRAVASCULAR HEMOLYSIS. The antibody-coated rbcs are cleared by the Reticuloendothelial system.

**Lab Tests**
- Hb ↓
- Bilirubin ↑

**Management**
- No specific therapy is required.

**NOTE:** Delayed HTR is diagnosed by the blood bank when a subsequent patient sample reveals a positive allo Ab screen or a new Ab in a recently transfused patient.
B. REACTION TO PLASMA COMPONENTS

1. **Allergic Reaction** (incidence 1–3%)
The recipient develops a hypersensitivity reaction to foreign plasma proteins in donor blood components.
S/S – Hives, erythema, pruritus

**Treatment**
**STOP TRANSFUSION AT ONCE**
– Give antihistamines
– Continue transfusion after S/S resolve
– Wash blood cell components to remove residual plasma

2. **Anaphylactic Reaction** (incidence 0.1–0.2%)
Anaphylactic reaction includes nausea, vomiting, coughing, dyspnea, hypotension, bronchospasm, respiratory arrest, unconsciousness and shock.
IgA deficient recipients if given IgA positive blood develop such reactions. They must be hence given IgA-ve units.

**Treatment**
**STOP TRANSFUSION AT ONCE**
– Epinephrine 0.5 to 1 ml inj. sub Q
– Steroids

C. REACTIONS TO WHITE BLOOD CELLS

1. **Febrile Non-Hemolytic Transfusion Reaction**
FNHTR is the **MOST COMMON** of all complications
S/S – Fever-temperature rise by 1°C or more
[Other causes of fever (patient’s disease, therapy, etc.) are ruled out]
– Chills and rigors/nausea, vomiting, discomfort

**Cause**
– Recipient’s serum contains Ab against donor leucocyte and HLA antigens.
– Cytokines released from donor lymphocytes during storage

**Management**
Use antipyretics/acetaminophen
– Pre-medicate if necessary
– Use leucocyte poor blood (filters remove WBCs)

2. **Transfusion Associated Graft vs. Host Disease**
– Donor T lymphocytes recognize host HLA antigens as foreign and mount an immune response
S/S – Develop at 8-10 days
  – Fever
  – Skin lesions
  – Liver damage
  – GI tract dysfunction (diarrhea)
  – B.M. – aplasia and pancytopenia
– Death 3-4 weeks post transfusion.
These reactions occur in immune incompetent hosts or immune competent recipients who share HLA antigens with the donor (family member, relative, etc.), and patients who have had BMT.

**Management**
- It is highly resistant to treatment.
- TA - GVHD can be prevented by irradiation of donor unit before transfusion

3. **Transfusion-Related Acute Lung Injury (TRALI)**
   Anti leucocyte or anti HLA antibodies in donor plasma bind with recipient’s leucocytes. The resulting immune complexes get trapped in pulmonary vasculature and release mediators that increase capillary permeability leading to PULMONARY EDEMA.
   Lipid moieties released from blood cell membranes also cause vascular endothelial damage.
   **S/S** (occur within 4 hours of transfusion)
   - Fever, chills
   - Dyspnea, respiratory distress
   - Bilateral pulmonary edema

**Management**
- Mechanical ventilation/support
  (Good recovery without sequelae)

**NOTE:** Donor’s (usually multiparous women) plasma will contain anti HLA antibodies.

D. **REACTION TO PLATELETS**

1. **Post Transfusion Purpura**
   This reaction presents as thrombocytopenia about 7–10 days after platelet transfusion (or rbc units with adequate contaminating platelets)
   - Patients who lack HPA-1a antigen on platelet receptor, upon exposure to a HPA-1a positive unit will develop antiplatelet Abs that react with platelets causing destruction and consequent low platelets.

**Management**
- Do not give additional platelets
- I.V. Ig is helpful
- Plasmapheresis to remove antibodies

2. **Refractoriness to Platelet Transfusion**
   Alloimmunization to antigens on leucocytes and platelets can cause refractoriness to platelet transfusions. Antiplatelet Abs are formed and these destroy the platelets, so that transfusion is ineffective.

   HLA matched platelets may or may not help.

**Management**
- Prevent sensitization by using leucocyte reduced platelet products/leuco-poor units
- Use single donor apheresis platelets

E. **IMMUNOMODULATION**

Allogeneic blood is immunosuppressive. Multiple transfusions in renal transplant patients enhance graft survival. However, immune suppression may be dangerous in cancer patients and post-operative cases. Leuko-poor blood units reduce immunosuppression.
NONIMMUNOLOGICAL REACTIONS

1. **Hypothermia**
   Administration of large amounts of blood at 1-10°C can cause discomfort, impaired hemostasis and cardiac arrhythmia due to effect of cold temperature on SA node. Use of blood warmers can avert this complication.

2. **Fluid Overload**
   Blood products are volume expanders and can cause volume overload – especially in patients with CHF or CRF
   S/S – S.O.B. chest pain etc.

   **Management**
   Monitor rate and volume of transfusion
   Use diuretics

3. **Iron Overload**
   Each rbc unit contains 200–250 mg of iron. When 100 or more units are transfused (e.g., rupture of aortic aneurysm) the excess iron is deposited in heart, pancreas, and liver (hemosiderosis).

   **Treatment**
   Iron chelating agents (deferoxamine)
   Plasmapheresis

4. **Potassium Toxicity**
   Potassium leak from Rbcs in stored blood increases K concentration. Such stored units can cause hyperkalemia in neonates (dangerous!) and patients with renal failure.

   **Treatment**
   Use fresh Rbc units
   Washing Rbc units removes extracellular ‘K’.

5. **Citrate Toxicity**
   Citrate chelates calcium ion and inhibits coagulation (anticoagulant effect). Citrate is normally metabolized to bicarbonate in the liver. Multiple rapid transfusions, hepatic diseases, etc., can cause citrate accumulation, hypocalcemia and tetany.
   S/S – Circumoral numbness
   Tingling sensations in toes/fingers
   Cardiac arrhythmias

   **Treatment**
   Use I.V. calcium

6. **2–3 Diphosphoglycerate (2–3 DPG) Depletion**
   Depletion of 2–3 DPG in “stored blood”, decreases the O₂ releasing capacity of Hb. This may cause tissue “hypoxia”. This may cause problems in neonates, but not in adults.
INFECTIONOUS COMPLICATIONS

Transmission associated infections from donor units are as follows:

1. **Viral** e.g., HCV, HBV, HGV, HIV, HTLV
   - **Parvovirus B-19**
     This virus suppresses rbc production and maturation. This may lead to red cell aplasia or chronic anemia.
   - **CMV**
     This virus is present in donor lymphocytes. Leucocyte reduced blood or CMV negative units are indicated for immune suppressed patients, neonates, and transplant recipients.

2. **Bacterial** e.g., gram-negative organisms such as yersinia and pseudomonas
   - Lyme disease, syphilis
   - Coagulase-ve staph (in platelets)
   - S/S – Septicemia, DIC, shock, etc.

3. **Parasites** e.g., Malaria, Babesiosis, Chagas disease

Table 114-3. Risks of Transfusion Complications

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Frequency, Episodes:Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute hemolytic</td>
<td>1:12,000</td>
</tr>
<tr>
<td>Fatal hemolytic</td>
<td>1:100,000</td>
</tr>
<tr>
<td>Anaphylactic</td>
<td>1:150,000</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>1:66,000</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1:103,000</td>
</tr>
<tr>
<td>HIV-1</td>
<td>1:676,000</td>
</tr>
<tr>
<td>HIV-2</td>
<td>None reported</td>
</tr>
<tr>
<td>HTLV-I and –II</td>
<td>1:641,000</td>
</tr>
<tr>
<td>Malaria</td>
<td>1:4,000,000</td>
</tr>
<tr>
<td>Other complications</td>
<td></td>
</tr>
<tr>
<td>RBC allosensitization</td>
<td>1:100</td>
</tr>
<tr>
<td>HLA allosensitization</td>
<td>1:10</td>
</tr>
<tr>
<td>Graft-versus-host diseases</td>
<td>Rare</td>
</tr>
</tbody>
</table>

*Infectious agents rarely associated with transfusion, theoretically possible or of unknown risk include:
- Hepatitis A virus
- Parvovirus B-19
- Babesia microti (babesiosis)
- Borrelia burgdorferi (lyme disease)
- Trypanosoma cruzi (Chagas’ disease)
- Treponema pallidum

|NOTE:| FNHTR, febrile nonhemolytic transfusion reaction; TRALI, transfusion-related acute lung injury; HTLV, human T lymphotropic virus; RBC, red blood cell. |
APHERESIS

Therapeutic apheresis is the removal of blood and subsequent separation of blood components (outside the body) and return of selective blood components to the individual.

1. **Plasmapheresis** (plasma exchange)
   Plasma is separated from the cellular blood components by apheresis instrument. The cells are returned to the patient and plasma is discarded.
   e.g., used to remove antibodies, immune complexes, paraproteins and protein-bound toxins.

2. **Cytapheresis**
   It is useful in removing one of the cellular elements of blood when present in abnormally high amounts causing “hyperviscosity syndrome” and stasis obstruction of microcirculation.
   e.g., leukemia, thrombocythemia

3. **Stem Cell Collections**

4. **Rbc Exchange** e.g., sickle cell anemia, neoplasia, etc.

ALTERNATIVES TO TRANSFUSION

– 0₂ carrying blood substitutes such as perfluorocarbons and aggregated Hb solution are available.
– Hematopoietic growth factors are also used, e.g., GM-CSF, Erythropoietin, and thrombopoietin.

REFERENCES

3. Lab Medicine: Clinical Pathology in the Practice of Medicine, by M. Lapasota, M.D., Ph.D., Chapter 12, pages 325-344.
**Case history**

A 65-year old 50 kg woman is seen for increased fatigue, weakness, and dyspnea on exertion. She had three uncomplicated pregnancies. She had no prior medical problems.

**Vital signs**
- Pulse: 106 bpm
- Blood Pressure: 110/76 mmHg sitting and supine
- Respiration: 24/min

**Physical Examination**
- Pallor; flow murmur; otherwise normal

**Laboratory**
- Hgb: 6.5 g/dl
- Hct: 19%
- Hypochromic, microcytic erythrocytes;
- Stools positive for occult blood
- Carcinembryonic antigen positive
- Serum electrolytes, liver enzymes, serum protein, BUN, serum glucose, bilirubin and coagulation tests all normal

**Radiology**
- Ascending colon tumor by barium enema

**Colonoscopy**
- Ascending colon tumor, biopsy proven adenocarcinoma

**Metastatic workup**
- Negative

**Questions**

1. Are this patient’s symptoms, signs, lab results and radiologic findings related? How?
   a. Is the patient anemic?
   b. What type of anemia? Differential of this type of anemia?
   c. What is the presumed patient diagnosis?
   d. What is your recommendation for therapy?

2. Is correction of Hgb deficit necessary? Why? (Criteria?) How? (Choose one or more)
   a. Transfuse three units of whole blood
   b. Initiate iron therapy
   c. Initiate vitamin B12 therapy
   d. Transfuse three units of packed red blood cells
   e. Transfuse three units of leukocyte poor packed red blood cells
   f. Initiate folate therapy
   g. Transfuse three units of frozen/thawed deglycerolized red blood cells
3. If you choose to transfuse this patient, which of the following orders would you write?
   
   a. Infuse components through a blood warmer
   b. Decrease viscosity of the component by adding lactated Ringer’s solution
   c. Infuse each component over four hours
   d. Decrease the viscosity of the component by adding no more than 50-100 ml of 0.9% (normal) saline
   e. Infuse components through a microaggregate filter
   f. Decrease viscosity of the component by adding 5% dextrose in water (D5W)
   g. Add penicillin to units to prevent bacterial overgrowth.

4. Your patient is taken to surgery for resection of the colon tumor. Which of the following options for intraoperative blood transfusion would you use?
   
   a. Allogeneic packed red blood cells
   b. Intraoperative blood salvage
   c. Intraoperative hemodilution
   d. Preoperative donation of autologous units

5. Resection of the colon tumor was completed without complication and with minimal blood loss. No intraoperative transfusion was needed. Postoperatively, the patient stabilized with a Hgb of 10 g/dl and Hct of 30%. Seven days postoperatively the patient becomes icteric. She has no other symptoms. Which of the following would you order?
   
   a. Hgb/Hct
   b. Serum electrolytes
   c. Serum bilirubin
   d. Urinary bilirubin
   e. Liver enzymes (ALT/SGPT, AST/SGOT, LD, Alkaline phosphatase)
   f. Serum glucose
   g. Direct and indirect antiglobulin tests
   h. Liver biopsy
   i. HBsAg (Hepatitis B surface antigen)
   j. HCV (Hepatitis C antibody)
   k. HAV-IgM (Hepatitis A-IgM)

6. With this information (to be given in class) what is your diagnosis of this patient’s icterus? Which are proper management options?
   
   a. Prednisone 60 mg daily
   b. Monitor Hct/Hgb
   c. Transfuse two units of whole blood
   d. Monitor patient’s urine output and renal function
   e. Cyclophosphamide 100 mg daily
   f. Infuse 0.9% (normal) saline at 100 ml/hr
   g. Monitor PT/aPTT
7. Nine weeks following surgery the patient returns complaining of fatigue and a return of icterus. Which of the following do you now order?

a. Hgb/Hct  
b. Bilirubin, serum and urine  
c. AST/SGOT, ALT/SGPT, LD and alkaline phosphatase  
d. Serum electrolytes  
e. HBsAg  
f. Liver scan  
g. HCV  
h. HAV-IgM  
i. CEA

8. With the above information (given in class) what is your diagnosis? What are your next steps?

a. Liver biopsy  
b. Administer immune serum globulin (ISG)  
c. Follow patient’s course and notify transfusion service  
d. Administer hepatitis B immune globulin (HBIG)  
e. Initiate penicillin therapy  
f. Monotherapy and interferon alpha  
g. Combination therapy with interferon alpha and ribavirin  
h. Monotherapy with PEG interferon alpha  
i. Combination therapy with PEG interferon alpha and ribavirin  
j. Monitor ALT/SGPT  
k. Monitor HCV RNA

9. What are the complications of this disease? What is this patient’s prognosis?
Medical Progress

TRANSFUSION MEDICINE
First of Two Parts

BLOOD TRANSFUSION

LAWRENCE T. GOODNOUGH, M.D.,
MARK E. BRECHER, M.D., MICHAEL H. KANTER, M.D.,
AND JAMES P. AU Ù BUCHON, M.D.

Blood transfusion and blood conservation (techniques or strategies to avoid the need for blood) are complementary activities that constitute the clinical arena of transfusion medicine. Recent improvements in the safety of the blood supply and the increasing costs associated with transfusion therapies have led to a reevaluation of the clinical practices of blood transfusion and blood conservation. Among the issues that have been reevaluated are the threshold for transfusion at which the benefits outweigh the risks and the identification of patients most likely to benefit from blood conservation. This review summarizes recent developments in transfusion medicine that have affected the clinical practices of blood transfusion and blood conservation and is intended to bring these issues into focus for physicians practicing in an era in which managed care is increasing.

TRENDS IN BLOOD USE AND COLLECTION

Issues concerning the safety of the blood supply1 in the past 15 years have been associated with changes in blood use. As summarized in Table 1, approximately 10 million red-cell units were transfused in the United States in 1980, with the number peaking at nearly 12.2 million units in 1986 and subsequently declining to 11.4 million units in 1997.2-5 However, the decline in the use of red-cell transfusions is even greater if the growth and aging of the population in the United States during this period are taken into account.

Trends in the collection of blood have reflected the same patterns noted for blood use. The blood supply in the United States totaled nearly 14 million units in 1986 and subsequently declined to 12.5 million units in 1997 (Table 2). The surplus of 1 million red-cell units (representing 8.6 percent of the total supply) in 1997, however, is misleading. In 1997, one third of the blood units collected from autologous donations (in which the patient's own blood is collected before surgery for possible use during or after surgery) was discarded, whereas only 7.4 percent of the units collected from allogeneic (volunteer and directed) donors was discarded. In addition, because blood group O (the blood group that can be transfused into any recipient regardless of the recipient's blood group) is highly desirable in situations requiring emergency transfusion, this blood is habitually in short supply. Nevertheless, the decline in the use of blood has allowed the United States to become less dependent on blood imported from the European Union, so that such blood now makes up less than 2 percent of the total blood supply. However, the predicted doubling of the proportion of the U.S. population that is over the age of 65 by the year 2050 will result in substantial demands on the blood supply in the future.6

Donor trends have changed appreciably since the 1970s. The rates of blood collection (the number of units collected per 1000 persons from 18 to 65 years of age) peaked in 1987 and declined by 9.3 percent from 1989 to 1994.54 Factors contributing to this decline include a reluctance to donate because of the misconception that the human immunodeficiency virus (HIV) can be transmitted by the process of blood donation,58 along with loss of blood donors because of enhanced screening and testing procedures. An estimated 500,000 donors are disqualified each year because of positive test results, representing over 8 percent of all blood units collected in 1994.57

Until recently, the decline in the number of voluntary donors has been offset by the increase in interest in autologous blood donation before elective surgery and directed donations. The percentage of total donations represented by autologous donations in the United States increased by a factor of more than 30, from only 0.25 percent of total donations in 19805 to 8.5 percent of total donations in 1992.4 Directed donations accounted for an additional 2 to 3 percent of all blood collected from 1989 to 1994.55 Together, these specialized blood units represented
TABLE 1. TRENDS IN THE USE OF BLOOD IN THE UNITED STATES, 1980–1997.*

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Voluntary donations</td>
<td>11,534 (95.0)</td>
<td>10,605 (93.4)</td>
<td>10,520 (94.7)</td>
<td>10,973 (95.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autologous donations</td>
<td>669 (3.1)</td>
<td>566 (5.0)</td>
<td>482 (4.3)</td>
<td>421 (3.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Directed donations</td>
<td>156 (1.3)</td>
<td>136 (1.3)</td>
<td>105 (0.9)</td>
<td>82 (0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>12,359</td>
<td>12,249</td>
<td>11,157</td>
<td>11,476</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data were adapted from Surgeon et al. and Wallace et al. with the permission of the publisher. Because of rounding, percentages may not total 100.
†The figures do not include units transfused to children. Data were obtained from the Blood Data Resource Center, courtesy of the American Association of Blood Banks.
§In autologous donations, blood is collected from a patient before surgery for possible use during or after surgery.
¶Directed donations are units donated for a specified recipient.

TABLE 2. TRENDS IN THE COLLECTION OF BLOOD IN THE UNITED STATES, 1980–1997.*

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Allogeneic donations — thousands of units (% of total)</td>
<td>11,146</td>
<td>13,601</td>
<td>13,574</td>
<td>12,677</td>
<td>12,327</td>
<td>11,938</td>
</tr>
<tr>
<td>Blood centers</td>
<td>9,675 (86.8)</td>
<td>12,054 (88.6)</td>
<td>11,925 (87.9)</td>
<td>11,480 (90.6)</td>
<td>11,328 (91.9)</td>
<td>11,246 (94.2)</td>
</tr>
<tr>
<td>Hospitals</td>
<td>1,207 (10.8)</td>
<td>1,312 (9.6)</td>
<td>1,256 (10.0)</td>
<td>997 (7.8)</td>
<td>779 (6.3)</td>
<td>692 (5.8)</td>
</tr>
<tr>
<td>European Union</td>
<td>266 (2.4)</td>
<td>235 (1.7)</td>
<td>285 (2.1)</td>
<td>206 (1.6)</td>
<td>220 (1.8)</td>
<td>NA</td>
</tr>
<tr>
<td>Autologous donations — thousands of units</td>
<td>28</td>
<td>206</td>
<td>655</td>
<td>1,117</td>
<td>1,015</td>
<td>611</td>
</tr>
<tr>
<td>Total — thousands of units</td>
<td>11,174</td>
<td>13,807</td>
<td>13,554</td>
<td>13,169</td>
<td>12,988</td>
<td>12,550</td>
</tr>
<tr>
<td>Percentage of units not transfused</td>
<td>11.1</td>
<td>11.9</td>
<td>11.0</td>
<td>14.1</td>
<td>14.0</td>
<td>8.6</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, data were adapted from Surgeon et al. and Wallace et al. with the permission of the publisher. Not available.
†Allogeneic donations consisted of voluntary and directed donations. In autologous donations, blood is collected from patients before surgery for possible use during or after surgery.
| Data were obtained from the Blood Data Resource Center, courtesy of the American Association of Blood Banks.
§This value has been adjusted for the number of units rejected after testing.

over 6 percent of all blood transfused in 1992. Since then, the contribution of these specialized blood products to the total has declined.

The percentage of the allogeneic blood collected by blood centers (American Red Cross and America’s Blood Centers) increased from 86.8 percent in 1980 to 91.9 percent in 1994, while the contribution from hospital collection facilities declined from 10.8 percent to 6.3 percent in this period (Table 2). Regional blood centers have also successfully adapted their charter for the generation of a national blood supply from volunteer donors to accommodate consumer (patient)-driven requests for blood units from specialized sources.

In a national health survey conducted in 1993, 46 percent of the population that was more than 18 years of age had donated blood at some time; however, only 5.4 percent had actually donated during the year of the survey. Persons who donate blood repeatedly are desirable because they are more easily persuaded to donate and have been repeatedly screened for risk factors for infectious diseases. Although an increasing proportion of donors are women, they are less likely than men to become regular donors, perhaps because of iron-restricted erythropoiesis. Members of minority groups also appear less likely to become regular donors. Persons over 65 years of age are now donating at some blood centers without any clinically significant complications, and this group represents an important and growing resource for blood.

RISKS OF BLOOD TRANSFUSION

When it was discovered in 1982 that HIV infection could be transmitted by blood transfusion, the rates of disease transmission could be calculated simply by following transfusion recipients over time. Since the current rates of transmission of viral infections are too low to measure, mathematical models...
The New England Journal of Medicine

TABLE 3. RISKS OF BLOOD TRANSFUSION.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Estimated Frequency</th>
<th>Deaths per Million Units</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viral</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>1</td>
<td>0</td>
<td>Dodd26</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>7–32</td>
<td>0.014</td>
<td>Schreiber et al.7</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>1–26</td>
<td>0.05–17</td>
<td>Schreiber et al.7</td>
</tr>
<tr>
<td>HIV</td>
<td>0.4–5</td>
<td>0.5–5</td>
<td>Schreiber et al.7</td>
</tr>
<tr>
<td>HTLV types I and II</td>
<td>0.5–4</td>
<td>0</td>
<td>Schreiber et al.7</td>
</tr>
<tr>
<td>Parvovirus B1</td>
<td>10</td>
<td>0</td>
<td>Dodd26</td>
</tr>
<tr>
<td>Bacterial contamination</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red cells</td>
<td>2</td>
<td>0.1–0.25</td>
<td>Dodd,37 Sazama34</td>
</tr>
<tr>
<td>Platelets</td>
<td>83</td>
<td>21</td>
<td>Dodd26</td>
</tr>
<tr>
<td>Acute hemolytic reactions</td>
<td>1–4</td>
<td>0.67</td>
<td>Sazama,37 Linden et al.36</td>
</tr>
<tr>
<td>Delayed hemolytic reactions</td>
<td>1000</td>
<td>0.4</td>
<td>Sazama,37 Linden et al.36</td>
</tr>
<tr>
<td>Transfusion-related acute lung injury</td>
<td>200</td>
<td>0.2</td>
<td>Linden et al.37, Popovsky and Moore38</td>
</tr>
</tbody>
</table>

*HIV denotes human immunodeficiency virus, and HTLV human T-cell lymphotropic virus.

are now needed to estimate the risks of blood transfusion. The models have been used to estimate the risks of transmission of HIV, hepatitis C virus (HCV), hepatitis B virus (HBV), and human T-cell lymphotropic virus types I and II (HTLV-I and HTLV-II) and are based on the fact that disease transmission is thought to occur primarily in the window period (the period soon after infection during which a blood donor is infectious but screening tests will be negative). It is also assumed that the timing of donation is independent of the time of infection; that the rate of transmission is close to 100 percent; and that laboratory error, infections due to variant viral strains that are not detectable by current tests, and infections characterized by a chronic, immunologically silent state do not occur. Models also disregard the fact that because of underlying disease, patients who receive transfusions have 1-year and 10-year mortality rates of about 24 percent and 52 percent, respectively, and may not live long enough for transfusion-transmitted disease to develop.22 The estimates of the window periods are based on relatively small numbers of persons and have wide confidence intervals, with some uncertainty in the rates of transfusion-related transmission.37

Nevertheless, the estimated risks of transfusion-transmitted diseases are lower than ever before and are listed in Table 3. These risks are expected to decrease even further when donors are screened by polymerase-chain-reaction assays,21 which should further shorten the window periods.

Transmission of HIV

The first descriptions of transfusion-associated HIV infection occurred in late 1982 and early 1983.45,52 In 1983 the Public Health Service recommended that persons at increased risk for HIV infection should not donate blood.23 Blood banks also began to ask potential donors about specific types of high-risk behavior24,25 and to give donors the opportunity to specify that their blood not be used after donation.26 Even before screening for antibodies to HIV was implemented, these measures resulted in an impressive decrease in transfusion-associated HIV infections (Fig. 1).27 After the implementation of HIV antibody testing in March 1985,28 only about 5 cases of transfusion-associated HIV infection per year were reported to the Centers for Disease Control and Prevention (CDC) during the subsequent five years, as compared with reports of 714 cases in 1984.29

The introduction of an additional test for antibodies to HIV type 2 has had only a small effect in the United States, since of 74 million donations tested only 3 positive donors were identified.30 Concern that HIV type 1 group O serotypes may be missed by current screening tests was aroused after the first case of infection was reported in the United States; most such infections have been reported in West Africa and France.31 In the United States, none of 1072 stored serum samples (which included some from high-risk persons) were positive for HIV type 1 group O infection.32

To decrease the risk of transfusion-transmitted HIV disease further, in late 1995 blood banks began to test donors for p24 antigen.33 In a little more than a year of screening of approximately 6 million donations, only 2 positive blood donors were identified (both were positive for p24 antigen but negative for antibodies to HIV).
Figure 1. The Risks of Transfusion-Related Transmission of Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), and Hepatitis C Virus (HCV) in the United States.

Each unit represents exposure to one donor. The risk of each of these infections has declined dramatically since 1983, the year the criteria for donor screening were changed; at that time the prevalence of HIV infection among donors was approximately 1 percent. Further declines have resulted from the implementation of testing of donor blood for antibodies to HIV beginning in 1985; surrogate testing for non-A, non-B hepatitis beginning in 1986–1987; testing for antibodies to HCV beginning in 1990; and testing for HIV p24 antigen beginning in late 1995. Adapted from Aubuchon et al.3 with the permission of the publisher.

Transmission of HBV and HBC

The labeling of blood from paid donors beginning in 1972 and the implementation of third-generation screening tests for hepatitis B surface antigen in 1975 led to a marked reduction in transfusion-transmitted HBV infection (Fig. 1), so that it now accounts for only about 10 percent of all cases of post-transfusion hepatitis.34 It is likely that further reductions in the rates will occur as vaccination against HBV becomes more widespread. Although acute disease develops in about 35 percent of persons infected with the virus, chronic infections develop in only 1 to 10 percent of patients.35

A reduction in the rates of non-A, non-B post-transfusion hepatitis occurred when efforts to exclude potential HIV-positive donors were implemented36 and again when donors began to be tested for surrogate markers of infection — alanine aminotransferase (an indicator of acute liver inflammation) and antibody to hepatitis B core antigen (an indicator of previous HBV infection).37 The risk of transmission of non-A, non-B hepatitis was greatly reduced after discovery of HCV and the implementation of a test for HCV antibody.38,39 The estimated risk of transfusion-transmitted HCV is now 1 in 103,000 transfusions.40 However, if one considers the unlikely possibility of a chronic, immunologically silent state of infection, the risk of HCV may be as high as 1 in
Nevertheless, although blood transfusions accounted for a substantial proportion of HCV infections that were acquired more than 10 years ago, it is now a rare cause of infection. The importance of post-transfusion HCV infection is that 85 percent of infections become chronic, 20 percent lead to cirrhosis, and 1 to 5 percent lead to hepatocellular carcinoma; the combined mortality from cirrhosis and hepatocellular carcinoma is 14.5 percent over a period ranging from 21 to 28 years.

**Transmission of Other Viruses**

The prevalence of hepatitis G viremia among U.S. blood donors is 1 to 2 percent. Although the virus can be transmitted by transfusion, there is no convincing evidence that it is particularly hepatotropic or causes disease. Currently, there is no approved test for donor screening, and there is no evidence that implementation of such a test would provide any benefit.

Transmission of hepatitis A virus by blood transfusion has been estimated to occur in the case of 1 in 1 million units. The absence of a chronic carrier state and the presence of symptoms that would rule out blood donation during the brief viremic phase of the illness explain why hepatitis A is so uncommonly associated with blood transfusion.

The risk of transfusion-related transmission of parvovirus B19 is quite uncertain, since it depends on the prevalence in blood donors, which is highly variable from year to year. Infection is generally not clinically significant except in certain populations such as pregnant women (in whom hydrodrops fetalis may develop), patients with hemolytic anemia (in whom aplastic crises may develop), and immunocompromised patients (in whom chronic aplastic anemia may develop).

Infection will develop in 20 to 60 percent of recipients of blood infected by HTLV-I or HTLV-II. The rate of transmission is affected by the length of time that blood has been stored and by the number of white cells in the unit. Blood that has been stored for more than 14 days and noncellular blood products such as cryoprecipitate and fresh-frozen plasma do not appear to be infectious. The risk of transfusion-related HTLV-I and HTLV-II infection listed in Table 2 does not account for the inefficient transmission of the virus, but it may be falsely low because an immunologically silent state of infection may exist. Myelopathy can occur in persons infected with HTLV-I or HTLV-II; one case of adult T-cell leukemia has been reported after transfusion-acquired disease. In 1988, a first-generation HTLV test was licensed for use in the screening of blood donors in the United States. Because these tests were able to detect only 46 to 91 percent of HTLV-II infections, use of a separate test for HTLV-II was recently implemented.

Advances in our ability to keep the blood supply safe from viral diseases now mean that, currently, deaths related to blood transfusion result as much from other risks, such as bacterial contamination, hemolytic reactions to transfusion, and transfusion-related acute lung injury, as from transmission of viral disease.

**Hemolytic Reactions**

Despite advances in our understanding of red-cell antigens and their clinical importance, fatal acute hemolytic reactions to transfusion continue to occur in the range of 1 in 250,000 to 1 in 1 million transfusions. Approximately half of all deaths from acute hemolytic reactions are caused by ABO incompatibility as a result of administrative errors. These most often occur outside the laboratory and are related to mismatching of the patient and the blood unit. Perhaps as a result of increased vigilance regarding the identification of patients and blood units, the number of reported deaths from ABO-incompatible hemolytic reactions has declined recently.

In addition, approximately 1 in 1000 patients has clinical manifestations of a delayed reaction to transfusion and 1 in 260,000 patients has an overt hemolytic reaction because he or she has antibodies to minor red-cell antigens that were not detected by a routine antibody assay before transfusion. The reaction rates are much higher in populations at increased risk, such as patients with sickle cell disease. Six deaths from delayed hemolytic reactions were reported in a 1-year period in the United States and have accounted for 10 percent of all deaths due to red-cell transfusion over a 10-year period.

**Contamination of Red Cells**

The organism most commonly implicated in bacterial contamination of red cells is *Yersinia enterocolitica*. Other gram-negative organisms have also been described. Bacterial contamination of blood units is directly related to the length of storage, but yersinia red-cell sepsis has been reported after the transfusion of red cells that had been stored for as few as 7 to 14 days. In the United States, a contamination rate of less than 1 per million red-cell units has been reported. From January 1987 to February 1996, 20 recipients of yersinia-infected red cells in 14 states were reported to the CDC, 12 of whom died. Clinical symptoms typically begin during transfusion; the median time to death was only 25 hours in the 12 patients who died. A recent report from New Zealand indicated that the rate of contamination by *Y. enterocolitica* was 1 per 65,000 red-cell units transfused, with a fatality rate of 1 per 104,000. Unrecognized cases, underreporting of cases, and regional variations may account for the differences in incidence. Red-cell units with gross
Contamination may in some cases be identified by comparing the color of the blood in the blood bag with the color of blood in the attached, segmented tubing; the blood in the bag will appear darker as a result of hemolysis and decreased oxygen supply.\(^6\)

Contamination of Platelets

The risk of platelet-related sepsis is estimated to be 1 in 12,000 but is greater with a transfusion of pooled platelet concentrates from multiple donors than with transfusion from a platelet unit obtained by apheresis from a single donor.\(^6\) Because of the increasing risk of bacterial overgrowth with time, the shelf life of platelets stored at 20 to 24°C is five days. In descending order, the organisms most commonly implicated in deaths (as reported to the Food and Drug Administration) are *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Staph. epidermidis*.\(^6\)

The clinical presentation of patients with platelet-related sepsis is more variable than that of patients infected by transfusion of bacterially contaminated red cells\(^6\) and can range from mild fever (which may be indistinguishable from febrile, nonhemolytic transfusion reactions) to acute sepsis, hypotension, and death. Sepsis due to the transfusion of contaminated platelets is underrecognized in part because the organisms found contaminating platelets are frequently the same as those implicated in catheter-related sepsis. The overall mortality rate for platelet-associated sepsis reported in the literature is 26 percent.\(^6\)

To date, there is no widely accepted test, method, or device to identify bacterially contaminated blood products. A promising approach is the use of psoralens and ultraviolet light to produce not only nonimmunogenic but also sterile blood products\(^6\); this method is discussed in part two of this article. In any patient in whom fever develops within six hours after platelet infusion, the possibility of bacterial contamination of the component should be examined and empirical antibiotic therapy should be considered.

Transfusion-Related Acute Lung Injury

Transfusion-related acute lung injury is an acute respiratory distress syndrome that occurs within four hours after transfusion and is characterized by dyspnea and hypoxia due to noncardiogenic pulmonary edema. Although the actual incidence is not well known and its occurrence is almost certainly underreported, its estimated frequency is approximately 1 in 5000 transfusions.\(^6\) Transfusion-related acute lung injury most likely results from several mechanisms. In some cases, blood-donor antibodies with HLA or neutrophil antigenic specificity react with the recipient’s neutrophils, leading to increased permeability of the pulmonary microcirculation.

Most recently, reactive lipid products from donor-blood-cell membranes that arise during the storage of blood products have been implicated in the pathophysiology of transfusion-related acute lung injury.\(^7\) Such substances are capable of neutrophil priming, with subsequent damage to pulmonary-capillary endothelium in the recipient, particularly in the setting of sepsis. As in other causes of the acute respiratory distress syndrome, therapy is supportive; at least 90 percent of patients with transfusion-related acute lung injury recover. The discordance between the estimated frequency of the disease\(^8\) and the actual mortality\(^9\) reported in Table 3 underscores the fact that this complication may evade clinical recognition, leading to the underreporting of deaths.

Transfusion-Mediated Immunomodulation

The immunosuppressive effect of allogeneic blood is related to exposure to leukocytes and subsequent sensitization and has been found to be clinically important in patients who are undergoing renal transplantation\(^2\) and in women who have multiple miscarriages.\(^2\) However, whether exposure to allogeneic blood causes clinically significant immunosuppression in other persons remains a subject of debate. A number of observational, retrospective reports have described an association between exposure to allogeneic blood and both earlier-than-expected recurrences of cancer and increased rates of postoperative infection.\(^8\)

Only a few prospective studies have attempted to clarify the potential immunomodulatory effects of allogeneic transfusion. A study of 120 patients undergoing curative resection of colorectal carcinoma failed to demonstrate a difference in either relapse-free survival or the prevalence of serious postoperative infections between patients who were randomly assigned to allogeneic transfusion and those assigned to autologous transfusion; however, the rate of all infections was three times as high in the group receiving allogeneic blood than in the other group.\(^2\) In a similar study of 423 patients, there was no difference in relapse-free survival or infectious complications between the two groups.\(^2\) Houbiers et al. compared the transfusion of leukocyte-reduced components (average leukocyte content, 0.2×10\(^6\)) with the transfusion of red cells from which the buffy coats had been removed (average leukocyte content, approximately 30 percent of the number in whole blood) and found no difference in the risk of recurrence of cancer after colorectal surgery.\(^7\) Van de Watering et al. found that leukoreduction had no effect on the rates of postoperative infection in patients who had undergone cardiac surgery, although the 60-day mortality rate in this group was approximately half that in the control group (3.4 percent vs. 7.8 percent).\(^7\) Jensen et al., however, noted markedly lower infection rates (by a factor of 10) after colorectal surgery when leukoreduced units were used for transfusion.\(^7\)

Although these prospective studies suffer from one
or more methodologic or statistical difficulties, in aggregate, they suggest that exposure to allogeneic blood increases the risks of a recurrence of cancer and postoperative infection. The recent pronouncement by the Blood Products Advisory Committee of the Food and Drug Administration that the benefits of universal leukocyte reduction of cellular blood components outweigh the risks is controversial. The annual cost of universal leukodepletion is estimated to exceed 5500 million and will need to be factored into any decision. Although the available data certainly raise questions about the immunosuppressive effect of allogeneic blood transfusion, they do not allow a definitive conclusion to be drawn as to its clinical importance and, consequently, as to whether changes in practice are required.

INDICATIONS FOR TRANSFUSION

Utilization Review

Audits of a facility's transfusion practices can improve the efficiency and appropriateness of transfusion if they are performed in a timely manner and if the results are communicated to physicians who order transfusions for their patients. Audits of the use of plasma and platelet products are particularly amenable to this approach and can reduce the use of blood components by up to 50 percent. However, a recent multihospital study found that a retrospective utilization review did not reduce the use of red-cell transfusions.

This lack of success may be a consequence of several factors. First, it is difficult to evaluate the appropriateness of the use of transfusion in patients with hemorrhage who are seen in emergency rooms and trauma units, operating rooms, and intensive care units. Second, some studies have found that fewer than 5 percent of red-cell transfusions are unjustified. One reason for this low rate is the use of clinical indicators for transfusion that are too generous. It is difficult to improve transfusion practices if over 95 percent of transfusions are found to be justified. Third, there is often no clearly documented information in a medical chart that explains why a transfusion was administered. In only two thirds of cases in which postoperative transfusions are administered on the day of surgery is blood loss or a change in vital signs noted in the medical record, and the rationale for transfusion is documented in fewer than a third of cases.

Intensive Care

A 1995 study of transfusion practices in 4875 consecutive patients who were admitted to six Canadian tertiary-level intensive care units found that 28 percent of all patients received red-cell transfusions, but the number of transfusions ranged from 0.82 to 1.08 per patient-day among the institutions. The most frequent reasons for administering red cells were acute bleeding (35 percent of patients) and the augmentation of oxygen delivery (25 percent of patients), rather than the patient's hemoglobin concentration. However, transfusion may not augment oxygen delivery in such patients. One study found that the transfusion of stored blood for up to six hours after infusion did not affect oxygen delivery in patients with sepsis.

In a multi-institutional Canadian study reported in this issue of the Journal by Hébert et al., critically ill patients with normovolemia were to receive red-cell transfusions when the hemoglobin level dropped below 7.0 g per deciliter, with hemoglobin levels maintained in the range of 7.0 to 9.0 g per deciliter, and 420 patients to receive transfusions when the hemoglobin level dropped below 10.0 g per deciliter, with hemoglobin levels maintained in the range of 10.0 to 12.0 g per deciliter. The 30-day mortality rates were similar in the two groups (18.7 percent vs. 23.3 percent, P = 0.11), indicating that a transfusion threshold as low as 7.0 g per deciliter is as safe as and possibly superior to a higher transfusion threshold of 10.0 g per deciliter in critically ill patients. Clearly, more data are needed to determine when transfusion in the intensive care unit is beneficial.

Surgery

The discharge hematocrit levels of patients who underwent orthopedic surgery ranged from 31 to 34 percent in the mid-1980s, suggesting that perioperative anemia was being treated too aggressively with transfusion. In the past 15 to 20 years, however, the overall rate of transfusions for patients undergoing hip and knee arthroplasty has declined by 15 to 35 percent. The patient's sex has been found to influence the outcome of transfusion in such patients and has been attributed to the fact that physicians use the same hematocrit value as a threshold for transfusion for both women and men, without taking into account that women have lower hematocrit levels. Two studies found substantial variability in the use of red-cell transfusions for patients undergoing total hip and knee arthroplasty, and the variability was attributed to the lack of clearly defined criteria for transfusion and to hospital-specific differences in the availability of autologous blood. A large, retrospective study of elderly patients who were undergoing hip repair found that the use of perioperative transfusion in patients with hemoglobin levels as low as 8.0 g per deciliter did not appear to influence 30-day or 90-day mortality, suggesting that this level is safe in patients who undergo orthopedic surgery.

There is considerable variation in transfusion practices among institutions with respect to patients who undergo cardiac surgery. A multicenter audit of 18 institutions demonstrated a wide range in the outcomes of allogeneic transfusions among patients.
who underwent primary coronary-artery bypass grafting. Two subsequent studies reported similar findings. The variability in transfusion outcomes in these patients is attributable to differences in training that are specific to hospitals and physicians rather than to differences in patient populations.

Guidelines for Transfusion

Guidelines for blood transfusion have been issued by several organizations including a National Institutes of Health consensus conference on perioperative transfusion of red cells, the American College of Physicians, and the Canadian Medical Association. These guidelines recommend that blood not be transfused prophylactically and suggest that in patients who are not critically ill, the threshold for transfusion should be a hemoglobin level of 7.0 to 8.0 g per deciliter. Adherence to these guidelines has raised questions about whether transfusion is now underused. In a recent study in which 84 patients who were undergoing repair of hip fracture were randomly assigned to receive transfusions either at a predetermined threshold (a hemoglobin level of 10.0 g per deciliter) or only if symptoms of anemia occurred (with the lower limit of the hemoglobin level set at 8.0 g per deciliter), the respective mortality rates at 60 days were 4.8 percent and 11.9 percent. Because of the small numbers of patients in the study, one should be cautious about drawing definitive conclusions regarding thresholds for transfusion.

Silent perioperative myocardial ischemia has been observed in patients undergoing noncardiac surgery as well as cardiac surgery. Hemoglobin levels ranging from 6.0 g to 10.0 g per deciliter — a range in which indicators other than the hemoglobin level may identify patients who may benefit from blood — therefore need to be the most closely scrutinized. A recent study of elderly patients who were undergoing elective, noncardiac surgery found that intraoperative or postoperative myocardial ischemia was more likely to occur in patients with hematocrits below 28 percent, particularly in the presence of tachycardia. In a prospective, randomized trial of two transfusion strategies in patients who were undergoing cardiac surgery, no significant differences in postoperative exercise endurance were found between patients who received transfusions in order to maintain a hematocrit of 32 percent and patients who received transfusions only if the hematocrit dropped below 25 percent. A hemoglobin level of 8.0 g per deciliter seems an appropriate threshold for transfusion in surgical patients with no risk factors for ischemia, whereas a threshold of 10.0 g of hemoglobin per deciliter can be justified for patients who are considered at risk. However, prophylactic transfusion of blood (i.e., in anticipation of blood loss) or transfusion to replace volume cannot be endorsed, particularly since studies have found that overuse of transfusion in patients undergoing cardiac surgery and critically ill patients may be associated with less favorable outcomes.

CONCLUSIONS

The use of blood transfusion has declined, in large part because of concern about the safety of the blood supply. It is unlikely that any level of hemoglobin can be used as a universal threshold for transfusion. The advent of a very safe blood supply suggests that outcomes should now be monitored to identify patients in whom transfusion may be underused in addition to identifying patients who receive unnecessary transfusions. Techniques or strategies to avoid blood transfusion will no longer be driven by the known risks of death from blood transfusion, since they are now so low that no alternative is currently as safe as a blood transfusion. Instead, blood conservation will be driven more by issues related to the costs and inventory of blood.

REFERENCES


Medical Progress

**Transfusion Medicine**

Second of Two Parts

**Blood Conservation**

Lawrence T. Goodnough, M.D.,
Mark E. Brecher, M.D., Michael H. Kanter, M.D.,
and James P. Aubuchon, M.D.

**Preoperative Autologous Donation**

Preoperative autologous donation was rarely used before the recognition that HIV could be transmitted by blood transfusion. Fifteen years ago, fewer than 5 percent of eligible patients who were scheduled for elective surgery chose autologous blood donation. When public awareness of the possibility of transfusion-transmitted HIV became widespread, however, there was concern that too few patients were choosing autologous blood donation as an option. Several states, including California, passed legislation requiring that whenever it was "reasonably" likely that transfusion would be needed, a patient should be informed of all of the options regarding and alternatives to allogeneic blood transfusion. Subsequently, the use of preoperative autologous donation increased substantially, with 50 to 75 percent of patients choosing this option before certain types of elective surgical procedures; in 1992, 1 in every 12 blood units collected in the United States was the result of autologous donation (Table 2).

Up to half the autologous blood that is collected is discarded. Reasons for the overcollection of autologous blood include local legislation, physicians' fear of legal liability, a perception that there are few or no adverse consequences to preoperative autologous donation, and an attempt to address patients' fear of contracting transfusion-transmitted diseases.

Moreover, preoperative autologous donation is used to cover the need for a range (up to 90 percent) of patients who might need blood, which results in the routine collection of more blood than is needed for the average patient. Since the use of surplus autologous units in patients other than the donor is not recommended, preoperative autologous donation is inherently wasteful. Increasing pressures to decrease the costs of medical care, along with the lack of reimbursement for preoperative autologous donation from Medicare and some private insurers, have also focused attention on the overcollection of autologous blood.

The decreased likelihood of the transmission of viruses by the transfusion of allogeneic blood has caused the practice of autologous blood donation to be reevaluated. Both autologous blood donation and transfusion are associated with risks. In one study, 1 in 16,783 autologous donations was associated with an adverse reaction severe enough to require hospitalization; this risk is 12 times as high as the risk associated with voluntary donations by healthy persons. Ischemic events have also been reported in association with but not necessarily as a result of autologous blood transfusion. The transfusion of autologous blood has many of the same complications as transfusion of allogeneic units, including the risk of bacterial contamination, hemolysis (ABO incompatibility due to administrative errors), and volume overload. Since 1992, the percentages of autologous blood collected and transfused have declined (Tables 1 and 2). Some advantages and disadvantages of autologous blood donation are summarized in Table 4.

Cost-effectiveness models also serve to illustrate the potential risks of autologous blood donation; even a very remote risk of death in patients with ischemic heart disease may entirely negate the benefits of having autologous blood available before coronary-artery bypass grafting. Key factors include the estimated postoperative life span of the patient and the likelihood of transfusion (Fig. 2). In a study of autologous blood donation before coronary-artery bypass grafting, the preoperative donation of two units was estimated to have a cost of $500,000 per quality-adjusted life-year. The risk of exposure to a hepatitis virus or to HIV has declined by at least an order of magnitude since the calculation of this estimate, and the current cost effectiveness would be significantly worse.
TABLE 4. ADVANTAGES AND DISADVANTAGES OF AUTOLOGOUS BLOOD DONATION.

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevents transfusion-transmitted disease</td>
<td>Does not eliminate the risk of bacterial contamination or volume overload</td>
</tr>
<tr>
<td>Avoids red-cell alloimmunization</td>
<td>Does not eliminate the risk of administrative error, resulting in ABO incompatibility</td>
</tr>
<tr>
<td>Supplements the blood supply</td>
<td>Costs more than allogeneic blood donation</td>
</tr>
<tr>
<td>Provides compatible blood for patients with alloimmunization</td>
<td>Results in discarding of blood that is not transfused</td>
</tr>
<tr>
<td>Prevents some adverse transfusion reactions</td>
<td>Causes perioperative anemia and increases the likelihood of transfusion</td>
</tr>
</tbody>
</table>

![Graph](image)

Figure 2. The Effect of the Likelihood of Transfusion and the Patient's Age on the Cost Effectiveness of Autologous Blood Donation before Coronary-Artery Bypass Grafting.

If two units of blood were collected preoperatively, younger patients, in whom projected postoperative survival is longer, and patients who are undergoing surgery in centers with a higher likelihood of perioperative transfusion derive more benefit from autologous blood donation. Adapted from Birkmeyer et al.\(^\text{109}\) with the permission of the publisher.

Estimates of the cost effectiveness of autologous blood donation are predicted according to known risks of transfusion. Should a new risk emerge, the estimates may become inaccurate. Similarly, should allogeneic transfusion be ultimately proved to be a cause of postoperative infection or recurrent cancer, the relative risks of allogeneic blood transfusion could change substantially.\(^\text{109}\) The use of leukoreduced allogeneic blood products might diminish these potential risks, albeit at a substantial increase in cost.

Erythropoiesis

Autologous blood donation may actually be harmful to patients. Figure 3 illustrates the effects of preoperative blood donation and the collection of two or four units of autologous blood on the preoperative hematocrit (before blood loss) and the final hematocrit on discharge from the hospital in a 70-kg patient with a blood volume of 5000 ml. In this model\(^\text{118}\) it is assumed that compensatory erythropoiesis results in the replacement of two thirds of the red cells donated.\(^\text{188}\) In the absence of autologous blood donation, the patient could sustain estimated losses of 2939 ml of blood before requiring a blood transfusion with the use of a hematocrit of 25 percent as a threshold for transfusion; however, with the preoperative collection of two or four units of blood a transfusion would be required after estimated blood losses of 2712 or 2473 ml, respectively. A study that analyzed blood transfusion in patients undergoing elective hysterectomy confirmed the accuracy of this model.\(^\text{124}\) In essence, preoperative autologous donation appears to increase the risk of postoperative anemia, as well as the likelihood of transfusion and its attendant risks (Table 3).

The degree of anemia induced by autologous blood donation varies, even though iron supplementation is routinely prescribed for patients who donate blood. This variability may be explained in part by the heterogeneity of patient populations and by differences in the timing of blood donations in relation to the date of surgery. Some studies have reported that the average decrease in the hemoglobin level was 1.0 g per deciliter per unit of autologous blood obtained (i.e., there was no compensatory erythropoiesis) before hysterectomy,\(^\text{126}\) radical prostatectomy,\(^\text{137}\) or colectomy.\(^\text{135,136}\) However, in a recent study, Kasper et al. estimated that compensatory erythropoiesis resulted in the replacement of 60 percent of the blood lost by weekly donations of three units of autologous blood over a period of three weeks.\(^\text{138}\) This rate of erythropoiesis was noted in other studies only when an aggressive strategy of phlebotomy (six units obtained over a period of three weeks) was used,\(^\text{188}\) or when intravenous iron therapy was given in addition to oral iron supplementation.\(^\text{138}\) The variability of compensatory erythropoiesis is dependent on initial iron status\(^\text{140}\) but not on the age or sex of the patient.\(^\text{141}\) Given that normal persons take many weeks to regenerate the blood lost in donation and that a lower hemoglobin level at admission is associated with an increased likelihood of transfusion, it would seem prudent to maximize the time between the last donation and the date of surgery.

Use in Managed Care

Guidelines have been published on the types of patients for whom autologous donation is most appropriate.\(^\text{142,143}\) Most commonly, the number of units of autologous blood obtained preoperatively is based on the number of units that would be crossmatched before surgery if allogeneic blood were being used.\(^\text{144}\) This approach was designed to allow the collection...
of enough autologous blood so that fewer than 10 percent of patients who were undergoing surgery would receive allogeneic blood transfusions. Not all countries adhere to this recommendation. A recent British consensus conference on autologous transfusion stated that autologous blood donation should be considered only if the likelihood of transfusion exceeds 50 percent. However, even for procedures such as joint replacement or radical prostatectomy, as much as 50 percent of autologous blood goes unused. When autologous blood is collected for procedures that seldom require transfusion, such as hysterectomy, vaginal delivery, and transurethral resection of the prostate, up to 90 percent of the units collected before these procedures go unused. In one study in a managed-care setting, the risks of autologous donation and the likelihood of transfusion were made clear to gynecologists and their patients who were scheduled to undergo hysterectomy. This approach resulted in the collection of fewer units of autologous blood, higher hematocrit levels, and fewer autologous transfusions (saving the hospital an estimated $16,000 in one year) without an increase in the rate of allogeneic transfusion.

Attempts to stratify patients according to the risk of transfusion on the basis of the baseline level of hemoglobin and the type of procedure planned have shown some promise. Using a system of points, Laroque et al. found that 80 percent of patients who were scheduled to undergo orthopedic procedures were at low risk for transfusion, so that autologous blood donation was not recommended. Algorithms that take into account the estimated blood loss and preoperative hematocrit also have the potential to identify patients at low and high risk for transfusion. One problem with these approaches is that blood losses are difficult to predict, and specific surgical procedures, even those performed by the same surgeon, can be accompanied by a wide range of blood loss.

ACUTE NORMOVOLEMIC HEMODILUTION

Acute normovolemic hemodilution entails the removal of whole blood from a patient immediately before surgery and simultaneous replacement with an acellular fluid, such as crystalloid and colloid, to maintain normovolemia. Blood is collected in standard blood bags containing anticoagulant, remains in the operating room, and is reinfused after any major loss of blood has ceased, or sooner if indicated.
percent guidelines state that acute normovolemic hemodilution should be considered when the potential surgical blood loss is likely to exceed 20 percent of the blood volume in patients who have a preoperative hemoglobin level of more than 10.0 g per deciliter and who do not have severe myocardial disease, such as moderate-to-severe left ventricular impairment, unstable angina, severe aortic stenosis, or critical left main coronary artery disease.\textsuperscript{152}

**Efficacy**

The value of hemodilution comes from the fact that the losses in red-cell volume are reduced during perioperative blood loss because of the attendant lowering of hematocrit levels preoperatively.\textsuperscript{149} Moderate hemodilution to maintain a preoperative hematocrit of 28 percent results in the preservation of 100 to 200 ml of red cells (the equivalent of one half to one unit of blood).\textsuperscript{154,156} Mathematical modeling has suggested that severe hemodilution in which the preoperative hematocrit is less than 20 percent, accompanied by substantial blood losses, would be required before the red-cell volume saved by hemodilution becomes clinically important.\textsuperscript{157}

Nevertheless, the clinical effect of acute normovolemic hemodilution is shown in Figure 4.\textsuperscript{144} Without hemodilution, an adult with an initial hematocrit of 45 percent could sustain surgical blood losses of up to 3939 ml without the need for transfusion yet have a hematocrit of at least 25 percent postoperatively. The use of hemodilution in this patient would still allow a surgical blood loss of up to 3036 ml, yet the hematocrit would remain at least 28 percent.

The aim of hemodilution is to protect patients who might have unpredictable or substantial blood losses,\textsuperscript{158} yet maintain perioperative hematocrit values that minimize the risks related to ischemia.\textsuperscript{150}

A prospective study of patients who underwent acute normovolemic hemodilution before radical prostatectomy found that 21 percent of patients received allogeneic blood; this rate is similar to the rate in patients who undergo autologous blood donation before radical prostatectomy\textsuperscript{160,161} and in patients who undergo autologous blood donation before elective orthopedic surgery.\textsuperscript{162,163} A retrospective European case–control analysis\textsuperscript{164} of hemodilution in more than 800 patients who underwent total joint arthroplasty concluded that acute normovolemic hemodilution reduced the need for allogeneic blood transfusions. The results of selected randomized, prospective studies comparing hemodilution with autologous blood donation are summarized in Table 5. Although the numbers of patients are small, there is no evidence that there is a meaningful difference in outcomes between autologous blood donation and acute normovolemic hemodilution for patients who undergo radical prostatectomy or total joint arthroplasty.

Acute normovolemic hemodilution has several advantages over autologous blood donation. First, the units procured by hemodilution require no testing, so that the costs are substantially lower than those of autologous blood donation.\textsuperscript{176} Second, since the units of blood are not removed from the operating

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**Figure 4. Maximal Allowable Blood Loss in a Patient with a Blood Volume of 5000 ml and an Initial Hematocrit of 45 Percent or 40 Percent in the Presence and Absence of Acute Normovolemic Hemodilution (ANH).**

The maximal allowable blood loss in a patient treated with acute normovolemic hemodilution can be calculated with use of the following equation: maximal blood loss = estimated blood volume × (initial hematocrit + target hematocrit - 1). The maximal allowable blood loss in a patient not treated with acute normovolemic hemodilution can be calculated with use of the following equation: maximal blood loss = estimated blood loss × ln (target hematocrit + initial hematocrit). The target hematocrit is the threshold for transfusion. Data were modified from Goodnough et al.\textsuperscript{150}
room, the possibility of an administrative error that could lead to an ABO-incompatible blood transfusion is theoretically eliminated, as is the risk of bacterial contamination. Third, blood obtained by hemodilution does not require an additional investment of time by the patient since it is done at the time of surgery, nor does it prolong the duration of surgery or anesthesia.168,170

INTRAOPERATIVE RECOVERY OF BLOOD

Intraoperative recovery of blood involves the collection and reinfusion of autologous red cells lost by a patient during surgery. Cell-washing devices can provide the equivalent of 10 units of banked blood per hour to a patient with massive bleeding. The survival of the red cells that are recovered appears to be similar to that of transfused allogeneic red cells.171 Relative contraindications include the potential for the aspiration of malignant cells, the presence of infection, and the presence of other contaminants such as amniotic or ascitic fluid in the operative field. Because washing does not completely remove bacteria from the recovered blood, intraoperative recovery should not be used if the operative field has gross bacterial contamination.152

As with other strategies of autologous blood procurement, the safety and cost effectiveness of intraoperative recovery of autologous blood should be carefully scrutinized. Four deaths related to the intraoperative recovery of blood were reported to the New York Department of Health from 1990 through 1995, for an estimated prevalence of 1 in 35,000 procedures.55 A controlled study of patients who were undergoing cardiothoracic surgery demonstrated that this approach had no benefit when transfusion requirements and clinical outcome were evaluated.152 A prospective, randomized trial of patients who were undergoing repair of abdominal aortic aneurysms also found that intraoperative recovery of blood did not result in the need for fewer blood transfusions. In the absence of cell washing, the equivalent of one unit of blood can be obtained relatively inexpensively; with the use of automated cell-washing devices, it is generally agreed that the equivalent of at least two units of blood needs to be recovered in order for the method to be cost effective.173-175 Even in the case of a patient with substantial blood losses during vascular surgery, intraoperative recovery of blood may be of value not because it reduces the requirements for blood transfusion, but because it provides blood that is less costly to obtain and immediately available in the event of rapid blood loss.

POSTOPERATIVE RECOVERY OF BLOOD

Postoperative recovery of blood involves the collection of blood from surgical drains followed by reinfusion, with or without processing. The blood recovered is dilute, is partially hemolyzed and defibrinated, and may contain high concentrations of cytokines. For these reasons, programs set an upper limit on the volume of unprocessed blood (1400 ml at one of the hospitals in which we work) that can be reinfused.

The evolution of cardiac surgery has been accompanied by considerable experience in the use of postoperative reinfusion of blood. Nevertheless, the practice of postoperative recovery and reinfusion of autologous blood varies among institutions.103-104 Prospective and controlled trials have reached disparate conclusions about the efficacy of postoperative recovery of blood from patients after cardiac surgery: at least three such studies demonstrated a lack of efficacy,176-178 whereas at least two have reported a benefit.179,180 The disparity in results may

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**Table 5. Results of Selected Prospective, Randomized Trials Comparing Acute Normovolemic Hemodilution with Autologous Blood Donation.**

<table>
<thead>
<tr>
<th>Type of Surgery and Study</th>
<th>Acute Normovolemic Hemodilution</th>
<th>Autologous Blood Donation</th>
<th>No Autologous Blood Donatjon</th>
<th>Acute Normovolemic Hemodilution</th>
<th>Autologous Blood Donation</th>
<th>No Autologous Blood Donatjon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostatectomy</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Nest et al.165</td>
<td>30</td>
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<td>-</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Monk et al.168</td>
<td>26</td>
<td>26</td>
<td>-</td>
<td>5</td>
<td>4</td>
<td>-</td>
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<tr>
<td>Orthopedic surgery</td>
<td></td>
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<tr>
<td>Lorez et al.167</td>
<td>16</td>
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<td>Goodnough et al.164</td>
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<td>White et al.169</td>
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<td>23</td>
<td>-</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

*There were no significant differences in the need for allogeneic blood transfusions between the groups assigned to acute normovolemic hemodilution and the groups assigned to autologous blood donation.*

†This group served as the control group.
be explained in part by the variability in transfusion practices among institutions. The safety and the benefit of the use of unwashed blood obtained from surgical drains after orthopedic surgery remain in question.181,182 One large group that initially found this approach to be beneficial182 subsequently reported that this costly practice is of no clinical benefit.184 Because the blood-cell volume of the fluid collected is low (hematocrit, 20 percent), the volume of red cells reinfused is often small.185 Selective use of the method in situations in which large postoperative blood losses are anticipated, such as in bilateral joint-replacement surgery, would improve the efficacy of the procedure, but such blood losses are difficult to predict.159

EMERGING DEVELOPMENTS IN TRANFUSION MEDICINE

Inactivation of Microbes in Platelet Units

The inactivation of viruses in a unit of platelets while retaining the viability and hemostatic properties of these blood cells has proved to be a formidable challenge. Inactivation of virus in units of platelets by means of exposure to psoralen derivatives followed by exposure to ultraviolet A has been intensely investigated and can greatly reduce the levels of HIV and hepatitis viruses.186 In order to limit the damage to platelets caused by irradiation, however, the process must be conducted in the absence of oxygen or in the presence of agents that remove damaging reactive intermediate compounds.187 In many systems, the proportion of plasma in the medium in which the platelets are suspended must be limited (to less than 15 percent) to prevent viruses from escaping inactivation.188

These treatments also appear to inactivate any contaminating bacteria186 and to reduce or eliminate immunomodulation due to lymphocytes.189 The potential toxicity of a viral-inactivation process that adds photoreactive dyes or other potentially carcinogenic or teratogenic compounds will require careful assessment.190 Since the current risks of blood transfusion are low, a small risk of an untoward effect of the inactivating agents could represent a larger health threat than the one that is being targeted.

Use of Plasma with Reduced Viral Infectivity

Efforts to inactivate viruses in plasma have proceeded more rapidly, and one technique is now licensed for use in the United States. Treatment of plasma with a solvent–detergent process provides a means to inactivate all viruses with lipid envelopes, including HIV and hepatitis B and C viruses.191 The process, accomplished on a commercial scale by pooling plasma from 2500 donors, yields units of standard size (200 ml) that are refrozen for distribution. The cost of a 200-ml unit of pooled plasma treated with the solvent–detergent process is two to five times as high as the cost of a 250-ml unit of untreated plasma from a single donor. The contents of the plasma appear to be unchanged except that procoagulant activity is reduced by about 15 percent and that levels of large multimers of von Willebrand factor and some other factors, including protein S, are decreased by over 50 percent.

The pooling of plasma from so many donors as part of the solvent–detergent process has aroused concern about the possible transmission of nonenveloped viruses that are not inactivated by the process (Table 6). The manufacturer and distributor have attempted to allay fears about the transmission of hepatitis A virus by documenting the presence of antibodies against this virus in their product. The transmission of parvovirus B19 is a potential problem for some transfusion recipients, such as patients with sickle cell disease or thalassemia, but it has not

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**TABLE 6. ADVANTAGES AND DISADVANTAGES OF THE USE OF POOLED PLASMA TREATED WITH A SOLVENT AND DETERGENT RATHER THAN THE USE OF PLASMA FROM A SINGLE DONOR.***

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kills viruses with lipid envelopes</td>
<td>Is ineffective against nonenveloped viruses</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>Hepatitis A virus</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>Parvovirus B19</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>TT virus</td>
</tr>
<tr>
<td>Eliminates the risk of transfusion-related acute lung injury because it dilutes the amount of donor antibody against specific HLA antigens</td>
<td>May not prevent transfusion-related acute lung injury mediated by biologically active lipids</td>
</tr>
<tr>
<td>May contain neutralizing antibodies against hepatitis A virus and parvovirus B19</td>
<td>May not contain neutralizing antibodies against unknown viruses</td>
</tr>
<tr>
<td></td>
<td>May be oversized, with few constraints, as is the case with albumin</td>
</tr>
</tbody>
</table>

*Pooled plasma is obtained from at least 2500 donors.
been reported among European recipients of plasma treated with the solvent-detergent process. However, if an HIV-like nonenveloped virus were to evolve, it could be present at an undetectably low frequency in donors (e.g., 1 in 100 million) and yet present a threat in a pooled product. The recent identification of a potential pathogen, T.T. virus, illustrates the validity of the concern about pooled blood products. This nonenveloped virus is present in 1 to 7.5 percent of blood donors in the United States and is transmissible by blood. Although it is not known to cause disease, the virus has been described in a preliminary report as present in 15 percent of patients with cryptogenic cirrhosis and in 27 percent of patients with idiopathic fulminant hepatic failure.

Other alternatives for increasing the safety of plasma through the selection of donors and various collection techniques have been proposed. Because plasma can be stored frozen for a year, units can be held in quarantine until the donor returns and is retested after a period that is longer than the window period of known viruses. The results of this test, if negative, would provide reassurance that the stored plasma unit did not contain certain infectious agents. This approach was approved in September 1998 by the Food and Drug Administration for units in which the donor is retested over a minimal period of 112 days. The costs and availability of plasma tested in this fashion are currently unknown.

Use of Red-Cell Substitutes

In recent years, there has been increasing interest in the development of red-cell substitutes. Efforts have included the development of cell-free hemoglobin solutions that approximate the oxygen-carrying and oxygen-delivery capacity of cellular hemoglobin and the development of perfluorocarbon emulsions (as synthetic oxygen carriers). The hemoglobin solutions are polymerized or cross-linked (or both) to maximize the length of time in which they are in circulation and to minimize nephrotoxicity. The potential advantages of such products include a prolonged shelf life, the fact that they can be stored at room temperature, universal biocompatibility (since ABO-blood-group testing is not necessary), and the fact that such products are subjected to viral-inactivation procedures. The disadvantages of such products include potential interference with the results of laboratory tests, their relatively short time in circulation (24 to 48 hours), and the fact that perfluorocarbons require a forced inspiratory oxygen concentration of 100 percent to be effective.

The two principal uses of red-cell substitutes currently under clinical investigation are for patients with acute trauma and patients who are undergoing surgery, with or without acute normovolemic hemodilution. The rationale for the use of red-cell substitutes with hemodilution is twofold: the cellular hemoglobin collected during hemodilution would be used to replace the hemoglobin solution or other synthetic oxygen carrier as it is eliminated, and the use of a red-cell substitute would permit more aggressive hemodilution with lower targeted cellular hemoglobin levels than would otherwise be tolerated. However, patients with preexisting anemia can be expected to derive only limited benefit from this approach, since there is less autologous cellular hemoglobin to begin with. Moreover, studies of some hemoglobin solutions that have been administered to anesthetized surgical patients in clinically relevant doses have demonstrated that the ability of hemoglobin-based oxygen carriers to increase oxygen delivery is limited by their vasoactivity. This vasoactivity is thought to be a direct effect of the free hemoglobin, since free hemoglobin has a different affinity for or proximity to nitric oxide than cellular hemoglobin.

Several of these products are in various stages of clinical development. They would most likely be used in military and trauma settings; their role in other arenas will most likely be determined by issues related to blood inventory and costs, rather than the safety of the blood supply.

CONCLUSIONS

Increased attention to the costs of health care delivery has caused the relative benefits and costs of blood conservation to be scrutinized. The prospective identification of surgical candidates who will need transfusion and therefore will truly benefit from blood conservation must be based on patient-specific factors, such as the baseline hematocrit and the anticipated blood loss during surgery. The challenge for physicians will be to educate their patients that the decision to conserve blood should no longer be based on the safety of the blood supply, but on evidence that blood conservation is safe and of value for individual patients.

REFERENCES

MEDICAL PROGRESS


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Volume 340 Number 7 - 533
BLOOD GASES AND ELECTROLYTES

Nasser Gayed, MD

FOR SELF-STUDY
Clinical laboratory Science

- Electrolytes
- Arterial Blood Gases

Renal Function

BUN, Creatinine

- BUN/Creatinine ratio
- Normally <10
- Increases in Prerenal azotemia (>20)
- Tubules are normal, increased reabsorption of urea.
- Acute on chronic.

Useful formula

- BUN/Creat today 34/2.1 ratio 16, but patient known to have CRF
- Previous BUN/Creat 20/1.6
- Calculate change in BUN and change in Creat
- The recent change has a ratio of 14/0.5=28
- So this patient has CRF but the recent insult is pre-renal
GFR
- Creatinine Clearance ≈ GFR
- Creatinine is secreted
- Creatinine Clearance = \[ \frac{U_{\text{creat}} \times U_{\text{vol}}}{\text{Serum creat.}} \]
  \[ = \frac{\text{Creat excreted in 1 min}}{\text{Serum creat.}} \]
  \[ = \frac{\text{Creat produced in 1 min}}{\text{Serum creat.}} \]

GFR
- Creatinine production ≈ 15 mg/kg/day.
- Related to muscle mass.
- Constant for a given individual.
- Declines with age.
- GFR declines with age.
- Serum creatinine does not change with age.

GFR
- \[ \text{GFR} = \frac{(140 - \text{age}) \times \text{wt in kg}}{72 \times \text{serum creat.}} \]
- Women 85%
- Beware of age!
- ? Acute renal failure
Decline in GFR

- In individual patient Decline in GFR with age and in CRF is linear.
- GFR = \text{Creat produced in 1 min}
  \text{Serum creat.}
- Constant / Serum creat. is linear
- 1 / Serum creat. is linear

CRF

- Creatinine 1.0 in 92 1.1 in 93
- 1.3 in 94 1.6 in 95
- 2.0 in 96 2.7 in 97
- 4.0 in 98 8.0 in 99
Hyponatremia

- Very common.
- Poor prognosis.
- Water metabolism.
- First exclude pseudo-hypertonic
- Next step in work up and treatment is to classify volume status.
- Hypovolemic, hypervolemic, euvoletic
- Symptoms only if acute and <125.

Hypernatremia

- Symptoms only if acute and osm > 320.
- Osmolality =
  - (Na+K) X 2 + glucose/18 + BUN/2.8
- BUN vs. others.
- Calculated vs. measured (<10).
  - Osmolar gap.

Hyperkalemia

- >5 evaluate, observe
- >5.5 treat the cause, repeat
- 6-6.5 treat. (Kayexylate)
- >6.5 check EKG.
Hyperkalemia, contd.

- 6.5-7 Peaked T waves
- 7-8 Prolonged P-R interval, loss of P wave, prolonged QRS. Keep on monitor while treating.
- >8 Sine wave form EKG.
- ? Chronic

Hypokalemia

- 0.1 mEq/L drop = Loss of 35 mEq
- Arrhythmia and muscle symptoms < 2.5 mEq/L
- EXCEPT!

Bicarbonate

- High bicarb. = metabolic alkalosis or compensation for resp acidosis.
- Low bicarb. = metabolic acidosis or compensation for respiratory alkalosis.
- Kidney compensation takes 6-12 hours to be evident and 3-7 days to be complete.
Henderson-Hasselbalch

- \[ \text{pH} = 6.1 + \log \left( \frac{\text{bicarb}}{0.03 \times \text{pCO}_2} \right) \]
- Chemically pH determines ratio of bicarb to CO$_2$.
- Time to think different.
- Bicarb and pCO$_2$ determine pH.
- And not the ratio.

Normal

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb</th>
<th>AG</th>
<th>pCO$_2$</th>
<th>pH</th>
<th>PO$_2$</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>103</td>
<td>27</td>
<td>5</td>
<td>30</td>
<td>7.40</td>
<td>100</td>
<td>4.2</td>
</tr>
<tr>
<td>135-145</td>
<td>108-110</td>
<td>24-30</td>
<td>5-12</td>
<td>25-40</td>
<td>7.25-7.45</td>
<td>75</td>
<td>3.5-5.5</td>
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<td>140</td>
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<td>5</td>
<td>40</td>
<td>7.40</td>
<td>100</td>
<td>4.2</td>
</tr>
</tbody>
</table>

- Serum electrolytes or BMP.
- Arterial blood gases (calculated bicarb).
- You have to calculate anion gap.

Check pH Normal 7.35-7.45

High pH

- \[ \text{pH} = 7.48 \]
- Alkalosis

Check pCO$_2$

- Check bicarb

Low pH

- \[ \text{pH} < 7.35 \]
- Acidosis

Check pCO$_2$

- Check bicarb

Both high
- Metabolic alkalosis

Both low
- Respiratory alkalosis

Both high
- Respiratory acidosis

Both low
- Metabolic acidosis
Practice

- pH 7.48
- pCO₂ 48
- Bicarb 32

Practice

- pH 7.48
- pCO₂ 30
- Bicarb 22

Practice

- pH 7.31
- pCO₂ 30
- Bicarb 22
Metabolic Alkalosis

- Vomiting
- Diuretics (Except)
- Volume depletion (Na Cl depletion)

Respiratory Acidosis

Hypoventilation due to
- COPD with CO2 retention
- Neuromuscular disease (drug, myasthenia, Guillain-Barre)

Respiratory alkalosis

- Check pO2
  - pO2 low: Hypoventilation due to hypoxia, hypovolemia, etc.
  - pO2 normal: Hypoventilation due to "central" cause
Respiratory Alkalosis

- Hyperventilation.

Due to
- Hypoxia
- Any pulmonary disease
- Any CNS disease
- Any stress (fever, sepsis)

A – a gradient

- Arterial alveolar oxygen gradient.
  - Alveolar \( pO_2 \) – Arterial \( pO_2 \) = 5 to 20 mmHg
  - Alveolar \( pO_2 \) = % inspired \( O_2 \) X (atmospheric pressure – water vapor pressure) – (\( pCO_2 \) X 1.2)
  - \( pO_2 \) = alveolar \( pO_2 \) – A a gradient
    = 0.21 X 700 – 40 X 1.2
  - To have low \( pO_2 \). The patient must be either hypoventilating or have lung disease.
  - To improve \( pO_2 \), increase \( FiO_2 \), hyperventilate, or treat lung disease.

Unusual situations

- \( pH \) 7.25
- Low = acidosis. Respiratory or metabolic
- \( pCO_2 \) 50
- Hi = Respiratory acidosis
- \( bicarb \) 22
- Low = Metabolic acidosis
- Metabolic acidosis and respiratory acidosis.
Unusual situations

- pH 7.29
- Low = acidosis. Respiratory or metabolic
- pCO₂ 50
- Hi = respiratory
- Bicarb 26
- Perfect = no compensation
- Either acute respiratory acidosis with no time to compensate
- Or there is a metabolic defect. What kind of defect?

Unusual situations

- pH 7.55
- Hi = alkalosis. Respiratory or metabolic
- pCO₂ 30
- Low = Respiratory alkalosis
- Bicarb 34
- Hi = Metabolic alkalosis
- Metabolic alkalosis and respiratory alkalosis.
Unusual situations

- pH 7.50
- Hi = alkalosis. Respiratory or metabolic
- pCO₂ 30
- Low = Respiratory alkalosis
- Base 28
- Perfect = No compensation
- Either acute respiratory alkalosis with no time to compensate.
- Or there is a metabolic defect. What kind of defect?

Metabolic Alkalosis

- Vomiting
- Diuretics (Except)
- Volume depletion (Na CI depletion)

Unusual situations

- pH 7.50
- Hi = alkalosis. Respiratory or metabolic
- pCO₂ 40
- Perfect
- Base 36
- Hi = Metabolic alkalosis
- Metabolic alkalosis and no compensation.
- There is a respiratory defect. What kind?
  Look under Respiratory alkalosis
Unusual situations

- pH 7.30
- Hi = acidosis. Respiratory or metabolic
- pCO₂ 40
- Perfect
- Bicarb 19
- Hi = Metabolic acidosis
- Metabolic acidosis and no compensation.
- There is a respiratory defect. What kind?
  Look under Respiratory acidosis

Unusual situations

- pH 7.40
- Perfect = ??
- pCO₂ ??
- Very low
- Bicarb 15
- Very low
- Respiratory alkalosis or Metabolic acidosis with perfect compensation?
- No. Compensation for a severe defect will not bring pH to perfect.
- Both Respiratory alkalosis and Metabolic acidosis

Unusual situations

- pH 7.40
- Perfect = ??
- pCO₂ ??
- Very hi
- Bicarb 40
- Very hi
- Respiratory acidosis or Metabolic alkalosis with perfect compensation?
- No. Compensation for a severe defect will not bring pH to perfect.
- Both Respiratory acidosis and Metabolic alkalosis.
Bicarbonate

- How to find combined defects?
- If bicarb is low and pCO₂ is high.
- If bicarb is high and pCO₂ is low.
- If one is abnormal and the other perfectly normal (no compensation) after ample time.
- If both are markedly decreased or both markedly increased an pH is perfectly normal.

Combined Defects

<table>
<thead>
<tr>
<th>pH</th>
<th>bicarb</th>
<th>pCO₂</th>
<th>Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>Low</td>
<td>Hi</td>
<td>Res and met acidosis</td>
</tr>
<tr>
<td>Hi</td>
<td>Hi</td>
<td>Low</td>
<td>Res and met alkalosis</td>
</tr>
<tr>
<td>Low</td>
<td>V. low</td>
<td>normal</td>
<td>Res and met acidosis</td>
</tr>
<tr>
<td>Hi</td>
<td>V. hi</td>
<td>normal</td>
<td>Res and met alkalosis</td>
</tr>
<tr>
<td>Low</td>
<td>normal</td>
<td>V. hi</td>
<td>Res &amp; met acidosis*</td>
</tr>
<tr>
<td>Hi</td>
<td>normal</td>
<td>V. low</td>
<td>Res &amp; met alkalosis*</td>
</tr>
<tr>
<td>perfect</td>
<td>Hi</td>
<td>Hi</td>
<td>Res alk &amp; met alk.</td>
</tr>
<tr>
<td>perfect</td>
<td>Low</td>
<td>Low</td>
<td>Res alk &amp; met acid.</td>
</tr>
</tbody>
</table>

Diabetic Ketoacidosis

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>105</td>
<td>27</td>
<td>8</td>
<td>40</td>
<td>7.40</td>
<td>90</td>
<td>4.2</td>
</tr>
<tr>
<td>135-145</td>
<td>100-110</td>
<td>24-30</td>
<td>6-12</td>
<td>35-45</td>
<td>7.55-7.45</td>
<td>&gt;75</td>
<td>&gt;3.5-5.5</td>
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<td>101</td>
<td>15</td>
<td>20</td>
<td>31</td>
<td>7.30</td>
<td>102</td>
<td>4.9</td>
</tr>
</tbody>
</table>

- Blood sugar elevated, and blood will have acetone.
- Ketones will lower bicarb and pH.
- Chloride unaffected (non hyperchremic).
- Anion gap increased.
- Hyperventilation lowers pCO₂ to compensate (Kussmaul).
- Serum potassium high. Body potassium depleted.
- Serum sodium low. Why?
Diarrhea

- Losing bicarb in diarrhea.
- Chloride goes up (hyperchloremic).
- Anion gap normal.
- Hyperventilation lowers pCO2 to compensate.
- Losing potassium in diarrhea.
- Serum sodium low. Why?

Vomiting

- Losing acid in vomit.
- Bicarb goes up.
- Hyperventilation raises pCO2 to compensate.
- Vomit very low in potassium. Losing potassium in urine. Why?
- Urine sodium also high. Why?
- Urine chloride is low. (differentiate from diuretic use).
- For each mmHg increase in pCO2, pO2 drops 1.2.

Acute asthma attack

- For pO2 consider age.
- Hypoxia due to lung disorder (increased A-a gradient)
- Hyperventilation to improve pO2 lowers pCO2
- No compensation (acute)
- Low pO2 and low pCO2 together always means increased A-a gradient. I.e. lung disease
- In respiratory alkalosis, first check pO2. Why?
**CPR**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
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<tr>
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<td>27</td>
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<td>7.40</td>
<td>180</td>
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<tr>
<td>135-145</td>
<td>100-110</td>
<td>24-26</td>
<td>8-12</td>
<td>35-45</td>
<td>7.30-7.45</td>
<td>7.75</td>
<td>3.5-6.0</td>
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<tr>
<td>140</td>
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<td>20</td>
<td>55</td>
<td>7.19</td>
<td>54</td>
<td>4.0</td>
</tr>
</tbody>
</table>

- Acidosis.
- Metabolic and respiratory.
- Increased anion gap.
- Severe hypoxia with hi pCO₂

**Patient on diuretic develops PE**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
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<td>35-45</td>
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<td>7.75</td>
<td>3.5-6.0</td>
</tr>
<tr>
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<td>90</td>
<td>33</td>
<td>10</td>
<td>30</td>
<td>7.55</td>
<td>66</td>
<td>3.6</td>
</tr>
</tbody>
</table>

- Alkalosis.
- Metabolic and respiratory.
- Hypoxia explains the respiratory alkalosis.
- The metabolic alkalosis is either vomiting or diuretic.

**COPD with CO₂ retention and diuretic**

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
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<td>3.5-6.0</td>
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<td>35</td>
<td>9</td>
<td>57</td>
<td>7.40</td>
<td>54</td>
<td>3.6</td>
</tr>
</tbody>
</table>

- Respiratory acidosis or metabolic alkalosis?
- Both?
- Respiratory acidosis and low pO₂ means COPD with CO₂ retention (only 1 other alternative)
- Metabolic alkalosis likely due to diuretic. Why?
Early septic shock

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb.</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
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<td>3.5-3.8</td>
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<td>132</td>
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<td>20</td>
<td>25</td>
<td>7.40</td>
<td>105</td>
<td>4.0</td>
</tr>
</tbody>
</table>

- Respiratory alkalosis or metabollic acidosis?
- Both.
- The respiratory alkalosis is not due to hypoxia.
- The metabolic acidosis is anion gap type.
- Very common cause of AG acidosis explains both.

Vomiting going into shock

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb.</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
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<td>125-145</td>
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<td>8-12</td>
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<td>7.35-7.45</td>
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<td>3.5-3.8</td>
</tr>
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<td>15</td>
<td>44</td>
<td>7.40</td>
<td>105</td>
<td>3.0</td>
</tr>
</tbody>
</table>

- Metabolic alkalosis.
- What else?
- Increased anion gap = metabolic acidosis.

Diarrhea going into shock

<table>
<thead>
<tr>
<th>Sodium</th>
<th>Chloride</th>
<th>Bicarb.</th>
<th>AG</th>
<th>pCO₂</th>
<th>pH</th>
<th>PO₂</th>
<th>Potassium</th>
</tr>
</thead>
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<td>7.38</td>
<td>105</td>
<td>3.0</td>
</tr>
</tbody>
</table>

- Metabolic acidosis.
- Anion gap type.
- What else?
- Non anion gap type also.
SEPSIS

Steve Nandkumar, MD

(for self-study)
SEPSIS

751,000 cases/year
220,000 deaths/year (10th leading cause of mortality)

Average hospital cost estimated $22,100 per case
Hospital stay about 19.5 days

Table 124-1. Definitions Used to Describe the Condition of Patients with Sepsis

<table>
<thead>
<tr>
<th>Condition</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteremia</td>
<td>Presence of bacteria in the blood, as evidenced by positive blood cultures</td>
</tr>
<tr>
<td>Septicemia</td>
<td>Presence of microbes or their toxins in blood</td>
</tr>
<tr>
<td>Systemic inflammatory response syndrome (SIRS)</td>
<td>Two or more of the following conditions: (1) fever (oral temperature &gt; 38°C) or hypothermia (&lt;36°C); (2) tachypnea (&gt;20 breaths/min); (3) tachycardia (heart rate &gt;90 beats/min); (4) leukocytosis (&gt;12,000/µL), leucopenia (&lt;4,000/µL), or &gt; 10% bands; hypocapnia pp of Co2 &lt; 32 mm of Hg. May have an infectious or a noninfectious etiology</td>
</tr>
<tr>
<td>Sepsis</td>
<td>SIRS that has a proven or suspected microbial etiology</td>
</tr>
<tr>
<td>Severe sepsis (similar to “sepsis syndrome”)</td>
<td>Sepsis with one or more signs of organ dysfunction (such as metabolic acidosis, acute encephalopathy, oliguria, hypoxemia, or disseminated intravascular coagulation) or hypotension</td>
</tr>
<tr>
<td>Septic shock</td>
<td>Sepsis with hypotension (arterial blood pressure of &lt;90 mmHg systolic or 40 mmHg less than patient’s normal blood pressure) that is unresponsive to fluid resuscitation, along with organ dysfunction (see severe sepsis)</td>
</tr>
<tr>
<td>Refractory septic shock</td>
<td>Septic shock that lasts for &gt; 1 h and does not respond to fluid or pressor administration</td>
</tr>
<tr>
<td>Multiple-organ dysfunction syndrome (MODS)</td>
<td>Dysfunction of more than one organ, requiring intervention to maintain homeostasis</td>
</tr>
</tbody>
</table>

SOURCE: Adapted from American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee.

NOTE: SIRS – systemic inflammatory response syndrome
CARS – compensatory anti-inflammatory response syndrome
MARS – mixed antagonistic response syndrome
CURRENT DIAGNOSIS

- Infection (documented or suspected) plus some of the following.
  
  General Variables
  - Fever (core temperature >38.3°C [101°F])
  - Hypothermia (core temperature <36°C [96.8°F])
  - Heart rate >90 bpm or >2 SD above the normal value for age
  - Tachypnea
  - Altered mental status
  - Significant edema or positive fluid balance (>20 mL/kg for longer than 24 h)
  - Hyperglycemia (plasma glucose >120 mg/dL or 7.7 mmol/L in absence of diabetes)

Inflammatory Variables

- Leukocytosis (WBC count >12,000/µL)
- Leukopenia (WBC count <4000/µL)
- Normal WBC count with 10% immature forms (bands)
- Plasma C-reactive protein >2 SD above the normal value
- Plasma procalcitonin >2 SD above the normal value

Hemodynamic Variables

- Arterial hypotension (systolic BP <90 mm Hg, MAP <70 mm Hg, or a systolic BP decrease >40 mm Hg in adults or <2 SD below normal for age)
- Svo2 >70%
- Cardiac index >3.5 L/min/m²
- Organ dysfunction variables
  - Arterial hypoxemia (Paco2/Fio2 <300)
  - Acute oliguria (urine output <0.5 mL/kg/h or <45 mmol/L for at least 2 h)
  - Creatinine increase >0.5 mg/dL
  - Coagulation abnormalities (INR >1.5 or aPTT >60 sec)
  - Ileus (absent bowel sounds)
  - Thrombocytopenia (platelet count <100,000/µL)
  - Hyperbilirubinemia (plasma total bilirubin >4 mg/dL or >70 mmol/L)

Tissue Perfusion Variables

- Hyperlactatemia (>1 mmol/L)
- Decreased capillary refill or mottling

NOTE: LODS – Logistic organ dysfunction syndrome
SOFA – Sepsis related organ failure assessment

BOX 1 PIRO Staging of Sepsis

| Predisposition | Predisposing conditions that influence likelihood of infection, sepsis, morbidity, survival (age, gender, hormonal state, genetic polymorphisms for immune response and coagulation proteins) |
| Infection | Organism associated with the sepsis response (type of organism, virulence potential, toxins, community or nosocomial acquisition) |
| Response | Clinical and immunologic manifestations of the septic response (either hypoinflammation or hyperinflammation) (e.g., procalcitonin, IL-6, HLA-DR, TNF, PAF) |
| Organ Dysfunction | Type and number of dysfunctional organs (reversible versus irreversible dysfunction), severity of dysfunction |

Abbreviations: HLA-DR = human leukocyte antigen-D related; IL = interleukin; PAF = platelet activating factor; TNF = tumor necrosis factor.

The validity and practical usefulness of this proposed staging system to further the understanding of sepsis remains to be demonstrated.

Risk Factors for Sepsis:
1. Aging (geriatric patients)
2. Immunocompromised patients, e.g., AIDS
3. Invasive procedures
4. Intravascular devices, catheters, etc.
5. Antibiotic resistance
6. Diabetes Mellitus
7. Alcoholism
8. I.V. drug abuse
9. Neutropenia
10. Genetics/Gender

NOTE: LODS – Logistic organ dysfunction syndrome
SOFA – Sepsis related organ failure assessment
Figure 4-22. Effects of lipopolysaccharide (LPS) and secondarily induced effector molecules. LPS initiates the cytokine cascade described in Figure 4-21; in addition, LPS and the various factors can directly stimulate downstream cytokine production, as indicated. Secondary effectors that become important include nitric oxide (NO) and platelet-activating factor (PAF). At low levels, only local inflammatory effects are seen. With moderate levels, more systemic events occur in addition to the local vascular effects. At high concentrations, the syndrome of septic shock is seen. DIC, disseminated intravascular coagulation; ARDS, adult respiratory distress syndrome. (Modified from Abbas AK, et al: Cellular and Molecular Immunology, 4th ed. Philadelphia, WB Saunders, 2000).
**NOTE:** Toxic insult maybe be due to
1. Gram negative organisms → Endotoxins
2. Gram-positive organisms → Exotoxins (act as superantigens)

Pro-inflammatory mediators . . .  e.g., IL-1, IL-2, IL-6, IL-8, TNF-α, IFN-γ
Anti-inflammatory mediators . . .  e.g., IL-4, IL-10, IL-13, TGF-β
LAB TESTS

A. **Nonspecific Evidence of Infection**

1. **WBC count**  
   Leucocytosis with left shift (bacterial infection)  
   Leucocytosis without left shift (usually viral infection)  
   Leucopenia – indicates poor prognosis.

   **NOTE:** White blood cells may show toxic granules and Dohle bodies

2. **E.S.R. elevated**

3. **C.R.P. elevated** (cytokines cause increased hepatic production of CRP)

4. **Coagulation System**  
   D.I.C. may be present due to tissue damage/tissue factor release activating extrinsic pathway

   **P.T.**  
   **Fibrinogen**  
   **P.T.T.** Elevated Platelets Decreased  
   **Thrombin Time (T.T.)** Other Clotting Factors  
   **Fibrin degradation products**

   **NOTE:** Antithrombin level is decreased.  
   Protein C-S inhibitory pathway is inhibited

   D-dimer test (indicates the presence of fibrin degradation products) is **POSITIVE**

5. **Hepatic Tests**  
   Due to liver damage hepatic enzymes such as ALT, AST, LDH and alkaline phosphatase are elevated.  
   T. Bilirubin increases (jaundice)  
   S. Albumin and total proteins are decreased  
   Hyperglycemia

6. **Renal Tests**  
   BUN and S. creatinine are elevated  
   “Urine” analysis may indicate evidence of tubular damage (proteinuria)

7. **Blood Gases**  
   Respiratory alkalosis initially due to hyperventilation  
   Metabolic acidosis follows due to anaerobic glycolysis and lactic acid production  
   Anion gap is increased

8. **Cytokine Levels**  
   IL-6 (> 1,000 pg/ml indicates poor prognosis). Limited clinical value as test is poorly standardized.
B. Specific Evidence of Infection

1. Blood, body fluid, tissues for microbial and/or toxin identification, e.g., direct visualization, serology, cultures, nucleic acid probes, etc.

   **Organisms isolated**
   Gram-ve 37%  Polymicrobial  low  Fungus  6%
   Gram+ve 52%  Classic pathogens  < 5%

   Gram-ve: Enterobacteria, Pseudomonas, Haemophilus
   Gram+ve: Staph aureus, enterococcus, S. pneumoniae
   Classic pathogens: N. meningitidis, H. influenzae, S. pyogenes

   **NOTE:**
   Positive blood culture occurrence 20-40%  40-70%

2. **Limulus Lysate test** (Limulus Amebocyte Lysate Assay)

   Hemolymph of horseshoe crab contains numerous circulating cells called amebocytes. Lysate of these amebocytes undergoes gel transformation in the presence of endotoxins from the cell wall of gram-negative bacteria. This test may be useful in detecting gram-negative infections (not commonly used, except to assess sterility of biological fluids/solutions).
CHRONIC LIVER DISEASE
LIVER FUNCTION TESTS

Steve Nandkumar, MD
CASE

PRESENT COMPLAINT:
A 52-year-old African American, a known chronic alcoholic, was found unconscious lying in a pool of blood at his home. He was rushed to the Emergency Room.

FAMILY HISTORY:
No “relevant” history could be obtained.

PHYSICAL EXAMINATION:
The patient was unconscious. His skin was cold and clammy.

VITAL SIGNS:
Blood Pressure: 40/10 mm Hg
Pulse: 110/min
Respiratory Rate: 24/min
Pupils: ECCRL (equal. central. circular reacting poorly to light).

The sclera was deep yellow, and blood was noted in the oral cavity.

EXAMINATION OF SYSTEMS
Examination of the CVS and RS was noncontributory.

CNS
Patient was unconscious

AS
Abdomen soft
Bowel sounds heard (borborygmi)
Liver edge felt 4 fingerbreadths below ® costal margin
Spleen – moderately enlarged
Rectal exam – Unremarkable

The patient was intubated and a nasogastric tube was inserted and suction yielded copious amounts of blood (about 1,500 cc).

QUESTIONS:
1. What is the possible diagnosis?
2. What are the differential diagnoses?
3. What lab tests must be ordered? (Most important and relevant to least important)

COURSE AND MANAGEMENT:
Attempts to pass a Blake-Moore tube were unsuccessful. The patient received 12 units of packed red blood cells and vasopressors. He was then transferred to the ICU (Intensive Care Unit) where he remained unresponsive to treatment. His pulse and blood pressure could not be recorded. The pupils were dilated and non-reactive to light. He was subsequently pronounced dead 40 minutes after admission to the ICU.

An autopsy was requested and performed. Images will be shown.
HEPATIC FUNCTION TESTS
(Liver Panel)

I. ALKALINE PHOSPHATASE (alk. phos.)

NORMAL RANGE: 20–130 U/L

Alkaline phosphatase is bound to canalicular cell membrane. It is involved in cleavage of Phosphate-containing compounds and helps in transferring substances across cell membranes.

Isoforms of alkaline phosphatase: There are five isoforms, detected by electrophoresis.

Coded by a single gene on chromosome 1
1. liver
2. Kidney
3. Bone

Coded by two genes on chromosome 2
4. Placenta
5. Intestine

Coded by a separate gene on chromosome 2
Placenta-like or germ cell isoform

Physiologic elevation of enzyme
1. Children
2. Pregnancy

Pathologic elevation of enzyme
1. Liver disease<br>  < Parenchymal<br>  < Cholestatic<br>  2. Bone disease<br>  < Metabolic<br>  < Malignancy<br>  < Paget’s disease<br>  3°
1° 2°
3. Placental disease
4. Intestinal disease/Diabetes Mellitus
5. Renal diseases

NOTE: Placental or placenta-like isoenzymes (Regan isoenzymes) are elevated in germ cell tumors, leukemias, cancer of cervix, uterus, lung, breast, colon, pancreas, etc.

Decreased alkaline phosphatase enzyme occurs in:
1. hypophosphatasia
2. zinc deficiency
3. oral contraceptive pills
4. Fibric acid drugs

ALKALINE PHOSPHATASE IS THE BEST INDICATOR OF BILIARY OBSTRUCTION.
II. GAMMA GLUTAMYL TRANSFERASE (GGT)

GGT or GGTP  gamma glutamyl transpeptidase  
NORMAL RANGE  5–40 U/L

This enzyme helps transfer of γ-glutamyl moiety from a peptide glutathione to other peptides, amino acids, or water. It is bound to the plasma membrane of bile canaliculi, intestinal epithelial cells, proximal renal tubular cells, and prostate ductal cells. Liver is the MAJOR source of enzymatic activity. GGT’s half life is about 7–10 days. In alcoholic liver disease, it increases to about 28 days.

CAUSES OF ABNORMAL RESULTS
1. Mainly LIVER DISEASE (hepatitis, obstructive liver disease, cirrhosis, cancer, and metastatic tumors)
2. Test for alcohol abuse: there is a rough correlation between alcohol consumption and GGT levels. However, GGT is abnormally elevated in only 30–50% of cases. Following abstinence, it may take weeks to months for the enzyme to return to normal levels.
3. Drugs (Alcohol, Acetaminophen, Barbiturate, Chlorpromazine, Dilantin) are conjugated with glutathione and then excreted; GGT levels are elevated due to microsomal induction by drugs.

NOTE: This test is not used for screening or initial evaluation of patients with liver disease.

III. AMINOTRANSFERASE (ASPARTATE AND ALANINE)

AST (SGOT – serum glutamic-oxaloacetic transaminase)  
NORMAL RANGES:  AST = 8–33 U/L
ALT (SGPT – serum glutamic-pyruvic transaminase)  
ALT = 4–36 U/L

These are catalytic enzymes participating in Krebs cycle (synthesis of oxaloacetic and pyruvic acid respectively). Pyridoxal phosphate (Vitamin B6) acts as a co-factor.

Enzymes present in cytoplasm of cells in organs such as liver, kidney, heart, skeletal muscle, spleen, rbc

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mitochondrial</th>
<th>Extra-mitochondrial (cytosol)</th>
<th>Extra mitochondrial (cytosol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>87 hours</td>
<td>17 hours</td>
<td>47 hours</td>
</tr>
<tr>
<td>ALT</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NORMAL AST:ALT = 0.8 to 1

In acute liver injury both AST and ALT are elevated (AST > ALT). After 24-48 hours ALT > AST due to longer ½ life.

In alcoholic hepatitis, AST/ALT ratio is greater than 2 or 3 due to

1. Mitochondrial induction of AST by alcohol.
2. Pyridoxal phosphate deficiency affects ALT synthesis

NOTE: 1. There is more AST than ALT (quantitatively).
2. ALT IS MORE SPECIFIC FOR LIVER DISEASES THAN AST.

CAUSES OF ENZYME ELEVATION –
1. Liver disease (hepatitis, cirrhosis, etc.)
2. Renal infarcts
3. Muscle injury
4. Malignancy
5. Hemolysis

CAUSE OF ENZYME DECREASE – Renal failure
IV. **LACTATE DEHYDROGENASE (LDH)**  
**NORMAL RANGE:** 100–250 U/L

Also known as LD, it is a zinc-containing enzyme that is part of the glycolytic pathway. It catalyzes the reversible oxidation of lactate to pyruvate (lactate + NAD $\rightarrow$ pyruvate + NADH +H). It is found in the cytoplasm of cells and has two subunits:

1. H = heart  
2. M = muscle

Combinations of these subunits result in isoenzymes:

- LD₁ (HHHH)  
- LD₂ (HHHM) seen in heart, kidney, red cells, and brain  
- LD₃ (HHMM) seen in lung and spleen  
- LD₄ (HMHM) seen in liver and skeletal muscle

**NOTE:** LD₆ seen on electrophoresis represents alcohol dehydrogenase (metabolizes lactate). Plasma LDH is mainly derived from red blood cells and platelets.

**CAUSES OF ABNORMAL RESULTS**

1. Damage/disease of kidney, lung, lymph nodes, red blood cells, and white blood cells (anemias, lymphomas)  
2. Cardiac or skeletal muscle injury  
3. Shock and sepsis

For normal serum LD₂ > LD₁ > LD₃ > LD₄ > LD₅ (35% > 25% > 20% > 15% > 5%)  
If LD₁ is > LD₂ (FLIPPED PATTERN), the causes are MI, anemias, renal infarcts, and tumors.  
If LD is elevated but relative amount of each isoenzyme is the same (LD₁ = LD₂ = LD₃ = LD₄ = LD₅) → TOMBSTONE pattern seen in shock, sepsis, etc.

**NOTE:** LDH is non-specific. It is **NOT** used as a marker for cardiac injury/hepatic injury/disease.

*Normal range depends on the methodology and temperature of reaction (depends on the lab!).*

V. **SERUM ALBUMIN**  
**NORMAL RANGE:** (3.5–4.6 gm/dl)

Constitutes 2/3 of total plasma proteins; synthesis is 120 mg/kg body wt./day.

**FUNCTIONS:**

1. Maintains normal intravascular oncotic pressure  
2. Serves as a repository of amino acids  
3. Serves as a transport or carrier protein (for thyroxine, bilirubin, Ca, Mg, cortisol, drugs, etc.)

**CAUSES OF ELEVATION:** Dehydration

**CAUSES OF DECREASED LEVELS:**

1. Impaired synthesis (malnutrition, malabsorption, and liver disease)  
2. Increased loss (ascites, protein losing enteropathy, or nephropathy)

**NOTE:** (a) Calcium and magnesium ions are bound to albumin and hence Ca and Mg levels may be affected by albumin levels. (b) Decreased albumin causes compensatory increase of other proteins (polyclonal gammopathy)!
ALBUMIN AND DIABETES MELLITUS
1. 25% of albumin is glycosylated during hyperglycemia
   Albumin + glucose → glycosylated albumin (also called fructosamine)
   Normally, Hb + glucose → glycosylated Hb (GHb) is used to monitor diabetes mellitus.
   In case of hemoglobinopathies, measurement of GHb is unreliable and hence, FRUCTOSAMINE LEVELS are used to monitor diabetes mellitus.

2. Diabetic nephropathy
   Albumin escapes in urine, causing “albuminuria” in this disorder. Measurement of “microalbumin in urine” is a standard of care for management of diabetes mellitus and the early detection of its complications.

<table>
<thead>
<tr>
<th>Albumin Excretion Rate</th>
<th>Normal</th>
<th>Microalbuminuria</th>
<th>Advanced Nephropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg/min</td>
<td>&lt; 10</td>
<td>20–200</td>
<td>&gt; 200</td>
</tr>
<tr>
<td>mg/day</td>
<td>&lt; 15</td>
<td>30–300</td>
<td>&gt; 300</td>
</tr>
</tbody>
</table>

VI. TOTAL PROTEINS
NORMAL RANGE: 6.3–8.5 gm/dl

Liver produces most proteins except γ-globulins and vWF.
The protein fractions are:
- albumin
- α₁-globulin
- α₂-globulin
- β-globulin
- gamma (γ) globulin

Other protein fractions include: complements, fibrinogen, prealbumin, etc.

Normal albumin:globulin ratio is about 2:1.

CAUSES OF ABNORMAL ELEVATION
1. Chronic inflammation (RA, SLE, and Rheumatic fever)
2. Infections (acute and chronic)
3. Liver disease (cirrhosis)
4. Leukemias and Hodgkin’s disease
5. Monoclonal gammopathies (e.g., multiple myeloma)

CAUSES OF ABNORMAL DECREASE
1. Malnutrition
2. Malabsorption
3. Nephrotic syndrome
4. Drugs
5. Burns
6. Cirrhosis
7. Hypogammaglobulinemia

NOTE:
- Serum protein electrophoresis (SPE) help in protein fractionation,
- Urine protein electrophoresis (UPE) useful in multiple myeloma;
- Immunoelectrophoresis (IEP) other gammopathies

Oligoclonal bands in CSF occur in CNS infections, autoimmune disorders, and demyelinating diseases.
VII. **AMMONIA**  

NORMAL RANGE: less than 40 µmol/L

**AMMONIA IS DERIVED FROM:**
1. Bacterial degradation of dietary proteins and urea present in GI secretions.
2. Metabolism of amino acids in the liver
3. Renal ammoniagenesis

```
Glutamic acid → Glutamine → Plasma
               ↓
Liver          
Excretion in urine
                        
Ammonium ions
Glutaminase
Renal uptake
```

**NOTE: Toxic ammonia is converted to less toxic urea in Kreb’s cycle and excreted.**

**ABNORMAL ELEVATION OCCURS IN**
1. Liver diseases (acute failure and cirrhosis)
2. Reye’s syndrome
3. Enzyme deficiencies (urea cycle)
4. Renal disease
5. Acute leukemias and following BMT

**AMMONIA ACCUMULATION IS ASSOCIATED WITH CEREBRAL DYSFUNCTION**  
(i.e., hepatic coma – mood and personality changes, ataxia, asterixis, convulsions, and coma)

**AMMONIA**

```
Direct effect

Increases glutamine in CNS (CSF)
Decreases GABA (γ-aminobutyric acid), an inhibitor of neurotransmission
```

Normally, glutamic acid → GABA

Decarboxylation

In heptatic failure

```
Ammonia + glutamic acid → glutamine
```

As glutamine increases, glutamic acid with consequent decreased GABA production; so there is decreased neuroinhibition (i.e. neuroexcitation).

**NOTE:** **Arterial** ammonia is more useful than venous!

**What are the drugs used in treating hyperammonemia?**
VIII. BILIRUBIN

Total bilirubin 0.3–1.2 mg/dl (Reference range)
Direct bilirubin 0.1–0.2 mg/dl
Indirect bilirubin 0.2–1.1 mg/dl

A. IN RES –
(Reticuloendothelial system)
SPLEEN – breakdown of old rbcs

B. LIVER – above Bilirubin traverses cell membrane with the help of anion transporter

BILIRUBIN + ( Y and Z PROTEINS) + LIGANDIN (carried to SER – smooth endo. reticulum)

G. transferase* GLUCURONIC ACID

CIS form

This bilirubin is Direct Conjugated Post hepatic Water soluble

BILIRUBIN MONOGLUCORONIDE BILIRUBIN DIGLUCORONIDE

Excreted in bile

C. IN GUT – Conjugated BILIRUBIN

UROBILINOGEN (also reabsorbed → enterohepatic circulation – 20%)

URINE STERCOBILIN (gives color to stools)°

STOOLS (80%)

NOTE:
* g-transferase (glucuronyl transferase) – mutations of the gene for this enzyme cause Gilbert’s syndrome and Crigler-Najjar syndrome.
° MOAT (multispecific organic anion transporter) – deficiency causes Dubin-Johnson syndrome (liver is dark in color due to pigment accumulation-lipofuscin).
Rotor syndrome → cause unknown; no liver pigmentation; impaired excretion of conjugated bilirubin
+ Loss of stereobilin causes “clay colored” stools. What does this imply?
**ELEVATED TOTAL BILIRUBIN**

- > 80% indirect
  - Hb < 12 gm/dl
    - Hemolysis/anemia
    - Pernicious anemia
    - Thalassemia
  - Gilbert’s syndrome
  - Crigler-Najjar syndrome

- > 50% direct
  - Liver function tests
    - Abnormal
      - Acute and chronic liver disease
      - Alcoholism
      - Obstructive diseases (intra or extra hepatic)
      - Tumors
    - Normal
      - Rotor’s syndrome
      - Dubin-Johnson syndrome

**NOTE:** GREATER THAN 2.5 mg/dl of total bilirubin causes CLINICAL JAUNDICE. BRAIN DAMAGE (KERNICTERUS) OCCURS IN NEONATES WHEN TOTAL BILIRUBIN EXCEEDS 15 mg/dl.
DIARRHEA

**DEFINITION:**
Passage of abnormally liquid or unformed stools at an increased frequency.

**Diarrhea**
- Stool weight exceeding 200 gm/day
- Increased frequency (2-3 bowel movements) per day.

I. **ACUTE DIARRHEA**
- Duration of diarrhea is < 2 weeks
- 90% of cases are due to infectious agents
- 10% of cases are due to drugs, toxins, ischemia, etc.

**Risk Groups:**
1. Travelers
2. Consumers of certain foods
3. Day-care, institutionalized persons
4. Immunodeficient persons

**Types of Diarrhea:**

<table>
<thead>
<tr>
<th>Watery</th>
<th>Bloody (Dysentery)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Watery with or without fecal matter</td>
<td>Unformed stool with blood/mucus</td>
</tr>
<tr>
<td>2. Site small bowel</td>
<td>Large bowel</td>
</tr>
<tr>
<td>3. Toxin mediated</td>
<td>Cytolytic</td>
</tr>
<tr>
<td>4. Inflammation absent</td>
<td>Inflammation present</td>
</tr>
<tr>
<td>5. Antibiotics not needed</td>
<td>Antibiotics are needed</td>
</tr>
</tbody>
</table>

**NOTE:** Most episodes of acute diarrhea are MILD and SELF LIMITING. Specific intervention is not necessary.

II. **CHRONIC DIARRHEA**
Defined as diarrhea lasting > 2 weeks. (Some consider > 4 weeks; between 2-4 weeks is considered as persistent diarrhea).

**Causes:**
1. **Osmotic**
   - e.g., lactase deficiency, cathartics, Mg containing antacids
2. **Secretory**
   - e.g., fluid electrolyte transport derangements, drugs, toxins
3. **Inflammatory**
   - e.g., IBD, collagenous colitis
4. **Abnormal intestinal motility**
   - e.g., irritable bowel syndrome (IBS)
5. **Infectious**
6. **Steatorrhea**
   - e.g., Celiac sprue, Whipple’s disease

**STOOL EXAMINATION**

<table>
<thead>
<tr>
<th>Normal Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bulk</td>
</tr>
<tr>
<td>100-200 gm</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>75%</td>
</tr>
<tr>
<td>Bacteria/cell debris</td>
</tr>
<tr>
<td>15%</td>
</tr>
</tbody>
</table>
Vegetable/food residues
Fat, etc.  10%
Total osmolality  290 osmol/kg H2O (same as plasma’s)
pH  7.0-7.5
Potassium  5-20 mEq/kg
Sodium  10-20 mEq/kg

Micros. Exam
Rbcs  absent
Wbcs  absent/rare
Epithelial cells  present
(Sudan stain)  Neutral fat globules
Color  Normal brown (due to stercobilin)
Smell is due to indole and skatole (protein breakdown products)

Osmolality – Osmotic Gap
Osmotic gap  =  measured stool osmolality – 2 [Na+k in stool]
            =  290 – 2[Na+k in stool]

If osmotic gap is > 100 mOsm/kg H2O  →  Osmotic Diarrhea
If osmotic gap is < 50-100 mOsm/kg H2O  →  Secretory Diarrhea

LABORATORY TESTING IN DIARRHEA

Hx Px Dx
  CMP, CBC, TSH, etc

  Stool exam  Endoscopy  Others
  a. Exam for ova/parasites  Biopsy  e.g., gastrin assay
  b. Exam for pus/blood
  c. Exam for pmns
  d. Culture (routine and special) for bacteria
  e. Toxin assays for bacteria c. difficile
    virus  Rotavirus, Norwalk virus
    protozoa  giardia
  f. Molecular tests (DNA sequences, etc.)
g. Fecal fat/day
  > 7 gm/day = steatorrhea
  > 14 gm/day = malabsorption syndrome

NOTE:  Infectious organisms are:
1. Traveler’s diarrhea  -  E. coli (0157:H7 serotype), giardia, salmonella, shigella
2. Infants  -  Rotavirus, E. coli
3. Elderly  -  C. difficile, salmonella, shigella, rotavirus
4. Food poisoning  -  Salmonella, shigella, campylobacter, E. coli
5. Sea food (undercooked)  -  Vibrio

NOTE:  Above notes on diarrhea are for self-study.
MOLECULAR PATHOLOGY

George Liu, MD

(SESSION TAUGHT IN SEP. 2014, AS PART OF NEOPLASIA)
Advances in genomics have had a major impact on the practice of clinical medicine

Sequencing of the human genome has aided in the discovery of many new disease-associated genes.

In addition, technological advances have permitted the identification of the molecular mechanisms of numerous diseases.

Molecular Pathology

- Molecular pathology is the application of the principles, theory, and technologies of molecular biology and molecular genetics to questions and problems in clinical medicine.
- The subspecialty of molecular pathology arose in parallel with the growth in knowledge about the molecular basis of diseases and the technological advances which made molecular diagnosis in the clinical laboratory possible.

The basic categories of analyses used to categorize DNA and RNA

- Nucleic Acid Extraction
- Electrophoretic separation
- Restriction enzyme digestion
- Hybridization
- Amplification methods: target and signal
**Target Amplification**

- Polymerase chain reaction (PCR)
- Ligase chain reaction (LCR)
- Nucleic acid sequence-based amplification (NASBA)
- Transcription-mediated amplification (TMA)

**Real-time PCR**

```
Fluorescence

Fluorescence Threshold

Amplification Cycle

CT = 25.2
```

**Hybridization Probes**

The peaks correspond to the melting temperatures for the wildtype sequence (higher temperature peak) and for the mutant sequence (lower temperature peak). The curve with the single peak is homozygous wildtype. The peak areas for the curve with the two peaks is approximately 1:1. This result is consistent with heterozygosity for the Factor V Leiden mutation.

**NASBA**

**Signal Amplification**

- Branch DNA (bDNA)
- Hybrid Capture
- Cleavage/Invader technology
**Branch DNA (bDNA) technology**

**HCV Genotyping – Invader Technology**

**In situ hybridization**
- DNA or RNA probes can be used
- Detects DNA or RNA in tissue
- Spatial and temporal localization of target sequence
- Requires tissue sections, probe and visualization system

**Fluorescent in situ Hybridization (FISH)**
FISH DNA Probe System

Human Diseases

Cause (etiology)

Mechanism (pathogenesis)

Structural alterations (morphologic/molecular)

Functional consequences (clinical significance)

Practical Applications of Molecular Diagnostics in Clinical Laboratory Medicine

Molecular Genetics
- Single gene disorders
- Polygenic disorders
- Chromosomal disorders

Molecular Oncology
- Diagnostic testing
- Disease prognosis
- Determination of predisposition

Practical Applications of Molecular Diagnostics in Clinical Laboratory Medicine (cont.)

Infectious Disease
- Qualitative and quantitative detection of infectious agents
- Microbial identity testing
- Genotyping/drug resistance testing

Practical Applications of Molecular Diagnostics in Clinical Laboratory Medicine (cont.)

Hematopathology
- Diagnostic testing
- Determination of clonality

Identity Testing
- Parentage
- Clinical testing

Types of Specimens for the Molecular Diagnostics Laboratory

- Whole blood
- Body fluids
  - CSF
  - Bronchial lavage
  - Amniotic
  - Sputum
  - Urine
- Tissue samples
  - Fresh/frozen
  - Paraffin-embedded
- Hair (shaft/root)
Anticoagulants

- EDTA
  - Lavender-top Vacutainer
  - Preferred specimen
- Heparin
  - Green-top Vacutainer
  - Inhibits several enzymes used in molecular assays

Specimen Storage Requirements — RNA

Blood, Bone Marrow, Other Fluids

- 22–25 °C  Not recommended within 2 hours
- 2–8 °C  Not recommended within 2 hours
- −20 °C  Not recommended 2–4 weeks
- NOTE: Do not freeze blood or bone marrow before centrifuging red blood cells (RBCs).
- −70 °C  Preferred storage condition

Molecular Genetics

Normal Chromosome Structure

- The telomeres, located at the ends of chromosomes, protect the chromosome from degradation and limit the number of times the replication process can occur.
- The centromere is the constriction region of a chromosome where the two sister chromatids are joined together. This region is essential for chromosome segregation during cell division. The centromere divides the chromosome into two parts: p (short arm) and q (long arm).
- A chromosome consists of two sister chromatids, each of which is composed of a single helix of DNA.

Structural: Translocation, inversion, deletion

- Translocation: Two strands of chromosome exchange pieces.
- Inversion: Strand of chromosome becomes inverted.
- Deletion: Part of chromosome is missing.

Inversion (left)

Characteristics of inversion organisms

Characteristic 1

Characteristic 2
Identifying Chromosomal Alterations

- Conventional Cytogenetics
- Fluorescence in situ hybridization (FISH)
- Reverse Transcriptase PCR (RT-PCR)
- Southern Blot
- Chromogen in situ hybridization (CISH)

Breast Cancer Susceptibility Genes

BRCA1
- Localized to chromosome arm 17q.
- Mutated in 45% of hereditary breast cancers and 90% of patients with breast/ovarian cancer.
- 120 different mutations have been identified.

Breast Cancer Susceptibility Genes

BRCA2
- Localized to chromosome arm 13q.
- Accounts for approximately 40-45% of early onset hereditary breast cancer.
- Mutation does not contribute significantly to risk for development of ovarian cancer.
- The BRCA2 gene is 70 kb in size, containing 27 exons.

Breast Cancer Susceptibility Genes (cont.)

Features of the BRCA1 gene include 23 exons, and a large exon 11 (100 kb) that contains 60% of the coding sequence.

Several families have been identified that contain individuals affected by breast cancer but lacking gene mutation, and others have been identified containing individuals with a mutated gene but no breast cancer.

Hereditary Breast Cancer

<table>
<thead>
<tr>
<th>Age</th>
<th>Risk BRCA1</th>
<th>Risk BRCA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 39</td>
<td>18%</td>
<td>15%</td>
</tr>
<tr>
<td>40-49</td>
<td>59%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Mutation detection
- Direct gene sequencing (gold standard)
- Mutations with unknown consequences

Critical Targets of Microsatellite Mutation In Human Cancer

- TGIFIRL, IGFRII—genes involved in suppression of cell proliferation
- BAX—gene involved in apoptosis
- MSH2, MSH6—mismatch repair genes
- APC—adenomatous polyposis coli tumor suppressor gene
Microsatellite Instability (MSI)

- Nucleotide mismatches that “normally” occur when DNA polymerase inserts the wrong base in the newly synthesized DNA are typically repaired by mismatch repair enzymes.

- Defects in the process of mismatch repair lead to MSI (instability in >40% of loci).

- Mutations in DNA mismatch repair (MMR) genes (primarily MSH2 & MLH1) are found in sporadic CRCs with MSI and in families with HNPCC.

Detection of MSI

Genomic DNA level
Gene mutations
Tumor level
Microsatellite analysis
Loss of expression of enzymes
MSH-2, MLH-1, MSH-6
50% of HNPC tumors have MSI
13% of all CRC have MSI
7% of CRC are associated with HNPC

MSI testing can be conducted for
$300-$1,000
Serves as a useful screening test prior to full MSI, MLH1 testing

**Interpretation of Results**

- **MSI High**
  - 2 or 5 loci
- **Recurrent loci**
- **MSI Low**
  - 1 of 5 loci
- **<30% loci**
- **Microsatellite Stable**
- **0 loci**

**Potential Targets of Mass Population Molecular Genetic Screening Programs**

- Cystic fibrosis (launched 2001)
- Thrombophilia Panel
  - Factor V - Leiden
  - Prothrombin 20210A variant
  - Protein S/C deficiency
  - Methyltetrahydrofolate reductase variant
  - Angiotensin converting enzyme polymorphism
- Hemochromatosis
- Alzheimer's disease (ApoE4 allele)
- HIV resistance (CKR-5 deletion)
- Congenital hearing loss
- Age-related macular degeneration
- Fragile X syndrome

**Solid Tumor**

**Chromosomal Translocation in Ewing's Tumor**

- Small round cell tumor of childhood
  - Ewing's sarcoma (common bone tumor)
  - Peripheral primitive neuroectodermal tumor (pPNET)
  - Neuroblastoma
  - Rhabdomyosarcoma
Ewing’s Tumors

- t(11:22)(q24;q12) occurs in 83% of cases.
- Translocation involves the EWS gene on chromosome 22 and the FLI-1 transcription factor gene on chromosome 11.
- Translocation results in the c-terminal FLI-1 coming under the control of the EWS gene promoter.
- Effective treatment and appropriate therapeutic approaches are based upon specific tumor type.

Chromosomal Translocations In Sarcomas: Detection Methods

- Conventional cytogenetics
- Fluorescence in situ hybridization (FISH)
- Southern blot
- RT-PCR
- Fluorescent in situ hybridization (FISH)

Sample types:
- Whole cells
- Tissue sections
- DNA
- RNA
- Protein

Involvement of EWS Gene Family In Sarcomas

- TAF1, 68
- TEC
- ATFI
- WT1
- FLI1
- ETVI
- EIAF
- FEV
- ERG
- CHOP

- Extraocular myxoid chondrosarcoma (25%)
- Clear cell sarcoma (>90%)
- Desmoplastic small round cell tumor (99%)
- Ewing sarcoma (1%)
- Ewing sarcoma / peripheral neuroectodermal tumor (PNET) (1%)
- Myxoid liposarcoma (99%)

Non-“EWS Family” Gene Fusions In Sarcomas

- SYT
- SSXI
- SSX2
- SSX4
- PAX3
- PAX7
- FKHR
- COLIA1
- PDGFB
- ETV6
- NTRK3
- ALK

- Synovial Sarcoma (60%)
- Alveolar soft part sarcoma (25%)
- PNET (10%)
- Dedifferentiated chondrosarcoma (99%)
- Pancreatic neuroendocrine tumor (99%)
- Congenital fibrosarcoma (99%)
- Inflammatory myofibroblastic tumor (99%)

Chromosomal translocations in sarcomas
Detection of chromosomal translocations by Southern blot analysis

Southern blot analysis
Practical Considerations
- Requires microgram quantities of DNA
- Often need multiple enzymes and probes
- High quality DNA - no degradation
- Not applicable to paraffin blocks
- Sensitivity to ~5%

Peripheral Primitive Neuroectodermal Tumors (pPNETs)

EWS (22q12)
FLI-1 (11q24)

EWS/FLI-1 Fusion transcript (mRNA)
(3;11)(p13;q24)

5' → Reverse Transcription → 3'

5' → Chimeric cDNA → 3'

PCR

PCR analysis
Practical Considerations
- Only need small amounts of RNA or DNA
- Variably degraded nucleic acid acceptable
- Applicable to paraffin blocks
- Sensitivity to 1/10⁶ - minimal disease assay

Interphase FISH using the EWSR1 Break-Apart Probes

Normal Cell
Tumor Cell

Breaks
EWSR1 gene

Chr 22 cen
~ 500,000bp
Red Probe
~ 1,100,000bp
Green Probe
**FISH analysis**

**Practical Considerations**
- Applicable to small samples: touch imprints
- Some nucleic acid degradation acceptable
- Applicable to paraffin blocks
- Useful when only know one of two involved loci (splitting assay)
- Sensitivity to 5-10%

**Chromosomal translocations in sarcomas: Clinical utility**
- Differential diagnosis
- Prognosis
- Minimal disease detection

---

**EWS-FLI1 fusion types correlate with survival in patients with localized Ewing sarcoma**

![Graph showing survival rates for EWS-FLI1 and other types](image)

- EWS-FLI1 type 1 (n = 49)
- Other types (n = 27)

**Minimal disease detection in Ewing’s sarcoma**

- 92 patients with clinically localized disease
- RT-PCR bone marrow for EWS-FLI1/ERG
- 18 positive
- 74 negative

<table>
<thead>
<tr>
<th>Relapse rate:</th>
<th>50%</th>
<th>11%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 yr DFS:</td>
<td>53%</td>
<td>80%</td>
</tr>
<tr>
<td>p</td>
<td>0.043</td>
<td></td>
</tr>
</tbody>
</table>

(Schäfer-Preuße et al., 2003)

---

**Importance of Chromosomal Translocations**
- Consistency and specificity
- Opportunities for advances in:
  - Biology
  - Diagnosis
  - Therapy

**Mechanisms of fusion protein function**

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Fusion protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aberrant transcription factor</td>
<td>PAX-FKHR, EWS &amp; FUS fusions, TFE3-AP1, SYT-SSX</td>
</tr>
<tr>
<td>Aberrant chromatin binding</td>
<td>COL1A1-PDGFB</td>
</tr>
<tr>
<td>Ectopic growth factor</td>
<td>ETV6-NTRK3 activation, ALK fusions</td>
</tr>
<tr>
<td>Constitutive tyrosine kinase</td>
<td></td>
</tr>
</tbody>
</table>
What Questions You Will Ask When BRCA Is Diagnosed

- What is the best treatment approach?
- What kind of therapy is needed?
- How much therapy?
- Finally, whether to choose the newer, more highly specific biological therapies such as Herceptin® for treatment of breast cancer?

Gene Amplification in Breast Cancer

- Amplification/overexpression of HER-2 seen in 25–30% of primary human breast cancer.
- Gene amplification is associated with worse clinical outcome in patients with node-positive patients.
- HER-2+ patients may benefit from chemotherapeutic and adjuvant therapy.
- Patients with HER-2+ advanced metastatic breast CA are likely to benefit from Herceptin.

HER2

- Member of growth factor receptor family
- Chromosome 17q21
- 185 kDa transmembrane tyrosine kinase growth factor receptor
- Overexpression due to gene amplification or enhanced transcription

Differences in Interpretation: HER-2 Assessment by FISH and IHC

- **High amplification**
- **Low amplification**
- **Normal**

- **3+**
- **2+**
- **1+**
- **Negative/0**
New “Equivocal” Result

Three categories of test results for each test:
- Positive:
  - FISH HER2 gene/CEP17 ratio of 3.2 or greater or
  - FISH HER2 gene copy number of >6.0
- Equivocal:
  - FISH HER2 gene/CEP17 ratio of 1.8-2.2 or
  - FISH HER2 gene copy number of 4.0-6.0
- Negative:
  - FISH HER2 gene/CEP17 <1.8 or
  - FISH HER2 gene copy number of <4.0

Sensitivity of Cytology for Urothelial Carcinoma by Grade
(Review of Literature)


FISH for the Detection of Bladder Cancer

FDA approved for:
- Detection of recurrent tumor in patients with a history of bladder cancer (2001)
- Evaluation of patients with hematuria for bladder cancer (2005)

UroVysion™

Relative Frequency of Different Types of Alterations

- Polysomy/tetrasomy: >95% of cases
- Homozygous 9p21 deletion: <5% of cases
- Trisomy: <5% of cases

Polysomy
- Gains of two or more of the four chromosomes
- Need to see 24 aseq on slide
- Abnormality is called case positive
- Most common abnormality seen (>50%)
- Correlates well with presence of higher grade tumor (i.e., grade 3 papillary, CIS, or invasive tumor)

Homozygous 9p21 Loss
- Loss of both copies of 9p21 (ie, of PTEN tumor suppressor gene)
- <3% of positive cases
- Correlates well with presence of high-grade papillary tumor
- Hemizygous 9p21 loss not currently used as positive criteria but could be in future
**UroVysion™ vs. Other Assays**

<table>
<thead>
<tr>
<th>Sensitivity (Overall and by Stage) and Specificity of Tests</th>
<th></th>
</tr>
</thead>
</table>


---

**UroVysion™ Comparison Studies**

**Sensitivity** (n=74*)

- Cystoscopy: 74%
- Cytology: 58%
- FISH: 81%
- Cystoscopy + Cytology: 88%
- Cystoscopy + FISH: 98%


---

**UroVysion™ Conclusions**

- **Negative FISH** result increases the confidence for safe extension of cystoscopy intervals.
- **Positive FISH** result should warrant a close follow-up with re-biopsy.

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**Molecular Hematology**

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**Hematopathology Diagnostic Approaches**

- H & E stain/EM - morphology
- Special histochemical Stains - cellular accumulation
- Immunohistochemistry - differentiation
- Flow cytometry - immunophenotyping
- Conventional cytogenetics (karyotyping)
  - chromosomal structure

**Molecular diagnostics - genotyping**

---

**Diagnostic Molecular Pathology in Hematopoietic Neoplasms**

- Determination of clonality
- Lineage assessment
- Detection of minimal or residual disease
- Detection of chromosomal translocation
  - t(9;22)(q34;q11): bcr-abl in CML/ALL
  - t(14;18)(q32;q21): bcl-2/ig11 in follicular lymphoma
Immune System

- Diversity: Must be able to recognize a wide variety of antigens
- DNA recombination: Large number of V, D, J and C segments can be transcribed and translated to millions of antigen receptors
- Somatic mutation: Allows for even more diversity

Determination of Clonality

- Definition: Population of cells with similar characteristics that are all derived from a single precursor cell.
- Morphology: Monomorphic cell population
- Immunopathology: K or L light chain
- Cytogenetics: Recurrent chromosomal alteration (e.g., translocation)
- Molecular genetics: Clonal B- or T-cell gene rearrangements (Southern blot or PCR)

Kappa and Lambda for Lymphoma

*Detection of Kappa and Lambda light chain mRNA in plasma cells and B-lymphocytes
*Each Immunoglobulin molecule contains either two
*Copies of Kappa or lambda light chains
*Kappa/lambda ratio 2:1 in Reactive Lymphoid Hyperplasia
*Kappa/lambda ratio 3:1 and greater or L/K 0.5 and smaller : B cell

*Ventana Benchmark (ISH)
60,000 people were diagnosed with a lymphoma in 2003.

Southern Blot

- Popular method for determining B- and T-cell clonality
- Uses DNA probes (large segments of DNA that recognize primarily J or C segments)
- May detect as little as a 1–5% clonal population

Polymerase Chain Reaction

- Increasingly popular method to evaluate for the presence of B- or T-cell clonality.
- Analogous to the Southern blot, involves evaluation of segments of DNA that code for the variable regions of the immunoglobulin and T-cell receptor (V and J segments).
- Uses consensus V and J segment primers (recognize shared DNA sequences).
- May detect a 0.1% clonal population.
Chromosome Translocations in Non-Hodgkin's Lymphoma

- A number of *nonrandom* translocations associated with specific subtypes of non-Hodgkin's lymphoma
- Examples: *bcl-1* in mantle cell lymphoma
  *bcl-2* in follicular lymphoma
- Demonstrate by Southern blot, PCR and FISH
- Identification may help confirm a diagnosis
- Used to monitor patients for evidence of minimal residual disease

Proto-oncogene *bcl-2*

- Normally resides on chromosome 18
- Involved in blocking apoptosis (programmed cell death)
- Expression limited to long lived cells
- In FCCL, *bcl-2* becomes overexpressed following t(14,18) extending lifespan of follicular center cells
- Translocation occurs in 80-90% of cases

Molecular Abnormalities in Some Lymphomas

PCR Detection of T-cell Receptor Gamma Gene Rearrangements

- Blank
- Negative
- Positive
- Polyclonal
- Patient
- Marker

180 bp

PCR Analysis of *bcl-2/IgH* Gene Rearrangements

- *bcl-2/IgH* fusion gene
- *mchi* primer
- *JH* primer
- *mcl* primer
- *JH* primer
Microarrays Designs and Applications

cDNA Microarrays in Gene Expression Studies

cDNA Microarrays:
- cDNA clone inserts are printed onto glass slides in high density.
- As many as 25,000 cDNAs can be applied to a single glass slide.
- Enables large-scale, high-throughput analysis.
- Does not require knowledge of cDNA sequence.
- Thousands of known genes and ESTs are available.
- Requires special instrumentation for generation of microarrays and for analysis of results.

Arrays allow simultaneous assessment of expression for all 30,000 human genes

 ✓ DNA array make it possible to survey patterns of expression for thousands genes simultaneously;
 ✓ The expression pattern of a cluster of genes in a given cancer will be correlated with the morphology clinical behavior and the treatment response.

Goal of DNA Array Studies

- Insights into pathogenesis
- Cancer diagnosis Molecular Profiling
- Prediction of clinical outcome of a diagnosis
- Identification of therapeutic targets
- Re-classification of cancer
Immediate potential Rx applications...

<table>
<thead>
<tr>
<th>Disease</th>
<th>Genotype</th>
<th>Rx relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric MALToma</td>
<td>AP2/MALT1 fusion</td>
<td>No value in treating H. pylori</td>
</tr>
<tr>
<td>PTLD</td>
<td>ABCD mutation</td>
<td>Will not regress with Rx withdrawal</td>
</tr>
<tr>
<td>DLBCL</td>
<td>ABC profile/NFκB activation</td>
<td>Rx with proteasome inhibitors</td>
</tr>
<tr>
<td>SLL/CLL</td>
<td>IGH/SHM/ZAP70</td>
<td>No need for aggressive Rx</td>
</tr>
<tr>
<td>ALL</td>
<td>BCR-ABL1 fusion</td>
<td>Only cure with SCT</td>
</tr>
</tbody>
</table>

Common Genotypic Abnormalities in AML

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Karyotype</th>
<th>Gene</th>
<th>Clinical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td>15/20</td>
<td>NPM1</td>
<td>good response to chemotherapy, monoblastic leukemia</td>
</tr>
<tr>
<td>M5</td>
<td>t(8;21)</td>
<td>RUNX1</td>
<td>monoblastic leukemia</td>
</tr>
<tr>
<td>M2</td>
<td>t(15;17)</td>
<td>PML-TEL</td>
<td>good response to chemotherapy, monoblastic leukemia</td>
</tr>
<tr>
<td>M6</td>
<td>inv(16)</td>
<td>t(16;16)</td>
<td>therapy-related or intrinsic, poor prognosis</td>
</tr>
<tr>
<td>AML-M3</td>
<td>t(15;17)</td>
<td>NPM1</td>
<td>therapy-related or intrinsic, poor prognosis</td>
</tr>
<tr>
<td>AML</td>
<td>Occult</td>
<td>FLT3-ITD</td>
<td>poor prognosis in AML with normal karyotype</td>
</tr>
<tr>
<td>AML</td>
<td>Occult</td>
<td>APL/ETO</td>
<td>poor prognosis in AML with normal karyotype</td>
</tr>
</tbody>
</table>

Refining the Prognosis of AML

- The patient is a 45 year-old male with a past medical history of gastroesophageal reflux disease and hyperlipidemia who presented to his primary care physician with a complaint of increasing fatigue. A routine laboratory assessment was performed and revealed a white blood cell count of 14 x 10^3/L and platelet count of 108,000/L. A bone marrow biopsy was performed and was consistent with acute myeloid leukemia.
Testing for the PML-RARα fusion gene product from the 15;17 translocation ((t(15;17)) in acute promyelocytic leukemia (PML).
**JAK2 1849G>T [V617F] Mutation is Specific for Chronic Myeloproliferative Disorders**

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycythemia vera (PV)</td>
<td>74%</td>
</tr>
<tr>
<td>Essential thrombocythemia (ET)</td>
<td>38%</td>
</tr>
<tr>
<td>Chronic idiopathic myelofibrosis (CIMF)</td>
<td>44%</td>
</tr>
<tr>
<td>AML, MDS, and other myeloid disorders</td>
<td>N/A</td>
</tr>
<tr>
<td>AML arising in JAK2-mutated MDS</td>
<td>N/A</td>
</tr>
<tr>
<td>Lymphoid neoplasia</td>
<td>0%</td>
</tr>
<tr>
<td>Normal or reactive tissue</td>
<td>0%</td>
</tr>
</tbody>
</table>

**CML**

- "leukemia"
- stem
- Ph (9;22) t(9;22)
- BCR/ABL
- CP → AP → BP
- Differential dx
- Rx: imatinib
- monitoring Rx

**Mechanism of Action of Gleevec**

- ATP
- Gleevec
- BCR-ABL1
- Substrate

**PRE**

- STI571 3 months
Applications of DNA FISH Probes in Hematopoietic Malignancies

- DNA FISH probes detect translocations that are defined in the W.H.O. classifications (JCO 1999; 17:3835-49)
  - AML with t(8;21)
  - AML with inv(16) or t(15;17)
  - AML with 11q23 (MLL) abnormalities
- In the t(15;17) the AML is detectable by FISH (N Engl J Med 1997; 337, 1024-30)
- FISH can be used to detect the BCR/ABL fusion (JCO 2000; 18(7):1533-8)
  - 19q13(BCR/ABL)
- FISH also accommodates Lymphoid breakpoint variation Better than PCR
  - examples: t(11;14), t(14;18), t(8;14), t(6;9), t(3;23), & t(4;8)

Molecular Microbiology

- Qualitative Testing-detection of pathogen presence or absence
- Quantitative Testing-viral load for management of infected individuals
- Drug Resistance Testing-detection of mutations associated with resistance
- Molecular Epidemiology-molecular strain identification to examine epidemic outbreaks

Recommended Genetic Monitoring of CML in patients on Gleevec or similar tyrosine kinase inhibitors

Measure minimal residual disease quarterly by Q-rTPCR on serial blood samples

Goal is to achieve Major Molecular Response (MMR) = ~3 log reduction in tumor burden

Hughes TP. Blood 100:29, 2002
**HSV Primary Infection**

*Acute necrotizing encephalitis*
- May be due to either primary infection or reactivation
- Infection of the brain by HSV
- Neurons of the temporal lobe are most commonly involved
- Infection is severe and necrotizing
- Clinical features include:
  - Sudden onset of fever
  - Headache
  - Confusion
  - Alteration in personality
  - High mortality rate

**HSV Primary Infection**

*Neonatal Infection*
- Exposure may occur
  - At birth if mother has genital herpes at time of delivery
  - Post-natal period when infant is handled by people with herpetic lesions

**Clinical Applications of HSV PCR**

Rapid diagnosis of HSV encephalitis—Current Gold Standard is PCR
- >95% sensitive in adults
- 70 – 100% sensitive in neonates
- Essential for appropriate use of antimicrobial therapy—Acyclovir and Valacyclovir
- Monitor response to antiviral therapy
- More rapid diagnosis of HSV meningitis or encephalitis

**Clinical Applications of HSV PCR**

Detect subclinical shedding of HSV during pregnancy
- ~70% of neonatal HSV result from asymptomatic shedding by the mother
- PCR considerably more sensitive than culture for detecting HSV in vaginal secretions

**METHICILLIN-RESISTANT S. AUREUS (MRSA)**

- Positive Blood Culture
- *Staphylococcus aureus*
- meca Gene Detection

- Direct Detection of MRSA from Blood Cultures by Real-Time Detection
- Detection of *S. aureus*-specific DNA and the meca gene equals presence of MRSA
Molecular Techniques for HPV Detection

- It is the second most common cancer among women worldwide.
- In 2004, the American Cancer Society estimates that there will be about 12,200 new cases of invasive cervical cancer in the U.S. About 4,100 women will die from this disease.
- Cervical cancer mortality has not declined in the U.S. since the 1980s.

ASCUS Management: The Dilemma

ASCUS diagnosis

- Disease present
- No disease present

<table>
<thead>
<tr>
<th>Disease present</th>
<th>No disease present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colposcopy/treatment</td>
<td>False positive. No colposcopy required.</td>
</tr>
</tbody>
</table>
Past Suggestions for Equivocal Cervical Cytology

- Repeat Pap test (3–6 months)
  - May delay or miss detection of significant disease
  - Patient anxiety while waiting for resolution of first Pap smear
  - Sensitivity of only 76.2%.

Past Suggestions for Equivocal Cervical Cytology (cont.)

- Colposcopy
  - Up to 78% of ASCUS reports are normal on colposcopy
  - As much as $300–$1200 is added to management costs
  - Patient anxiety over risk of cancer
  - Invasive procedure
  - Largest proportion of HSIL comes from women whose Pap smears are reported ASCUS

http://www.cytopathology.org/NIH/

- The Bethesda atlas figure number (chapter # and figure #)
- The Bethesda terminology pull out table
- Keywords
- Specimen preparation type: Conventional smear, ThinPrep
- Histology, SurePath
- Bethesda Interobserver Reproducibility Project (BIRP) images with interobserver variability histograms

Clinical Relevance of Hybrid Capture® II HPV Testing

- 97% Sensitivity: More effective in detecting HSIL than repeat Pap
- Detects the underlying cause of cervical cancer
- 99.9% negative predictive value for women with two negative HPV results

HPV

- Primary cause of cervical cancer.
- Over 70 site-specific types.
- 5–10% of women >35 years of age are persistent carriers of HPV.
- HPV E6 and E7 proteins inhibit p53 and pRB protein functions.

Digene HC 2 Assay

- Only FDA approved method currently available
- Two configurations
  - Low Risk HPV
  - High Risk
    - Types: 6, 11, 16, 31, 33, 35, 39, 45, 51, 52, 58, 59, 66
- Recommended Specimen Types
  - Cervical Swabs in proprietary collection device
  - Liquid Collection media
  - CytoThinPrep (FDA approved)
  - TruPath (BD) SurePath

(Caption for image)

24
Digene Hybrid Capture® DML 2000 System

- Commercially available
- FDA approved
- Two probe mixtures
  >High-risk: 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, 59 and 68
  >Low risk: 6, 11, 42, 43, 44
- Sensitivity 5,000 copies of HPV

Prognostic Value of Persistent High-risk Type HPV Infection

- 60–70% of women who are HPV positive and cytology negative will develop their first abnormal Pap smear within 4 years.
- HPV detection in women >30 is likely to represent persistent infection.
- The positive predictive value of HPV DNA for the detection of CIN rises with age, whereas that of cytology decreases.

Prognostic Value of High-risk Genotype HPV Infection

Prospective studies have shown that 28% of HPV DNA-positive women (cytology normal) developed SIL within 2 years compared to only 3% of HPV DNA negative women.

Patient Management Using HPV Triage

ASCUS

\[\text{Digene HPV Test}\]

\[\text{Low risk or HPV-}\]

Repeat Pap and/or HPV test in 6 mo or return to routine screening at discretion of clinician

Colposcopy

\[\text{Biopsy/Ablation}\]

Turnaround Time

- Viral Isolation
  - Positive result: average 108 hours
  - Negative result: average 154 hours
- PCR
  - Range: 36–72 hours
  - Mean estimated 48 hours
Pharmacogenetics

**Trends in Disease Management**
Pharmacogenomics a key driver — genetic variations that influence the response to therapeutic drugs.

**Pharmacogenetics — Warfarin**
CYP2C9 and VKORC1 variants influence variability in warfarin dosage requirements as do various environmental factors (body size, vitamin K status, concurrent disease, etc.)
CYP2C9*2 and *3 are 12% and 5% as efficient as the CYP2C9*1 enzyme; individuals with these 2 genotypes are at increased risk of bleeding.
Warfarin causes anticoagulation by inhibiting VKOR (Vitamin K epoxide reductase); polymorphisms in VKORC1 have been shown to contribute to both warfarin sensitivity (haplotype A1 as well as warfarin resistance (haplotype B5).

**Warfarin is metabolized in the liver by CYP2C9**
- CYP2C9 SNPs effects
  - CYP2C9*1 (WT) normal
  - CYP2C9*2 (Arg144Cys) low / intermediate
  - CYP2C9*3 (Ile359Leu) low

**Effect of CYP2C9 Genotype on Warfarin Maintenance Dose**

![Graph showing effect of CYP2C9 genotype on warfarin maintenance dose.](image)

**Pharmacogenetics — Irinotecan and UGT1A1**
- UGT1A1 (uridine diphosphate-glucuronosyl transferase 1A1)
  - Inactivates the irinotecan metabolite SN-38 (7-ethyl-10-hydroxy-camptothecin) to SN-38 glucuronide
- Wild-type promoter region: UGT1A1*M is six TA repeats
- Three other variants have been identified: five, seven and eight TA repeats
- UGT1A1*28 (TA7) is homozygous in ~10% of North Americans.
- The increase in TA repeats decreases SN-38 glucuronidation which leads to prolonged plasma levels of SN-38 and an increased risk of irinotecan-induced diarrhea and leukopenia.
- Third-Stage Technologies markets an in vitro assay for UGT1A1*1 and UGT1A1*28 that is FDA approved.
JOINT DISEASES AND IMMUNOLOGIC DISORDERS

Nasser Gayed, MD

(FOR SELF-STUDY – goes with early Jan. ’15 session)
Lab Evaluation of Immune Diseases

Systemic Lupus Erythematosus
- Multisystem autoimmune disease
- 90% in women, mostly childbearing age
- More common in blacks

Systemic Lupus Erythematosus 11 Criteria
1. Malar rash (distribution and type)
2. Discoid rash (type)
3. Photosensitivity (rash)
4. Oral ulcers
5. Nonerosive arthritis (2 or more joints)
6. Serositis (pericarditis, pleuritis)
7. Renal (proteinuria or cellular casts)
8. Neurologic (seizures or psychosis)

Systemic Lupus Erythematosus 11 Criteria
9. Hematologic (hemolytic) anemia, leukopenia, lymphopenia, or thrombocytopenia
10. Immunologic (anti-ds DNA, anti-Sm, or antiphospholipid, more on that one later)
11. ANA without exposure to drugs known to cause positive ANA

Drug induced Lupus
- Procainamide, Hydralazine, INH
- Cause positive ANA in some patients
- Clinical disease in a small minority of those with positive ANA
- Means: ALL patients with drug induced lupus will have positive ANA
Back to 11 Criteria

- Need 4 at any time, serially or simultaneously during any interval
- Approach to diagnosis versus criteria for research.

Antiphospholipid

- 3 types?
- 3 ways to be positive
  1. False positive test for syphilis
  2. Anticardiolipin antibodies
  3. Lupus Anticoagulant: definition
     Arterial and venous thrombosis!, miscarriage

ANA Sensitivity, Specificity

<table>
<thead>
<tr>
<th>Disease</th>
<th>ANA frequency sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>95-99%</td>
</tr>
<tr>
<td>Drug induce LE</td>
<td>100%</td>
</tr>
<tr>
<td>Is it specific?</td>
<td></td>
</tr>
<tr>
<td>Sjogren's syndrome</td>
<td>75%</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>50-60%</td>
</tr>
<tr>
<td>MCTD</td>
<td>99-100%</td>
</tr>
<tr>
<td>RA</td>
<td>20-40%</td>
</tr>
</tbody>
</table>

ANA Pattern

- May give clue to what particular antibodies are present
- Diffuse
- Peripheral or rim
- Speckled
- Nucleolar

Individual Antibodies

- Anti-ds-DNA and Anti-Sm are highly specific (hence included in criteria) but not sensitive.
- High titer of Anti-ds-DNA associated with severe nephritis.
- Anti-Ro (SS-A) crosses placenta, causes neonatal lupus: Congenital Heart Block.
- Drug induced lupus: Anti-histone: No anti-ds-DNA
Other labs
- CBC
- Complement levels low
- ESR correlates with disease activity
- CPR if markedly elevated >10mg/dl indicates bacterial infection

Useful Information
- ANA very sensitive for SLE. So a negative ANA can help rule out SLE (who needs that?)
- In what setting is it useful?
- Not specific. It is so nonspecific that it is sensitive for other diseases.
- The higher the titer the more specific.
- If a patient already has 3 criteria then...?

In what setting is it useless?
- Email
  - “I sent an ANA on a young female patient with unexplained weight loss but not much else. Her ANA was 1:80, species. Would you make much of this in the absence of other symptoms?”

Advice
- Before you order a lab you should know what to do with it when it comes back.
- If you don’t, don’t order it.

Rheumatoid Arthritis
- Persistent inflammatory synovitis
- Multisystem disease
Criteria

Four of seven criteria are required to classify a patient as having rheumatoid arthritis (RA).

Criteria
1. Morning stiffness: Stiffness in and around the joints lasting 1 h before maximal improvement.
2. Arthritis of three or more joint areas: At least three joint areas, observed by a physician simultaneously, have soft tissue swelling or joint effusions, not just bony overgrowth. The 14 possible joint areas involved are right or left proximal interphalangeal, metacarpophalangeal, wrist, elbow, knee, ankle, and metatarsophalangeal joints.
3. Arthritis of hand joints: Arthritis of wrist, metacarpophalangeal joint, or proximal interphalangeal joint.
4. Symmetric arthritis: Simultaneous involvement of the same joint areas on both sides of the body.
Criteria

5. Rheumatoid nodules: Subcutaneous nodules over bony prominences, extensor surfaces, or juxtaarticular regions observed by a physician.
6. Serum rheumatoid factor: Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in less than 5% of normal control subjects.
7. Radiographic changes: Typical changes of RA on posteroanterior hand and wrist radiographs that must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints.

Systemic manifestations

- Nodules
- Vasculitis
- Pleural effusion: very low glucose
- Felty’s syndrome = RA, splenomegaly, neutropenia...interesting

Synovial Fluid

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal</th>
<th>Group I (Vasculitis)</th>
<th>Group II (Inflammation)</th>
<th>Group III (Sjogren’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mL)</td>
<td>&lt;3 mL</td>
<td>&gt;3.5 mL</td>
<td>&gt;3.5 mL</td>
<td>&gt;3.5 mL</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Very high</td>
<td>High*</td>
<td>Low</td>
<td>Variable</td>
</tr>
<tr>
<td>Color</td>
<td>Clear</td>
<td>Stainless</td>
<td>Yellowish</td>
<td>Variable with organisms</td>
</tr>
<tr>
<td>Clarity</td>
<td>Transparent</td>
<td>Transparent</td>
<td>Translucent, opaque of tines</td>
<td>Opaque</td>
</tr>
<tr>
<td>Leukocytes/mm³</td>
<td>200</td>
<td>200-1000</td>
<td>3000-50000</td>
<td>&gt;500000</td>
</tr>
<tr>
<td>Polymorphonuclear cells/µL</td>
<td>&lt;12</td>
<td>&lt;22</td>
<td>&gt;100,000</td>
<td>&gt;200,000</td>
</tr>
<tr>
<td>Culture</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Usually positive</td>
</tr>
</tbody>
</table>

Still’s Disease

- A subtype of Juvenile RA
- Negative RF
- With systemic manifestations
- Can occur in adults

RF

- Ig (usually IgM) antibody against Fc portion of IgG

RF Sensitivity, Specificity

<table>
<thead>
<tr>
<th>Disease</th>
<th>RF frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA</td>
<td>50-80%</td>
</tr>
<tr>
<td>Is it specific?</td>
<td>15-35%</td>
</tr>
<tr>
<td>SLE</td>
<td>20-30%</td>
</tr>
<tr>
<td>Sjogren’s syndrome</td>
<td>75-95%</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>20-30%</td>
</tr>
<tr>
<td>MCTD</td>
<td>50-60%</td>
</tr>
<tr>
<td>Polymyositis</td>
<td>5-10%</td>
</tr>
</tbody>
</table>
Non-rheumatic

<table>
<thead>
<tr>
<th>Disease</th>
<th>RF frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>General public</td>
<td>5%</td>
</tr>
<tr>
<td>Aging (&gt;70)</td>
<td>10-25%</td>
</tr>
<tr>
<td>Bacterial endocarditis</td>
<td>25-50%</td>
</tr>
<tr>
<td>Parasitic diseases</td>
<td>20-90%</td>
</tr>
<tr>
<td>Viral infections</td>
<td>15-65%</td>
</tr>
<tr>
<td>Liver disease</td>
<td>15-40%</td>
</tr>
<tr>
<td>Cryoglobulinemia</td>
<td>40-100%</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>45-70%</td>
</tr>
</tbody>
</table>

RF summary
- Moderately sensitive and specific
- Higher titers have higher specificity
- Higher titers and more sensitive in more severe disease. But does not correlate with disease activity!
- 100% sensitive in patients with nodules or vasculitis.
- Higher titer in patients with vasculitis.
- Positive predictive value low in most non-specialty settings (<1/3)

RF summary
- A negative test does not rule out RA (20-35% of RA are seronegative)
- Seronegative RA (contrast to SLE ANA)
- Can be used to assess prognosis
- High titer RF without rheumatic disease think of SBE and cryoglobulinemia

Other labs in RA
- Anemia and thrombocytosis correlate with disease activity
- ESR and CRP also sensitive and correlate with disease activity

The future of RA
- Anti-CCP = Anti-cyclic citrullinated peptide
- Old names antikeratin and antiperinuclear factor, anti flgagrin
- The future is bright
- SPECIFIC 98%
- Sensitivity 50-70%
- Can be positive before clinical disease (the future is not so bright!)

Systemic Sclerosis
“Scleroderma”
- Accumulation of connective tissue causing thickening of skin, fibrosis of other organs
Scleroderma

- Caution!
- Diffuse cutaneous versus limited cutaneous
- Limited cutaneous: CREST syndrome = calcinosis, Raynaud’s phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia.
- Prognosis is good (no renal disease) except for patients who develop pulmonary hypertension.

<table>
<thead>
<tr>
<th>Features</th>
<th>Limited Cutaneous SSc (%)</th>
<th>Diffuse Cutaneous SSc (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin involvement</td>
<td>90</td>
<td>100</td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>99</td>
<td>88</td>
</tr>
<tr>
<td>Esophageal involvement</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>Pulmonary fibrosis</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>Pulmonary arterial hypertension</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>Myopathy</td>
<td>11</td>
<td>23</td>
</tr>
<tr>
<td>Cardiac involvement</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Scleroderma renal crisis</td>
<td>2</td>
<td>15</td>
</tr>
</tbody>
</table>

Scleroderma

Antibodies
- Antitopoisomerase = Anti Scl-70, specific for SSc but only 40% sensitive
- Anticentromere specific for CREST = limited, but 70% sensitive

Polymyositis/ Dermatomyositis
- Presents as proximal muscle weakness = difficulty climbing steps, getting up from chair.
- No inflammatory symptoms or signs
- Dermatomyositis is associated with malignancy: ovary, breast, melanoma, colon. Look for those! No extensive invasive tumor search.

Laboratory findings
- CK is markedly elevated (50X normal limit), but not 100% sensitive
- ALT, AST, LDH, aldolase
- EMG will show “myopathic” pattern
- Muscle biopsy diagnostic: “inflammatory myopathy”
- Anti-Jo-1 associated with interstitial pulmonary disease/fibrosis
MCTD

- Mixed connective tissue disease patients have features of SLE, SSc, Polymyositis, and RA.
- But it is a distinct disease.
- AND a very high titer of anti nuclear RNP, now called anti-U1 RNP. 100% sensitive.

Sjogren Syndrome

Autoimmune lymphocytic infiltration of exocrine glands

Sjogren Syndrome

I. Ocular symptoms:
   1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?
   2. Do you have a recurrent sensation of sand or gravel in the eyes?
   3. Do you use tear substitutes more than three times a day?

II. Oral symptoms:
   1. Have you had a daily feeling of dry mouth for more than 3 months?
   2. Have you had recurrent or persistently swollen salivary glands as an adult?
   3. Do you frequently drink liquids to aid in swallowing dry foods?

Sjogren Syndrome 3 probable, 4 definite

III. Ocular signs: one of the following two tests:
   1. Shirmer’s I test, performed without anesthesia (5 mm in 5 min)
   2. Rose Bengal score or other ocular dye score (4 according to van Bijsterveld's scoring system)

IV. Histopathology: In minor salivary glands focal lymphocytic sialoadenitis, with a focus score of 1

Sjogren Syndrome

V. Salivary gland involvement: one of the following diagnostic tests:
   1. Unstimulated whole salivary flow (1.5 mL in 15 min)
   2. Parotid sialography
   3. Salivary scintigraphy

VI. Antibodies in the serum to Ro/SS-A or La/SS-B antigens, or both

Sjogren Syndrome

HIV infection causes a “Sicca syndrome” almost identical to Sjogren’s except:
   1. No anti-Ro/SS-A, or anti-La/SS-B
   2. Lymphoid infiltrates are CD8+

Sjogren associated with Chronic hepatitis and primary biliary cirrhosis
Sjogren Syndrome
- Anti-Ro (SS-A), Anti-La (SS-B), indicate more severe disease

ESR
- Polymyalgia rheumatica, giant cell arteritis very sensitive, also useful to monitor disease course
- Also very sensitive for SBE
- Helpful in monitoring treatment and disease activity in IBD, osteomyelitis, PID, abscess
- Rises with age (normal = age/2), pregnancy, infection, malignancy especially liver cancer
- SUO (remember advice)

CRP
- Less sluggish than ESR
- Usually normal in SLE without bacterial infection

Monoclonal Gammopathies
- Multiple myeloma: Transformation of a lymphocyte into a plasma cell clone that produces Ig or Ig fragments
- Monoclonal protein (M protein)
- Hypogammaglobulinemia?
- Plasma protein electrophoresis will show a narrow band (M spike)
- The M component can be monitored for response to treatment

SPEP
- Will identify the type of Ig and quantify it
- That helps with diagnosis and staging

Immunoelectrophoresis
Why is MM interesting?
- Symptoms are non-specific
- Hyponatremia
- Decreased anion gap
- Hypercalcemia
- Normal bone scan
- Normal alkaline phosphatase
- Bence Jones protein

Why is MM interesting?
- Dipstick negative
- Acid precipitation with sulfosaliclyc acid required

Stage I
All of the following:
1. Hemoglobin >100 g/L (>10 g/dL)
2. Serum calcium <3 mmol/L (<12 mg/dL)
3. Normal bone x-ray or solitary lesion
4. Low M-component production
   a. IgG level <50 g/L (<5 g/dL)
   b. IgA level <30 g/L (<3 g/dL)
   c. Urine light chain <4 g/24 h

Stage III
Any of the following
1. Hemoglobin <85 g/L (<8.5 g/dL)
2. Serum calcium >3 mmol/L (>12 mg/dL)
3. Advanced lytic bone lesions
4. High M-component production
   a. IgG level >70 g/L (>7 g/dL)
   b. IgA level >50 g/L (>5 g/dL)
   c. Urine light chains >12 g/24 h

Stage II
Doesn’t fit I or II
Serum creatinine:
- A = creatinine < 2
- B = creatinine ≥ 2

Survival (in months)
- Based on stage I-III
- and serum creatinine:
  - A = creatinine < 2
  - B = creatinine ≥ 2
- IA: 61
- IIA, B: 55
- IIIA: 30
- IIIB: 15
MENINGITIS

Nasser Gayed, MD

(FOR SELF-STUDY)
MENINGITIS

CLS Case Study

A 30-year-old man was admitted to the hospital because of confusion and headache.

He was well until one month previously, when intermittent headaches developed. Two weeks before admission he began to have nausea, vomiting, and photophobia, and complained of “sinusitis”. During the next week, the symptoms worsened. One week before entry, a brief episode of confusion occurred and subsided spontaneously. His physician prescribed a mixture of phenobarbital and codeine, and the nausea and vomiting ceased. On the day before admission, the patient appeared well and he returned to his work as an electrical engineer, but was brought home later in the day by his associates because of recurrent confusion. He was taken to another hospital where he appeared agitated and combative and spoke gibberish. His temperature was 37.8°C, pulse 95/min, and the respiration 14/min; the hematocrit was 45%, and the white cell count was 7,400/µl with 82% neutrophils. The urea nitrogen was 10 mg/dl and the glucose 124 mg/dl. The sodium was 127 meq/l per liter. A CT scan of the head was reportedly negative. A lumbar puncture yielded colorless cerebrospinal fluid under an initial pressure of 300 mm; the fluid contained 7 red cells, 92 lymphocytes, and 10 neutrophils per cubic millimeter; the glucose was 26 mg/dl, and the protein 408 mg/dl. Microscopical examination of stained specimens of the fluid including an India-ink preparation, revealed no microorganisms. On the following day the patient was transferred to this hospital’s

There was a history of ulcerative colitis nine years earlier; it was asymptomatic during the six years before admission. His wife and two children were healthy. His mother had received pneumothorax for pulmonary tuberculosis in the 1930’s. Repeated tuberculin skin tests performed on the patient as a child and young adult were reported to be negative. There was no history of recent travel, use of medications or illicit drugs, head trauma, mucocutaneous herpes infection, or sexually transmitted diseases.

The temperature was 37.2°C, the pulse 100/min. and the respiration 16/min. The blood pressure was 110/70 mm Hg.

On examination the patient was disoriented, agitated, and intermittently stuporous, but arousable. No rash, ecchymoses, or herpetic lesions were observed. The head was normal, and the neck was supple. No lymphadenopathy was found. The lungs, heart, and abdomen were normal. The cranial nerves were normal. The patient responded to noxious stimuli in all extremities. Motor function was intact, and muscle tone was normal. Cerebellar functions could not be tested. The tendon reflexes were ++ and equal, and the plantar responses were flexor.

Urinalysis was normal; the sediment contained 2 white cells and 5 red cells per high-power field. The hematocrit was 39.3%; the white cell count was 13,000 with 81% neutrophils, 2% band forms, 11% lymphocytes, 5% monocytes, and 1% basophils. The erythrocyte sedimentation rate was 9 mm per hour. A stool specimen gave a negative test for occult blood. The urea nitrogen was 7 mg/dl, the glucose 183 mg/dl, the calcium 9.6 mg/dl, the bilirubin 0.6 mg/dl, and the protein 7.0 g (the albumin 4.1 g, and the globulin 2.9 g/dl. The sodium was 125 meq/l, the potassium 5 meq/l, the chloride 83 meq/l. The serum aspartate aminotransferase was 52 U per milliliter, the lactic dehydrogenase 131 U per milliliter, and the alkaline phosphatase 25 IU/l. HIV serology was negative. An electrocardiogram and x-ray films of the chest were normal. A computed tomographic (CT) scan of the brain revealed moderate, diffuse ventricular enlargement bilaterally. A lumbar puncture yielded slightly cloudy cerebrospinal fluid under an initial pressure of 300 mm; the fluid contained 6 red cells, 45 neutrophils, 50 lymphocytes, and 5 mononuclear cells per cubic millimeter, xanthochromia was graded 1 on a scale of 10; the glucose was 52 mg/dl, and the protein 480 mg/dl. Microscopical examination of specimens stained by Gram and
acid-fast methods and India-ink showed no microorganisms; tests on the cerebrospinal fluid and serum for cryptococcal polysaccharide antigens were negative.

Specimens of cerebrospinal fluid, blood, and urine were obtained for culture, and ceftriaxone and vancomycin were administered. On the evening of the first hospital day the temperature rose to 37.9°C. On the second hospital day the patient was alert, well oriented, and afebrile in the morning but disoriented with inappropriate speech during the remainder of the day. Another physical examination showed no change. The sodium was 130 meq/l, the potassium 4.4 meq/l, the chloride 91 meq/l. In the evening the temperature rose to 38°C. On the following day the patient complained of a mild headache. He was again afebrile and alert, but intermittently disoriented, with inappropriate speech and poor memory for recent events. Repeat cultures of blood, urine, and cerebrospinal fluid continued to be negative. Intake of fluids was limited to 1,000 ml daily. On the fourth hospital day the patient again complained of a mild headache and was disoriented. The hematocrit was 38.5%; the white-cell count was 10,300, with 77% neutrophils. The urea nitrogen was 13 mg/dl, and the glucose 122 mg/dl. A lumbar puncture yielded cerebrospinal fluid that contained 8 red cells, 176 lymphocytes, 60 neutrophils, and 16 mononuclear cells per cubic millimeter, xanthochromia was graded 2 on a scale of 10; the glucose was 33 mg/dl, and the protein 770 mg/dl; microscopical examination of a stained specimen disclosed no acid-fast bacilli; another test for cryptococcal polysaccharide antigen and cytologic examination for tumor cells were negative. Antibiotics were discontinued.

On the fifth hospital day the patient appeared lethargic. The neck was not stiff, no focal neurologic signs were found except for bilateral Babinski signs; the defect in recent memory was more prominent. All cultures continued to be negative. The patient became progressively lethargic. On the sixth hospital day the temperature was 36.8°C. The patient was obtunded and did not obey commands or speak, although he responded to noxious stimuli. A skin test with monilia antigen was positive at 24 hours; a skin test with tuberculin (PPD, 5 TU) was negative.

A diagnostic procedure was performed.
Meningitis
Cerebrospinal Fluid (CSF)

Definitions
- Hypoglycorrhachia: Low glucose in CSF
- Pleocytosis: Increased number of cells (>10 cells/μL)
- Albuminoctologic (Cytoalbuminous) dissociation: High protein, normal cell count
- Xanthochromia: yellow-orange pigment in supernate. Oxyhemoglobin and bilirubin from lysis of RBCs in CSF.
- Aseptic meningitis: negative bacterial cultures (TB causes aseptic meningitis)
- Pyogenic

Risk Factors
- Immunodeficiency (cryptococcal in HIV)
- Head trauma, surgery
- Concurrent infection (pneumonia, pharyngitis, sinusitis, otitis, mastoiditis)
- History of TB, or exposure
- Premature birth, premature rupture of membranes, birth trauma

Symptoms
- Headache
- Stiff neck
- Nausea, vomiting
- Photophobia, diplopia
- Confusion, lethargy
- Fever

Signs
- Stiff neck
- Fever
- Confusion
- RASH
- Kernig’s sign
- Brudzinski’s sign
- Papilledema is unusual

Causes
- Bacterial causes by age group
  Neonates:
  - Group B Strep 40-50%
  - E. coli, 40%
  - Klebsiella, Listeria
1 Month to 15 Years

Used to be
- H. flu 40-60%
- N. meningitidis 25-40%
- S. pneumoniae 10-20%

Since vaccine
Meningococcus > S. pneumo

> 15 Years

- S. pneumoniae 40-50%
- N. meningitidis 15-30%

Younger adults more N. meningitidis

Work-up

- Neurologic exam, fundus.
- If there is papilledema, coma, or focal signs need imaging first to rule out mass, hydrocephalus, herniation, etc.
- DON'T Delay LP if there is no indication for CT.
- If NEED CT, start antibiotics before CT/LP, obtain blood cultures before antibiotics.
- Obtain blood cultures, CBC, BMP.
- Lumbar puncture (spinal tap)

Lumbar Puncture

- Position: lateral decubitus or sitting bending with feet supported and chest on knees (better than bent over bedside stand).
- Palpate for iliac crests (L4 or L4/5 pace).
- Use L3/4 or L4/5.
- Clean, anaesethize.
- Use atraumatic needle 20-22 gauge. May need introducer.
- Show needles.

Lumbar Puncture

- 1 cm below spinous process.
- Advance needle toward umbilicus, hit bone withdraw but stay within skin realm.
- Pain in legs means too lateral.
- Feel pop, withdraw stylet. If no fluid rotate needle.
- Note opening pressure.
- Queckensted maneuver.
- Collect 4 bottles, at least 2 cc each.

Lumbar Puncture

- 1st bottle chemistry
- 2nd bottle micro
- 3rd other
- 4th cell count
- Reinsert stylet, Why?
- Less headache, Why?
- Supine 6 hours?
- NO
Atraumatic needle

- Less headache
- May need more attempts
- Smaller needles (less than 22 gauge) also less headache but require introducer

Lumbar Puncture Complications

- Headache
  - 10% - 60%
  - More common in young and in females.
  - Starts 5 minutes to 4 days after LP.
  - Worse when upright.
  - Resolves spontaneously within 1 week.
  - Treatment: bed rest, hydration, caffeine.
  - If persistent: epidural blood patching.

Lumbar Puncture Complications

- Backache
- Brain herniation
- Hemorrhage
  - subarachnoid, subdural, epidural
- Diplopia
- Radiculopathy
- Infection

NORMAL VALUES

<table>
<thead>
<tr>
<th>Age</th>
<th>Protein mg/dl</th>
<th>Glucose mg/dl</th>
<th>Cell Count/mm³</th>
<th>Lymph /PMN</th>
<th>Pressure mm CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>90</td>
<td>50</td>
<td>8</td>
<td>40/60</td>
<td>80 -100</td>
</tr>
<tr>
<td>Child</td>
<td>5 - 40</td>
<td>40 - 80</td>
<td>0 - 5</td>
<td>60 -200</td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>20 - 40</td>
<td>50 - 70</td>
<td>0 - 5</td>
<td>100/0</td>
<td>60 -200</td>
</tr>
</tbody>
</table>

Disease | Protein mg/dl | Glucose mg/dl | Cell Count/mm³ | Lymph /PMN | Pressure mm |
--------|---------------|---------------|----------------|------------|-------------|
Bacterial| N-2000        | < 20          | 100-10,000     | 5-10       | >200        |
| Viral   | N-400         | N             | 10-1000        | Var.       | Var.        |
| Fungal  | N-100s        | <1/2 serum    | >10            | Var.       | N           |

Disease | Protein mg/dl | Glucose mg/dl | Cell Count/mm³ | Lymph /PMN | Pressure |
--------|---------------|---------------|----------------|------------|----------|
TB      | 100-1000      | 20-40         | 50-500         | Var        | >200     |
Neoplasm| Var.          | N             | N-500          | Var.       | Var.     |
Subarachnoid Hemothage | Var. | N | N | Xanthocromia | Var. | >200 |
Increased Pressure

>200 mm CSF
CAUSES:
• Hydrocephalus (communicating)
• Cerebral edema
• Meningitis (Increased permeability of BBB)

Appearance

• Turbid when WBC>200.
• Or RBC> 400.
• Tyndall’s effect.
• Grossly bloody if RBC > 6000
• 500-6000: Xanthochromia appears 2-4 hours after SAH. Max in 2-5 days and lasts 7-10 days.

Blood in CSF

Differentiate bloody (traumatic) tap (very common) from subarachnoid hemorrhage
Traumatic tap will have:
1. Less blood in each tube.
2. Centrifuge → Clear supernate.
3. Let stand → clot.
4. RBCs appear normal.
5. May repeat (caution: may have blood up to 5 days after traumatic tap).

Cell Count and Differential

• Should be done immediately (less than 1 hour) (Take CSF specimens to lab yourself). PMN count can decrease 50% in 2 hours.
• 1 PMN is OK.
• If tap is traumatic subtract 1 WBC for each 700 RBCs.
• There may be PMNs without meningitis (brain abscess or mastoid abscess).

Protein

• Non specific indicator of disease.
• Means disrupted BBB.
• Albuminocytologic dissociation (High protein with normal cell count): Guillain-Barre Syndrome, tumors, polio.

Glucose

• Need simultaneous blood glucose (2 hours ago would be best).
• Decreased in bacterial meningitis because Glucose is used up by PMNs, and parameningeal tissues and impaired transport.
• Very low in pyogenic meningitis.
• Low in TB, and fungal.
• Rare causes of low glucose: carcinomatous, leukemia.
High Specificity

- WBC count >500. LR+ = 15, LR- = 0.3
- Glucose < 0.4 of blood glucose. LR+ = 18
  Normal (0.6-0.7 of blood) LR- = 0.3
- Lactate > 31.53 mg/dL (>3.5 mmol/L)
  LR+ = 21, LR - = 0.10

High Sensitivity

You can rule out bacterial meningitis if the patient does NOT have ANY of these:
1. Positive CSF Gram stain
2. CSF ANC ≥ 1000/μL
3. CSF protein ≥ 80 mg/dL
4. Periphera blood ANC ≥10,000/μL
5. Seizures
(ANC = Absolute neutrophil count)

Smears

- Gram Stain: Positive in 60-86% of untreated bacterial meningitis.
- Less chance if partially treated.
- Poorer prognosis compared to negative smear.
- Lots of organisms and few cells: neutropenia, overwhelming infection.
- Acid fast stain positive in TB 20%.
- India ink positive in Cryptococcal 50%.

Latex Agglutination

- Identifies specific bacterial antigens in CSF even if antibiotics have been given, Sensitivity 80%.
- Cryptococcal antigen positive in >90%.

Cultures

- May be negative if antibiotic treated.
- In TB takes 6 weeks (Patient may be dead by then).

Blood Culture

- 80% positive in H. flu
- 50% in pneumococcal
- 30-40% in meningococcal
TB Meningitis

- Reactivation
- 30-66% have active pulmonary TB
- 75% have neck rigidity
- CSF very high protein, low glucose
- Pathologic findings: Thick exudate at base of brain causes: cranial nerve palsies, obstruction, hydrocephalus

TREAT

- Bacterial meningitis is a medical emergency
- Treat with antibiotics before patient leaves ER.
- Positive gram stain, very low glucose, petechial rash are absolute indications for antibiotics.
- You will save a life if you give penicillin to a patient with a rapidly progressive petechial rash.
TUMOR MARKERS

Steve Nandkumar, MD

(FOR SELF-STUDY)
TUMOR MARKERS

Definition:
A substance produced by a tumor or by the tumor’s host – in response to the tumor.

Some of the common tumor markers are proteins, glycoproteins, enzymes, hormones, and their metabolites. Cell receptors, DNA, RNA, and genes are also helpful.

CELL BIOLOGY

Tumor markers are found in cells, tissues and body fluids (serum, urine, etc.) and can be detected by qualitative and quantitative methods (e.g., chemical, immunologic, molecular biologic methods).

CELLS

Hyperplasia
(control of growth, differentiation)

- Elevated levels of normal proteins and metabolites

Neoplasia
(loss of growth control and regulation)

- Elevated levels of normal proteins and metabolites (cell proliferation)
- Abnormal proteins e.g., CEA
- Ectopic markers (Dedifferentiated cells)

TUMOR HETEROGENEITY

Mutations yield clones of cells with different phenotypes. Cells vary with respect to growth rate, cell surface receptors, immunogenicity, expression of tumor markers, invasive potential, etc.

Tumour markers are associated with

a. cell proliferation, e.g., HCG (pregnancy vs. GTDs).
b. cell differentiation, e.g., carcinoembryonic proteins (CEA)
c. metastases, e.g., detect tumour cells in circulation.
d. tumour associated events, e.g., AFP (benign liver disease vs. Ca)
e. malignant transformation, e.g., oncoproteins, Her 2-neu).
f. inherited mutations, e.g., BRAC1 and BRAC2 genes in breast Ca, etc.
**POTENTIAL USES OF TUMOR MARKERS**

- Screening in general population
- Differential diagnosis in symptomatic patients
- Clinical staging of cancer
- Estimating tumor volume
- Prognostic indicator for disease progression
- Evaluating the success of treatment
- Detecting the recurrence of cancer
- Monitoring responses to therapy
- Radioimmunolocalization of tumor masses
- Determining direction for immunotherapy

**CLINICAL USEFULNESS**

1. **Screening of cancer**
   
   Tumor markers are usually neither sensitive nor specific. Screening tests are, however, useful, e.g., PSA in prostate cancers and AFP in liver cancers in China.

2. **Sensitivity**
   
   There is OVERLAP of values in physiologic and neoplastic diseases, e.g., HCG in pregnancy and GTDs.

   Used as an adjunct test in cancer detection. Multiple tumor markers used (Disadvantage – COST!).

3. **Specificity as follows (on tissue diagnosis)**
   
   Epithelial membrane Antigen – CARCINOMA
   Cytokeratins – CARCINOMA
   CD for hematopoietic cells – LEUKEMIAS/LYMPHOMAS
   HMB-45, Melan A – MELANOMA
   Vimentin, Desmin, Actin – SARCOMA
   PSA – PROSTATE CANCER

   NOTE: HMB = Human Melanoma Black

4. **Monitoring course of disease/treatment**
   a. Response to surgery/chemotherapy
   b. Detection of recurrence or metastases

5. **Detection of recurrence**
   
   Tumor markers are usually elevated 3-6 months before there is clinical evidence of tumour

6. **Prognosis**
   
   E.g., ER, PR, Her 2-neu in breast cancer

**RECOMMENDATIONS FOR ORDERING TUMOR MARKERS**

1. Never rely on the result of a single test.
2. In serial testing, order every test from the same lab using the same assay kit.
3. Consider ordering multiple markers to improve both the sensitivity and the specificity for diagnosis.
4. Make sure that the tumor marker used in prognosis/monitoring recurrence or therapy effect was elevated before treatment.
5. Consider half-life of the tumor marker when interpreting test result.
7. Be aware of “ectopic” tumor markers. Non-neoplastic and neoplastic diseases can produce tumor markers.
8. Be aware of methodologic problems in tumor marker assays.
9. Consider non-specific markers for cost saving. They are sensitive to any changes in tumor activity; the tests are rapid, economical and simple to perform.
   E.g., β2M, lipid associated sialic acid P (LASA-P), etc.

<table>
<thead>
<tr>
<th>Table 79.5 Tumor Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor Markers</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>HORMONES</strong></td>
</tr>
<tr>
<td>Human chorionic gonadotropin</td>
</tr>
<tr>
<td>Calcitonin</td>
</tr>
<tr>
<td>Catecholamines</td>
</tr>
<tr>
<td><strong>ONCOFETAL ANTIGENS</strong></td>
</tr>
<tr>
<td>Alpha fetoprotein</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
</tr>
<tr>
<td><strong>ENZYMES</strong></td>
</tr>
<tr>
<td>Prostatic acid phosphatase</td>
</tr>
<tr>
<td>Neuron-specific enolase</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td><strong>TUMOR-ASSOCIATED PROTEINS</strong></td>
</tr>
<tr>
<td>Prostate-specific antigen</td>
</tr>
<tr>
<td>Monoclonal immunoglobulin CA-125</td>
</tr>
<tr>
<td>CA 19-9</td>
</tr>
<tr>
<td>CD30</td>
</tr>
<tr>
<td>CD25</td>
</tr>
</tbody>
</table>

* MGUS, monoclonal gammopathy of uncertain significance.
PROSTATIC ACID PHOSPHATASE  
NORMAL VALUE < 3.7 ng/ml

PAP hydrolyses phosphate esters at a pH of < 7.0; it is needed by sperms for metabolism.

Acid phosphatase is present in the lysozyme of secretory epithelial cells. It is found primarily in the prostate but is also present in rbcs, wbcs, platelets, macrophages, bone marrow, bone, liver, spleen, kidney and intestines (various isoenzymes).

Prostatic acid phosphatase can be distinguished from other phosphatases (tartrate inhibits PAP).

USE
1. It was used as a tumor marker for prostate cancer, but has been replaced by PSA (prostate specific antigen).
2. Elevated levels are also found in bone cancers (primary or metastatic), multiple myeloma, etc. and benign conditions such as BPH, osteoporosis and hyperparathyroidism.

PAP is NOT used nowadays as a tumor marker but may be helpful in staging of prostate cancer (elevated PAP may be associated with metastases); correlate with the prognosis of disease and in the monitoring of therapy. It is a useful marker for prostate carcinoma treated with anti-androgen therapy (PSA is androgen dependent and hence not useful as a tumor marker).

3. Rape detection – presence of PAP in semen (12 hours – 4 days after assault); half-life is 1-3 hours.

PROSTATE SPECIFIC ANTIGEN  
NORMAL VALUE < 4ng/ml

It is a glycoprotein encoded by a gene on chromosome 19. A serine protease of the kallikrein family, it is produced by the prostatic ductal/acinar cells and is regulated by androgens.

It helps liquefy seminal vesicle fluid (coagulum).

\[ \text{PSA} \xrightarrow{\text{Bound (complex) form (90%)}} \text{PSA} + \text{ACT (} \alpha_1 \text{ antichymotrypsin)} \xrightarrow{\text{PSA} + \alpha_2 \text{ Macroglobulin}} \text{free form (10%)} \]

Total PSA is bound form + free form

Free PSA occurs in 3 forms:
1. BPSA (BPH associated PSA)
2. pro PSA (inactive precursor); MORE SPECIFIC FOR CANCER
3. i PSA (intact PSA).

USE
1. PSA is elevated in prostatitis, infarction, BPH, following digital rectal exam, prostate cancer and any surgery of the prostate gland. Thus it is prostate specific but NOT cancer-specific.
2. Serum PSA, together with digital rectal examination and ultrasound, provides accurate and sensitive diagnosis.
3. PSA sensitivity = 80% sensitivity; specificity = 50%.
   PSA values may be False positive (20%) or False negative (20%) of cases.
4. PSA velocity – rate of increase as a function of time.
   Rapid rate of increase (> 0.75 ng/ml/year) favors early cancer; need 3 measurements over a 2-year period. European guidelines indicate >0.6 ng/ml.
5. PSA density = PSA/prostate volume as determined by ultrasound. Low density is unlikely to indicate cancer; increased density (> 0.15) is likely to represent cancer.
6. PSA doubling time. Time taken for PSA value to double itself (useful in post-surgical cases of cancer).
7. PSA correlates with clinical staging/pathologic staging. Tumor spread and metastases are associated with high PSA levels (usually > 50 ng/ml).
8. PSA levels help in monitoring treatment. Following surgery for cancer, PSA levels fall below the detection limit of assay in 2-3 weeks. If levels persist or increase, there is a possibility of tumor recurrence or persistence. PSA levels may also be useful following radiation therapy and antiandrogen therapy, (however PSA is androgen dependent and hence PAP may be more useful).

FREE PSA

\[
\text{% Free PSA} = \frac{\text{Free PSA}}{\text{Total PSA}} \times 100
\]

Higher % free PSA (> 23%) indicates BPH (benign diseases); Prostate cancers are associated with low % free PSA (< 6%). Screening for cancer by PSA lowers death rate by 20%.

**NEURON SPECIFIC ENOLASE (NSE)**

NORMAL VALUE < 12.5 ng/ml

It is a glycolytic enzyme found in neuronal tissue and cells of the APUD (amine precursor uptake and decarboxylase) neuroendocrine system.

**Elevated serum levels occur in:**
1. small cell carcinoma of lung (sensitivity 80%, specificity 80-90%).
2. neuroblastoma (90% sensitive)
3. pheochromocytoma
4. carcinoid
5. medullary thyroid carcinoma
6. pancreatic endocrine tumors
7. melanoma

**NSE levels**
1. correlate with stage of disease.
2. useful in monitoring disease following treatment
3. provides prognosis for disease progression.

**CALCITONIN (CT)**

NORMAL BASAL LEVELS

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt; 19 pg/ml</td>
<td>&lt; 14 pg/ml</td>
</tr>
</tbody>
</table>

A polypeptide, produced by the C cells of the thyroid in response to an increase in serum calcium level. It prevents calcium release from bones and thus lowers serum calcium levels.

1. It is most useful as a tumor marker for MTC (medullary thyroid carcinoma – levels 500-2000 pg/ml); may help in islet cell tumors, carcinoid, pheochromocytomas.
2. Used as a screening test in familial MTC and for asymptomatic family members.
3. Calcitonin levels correlate with tumor volume and stage.
4. It is elevated in cancers of lung, breast, kidney, liver, ovary etc. (ectopic production)
5. Benign conditions such as pancreatitis, hyperparathyroidism, P.A., Paget’s disease of bone, pregnancy, renal failure also reveal elevated CT levels.
**HUMAN CHORIONIC GONADOTROPIN (hCG)**

<table>
<thead>
<tr>
<th>NORMAL VALUES</th>
<th>Females</th>
<th>&lt; 5 U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Menopausal</td>
<td>&lt; 9 U/L</td>
<td></td>
</tr>
</tbody>
</table>

hCG is a glycoprotein produced by the placental syncytiotrophoblasts.

**There are 2 subunits:**
1. \( \alpha \) (alpha)
2. \( \beta \) (beta) – unique for placenta

Serum contains free \( \alpha \) units, free \( \beta \) units and intact whole hCG molecules (half-life is 12 – 20 hours). \( \alpha \) unit is common to other hormones such as FSH, LH, TSH and hence cross reaction minimizes its usefulness. \( \beta \)-units are more useful.

**USE**

Elevated levels occur in
1. pregnancy
*2. gestational trophoblastic disease (GTD)
*3. germ cell tumors (NSGCT 60%, SGCT 30%)
4. cancer of breast, lung, GI tract, ovary, skin (melanoma), urothelial cancers (30%).
5. \( \alpha \) hCG is useful for pancreatic endocrine tumors.
6. Benign conditions such as IBD, cirrhosis, duodenal ulcers.

**NOTE:**
hCG is most useful in detecting and monitoring the progression of GTD. hCG levels correlate with tumor volume, staging and prognosis of the disease. Following successful Rx, levels return to normal in about 12 weeks.

hCG, along with \( \alpha \)-FP (\( \alpha \)-fetoprotein) is helpful in detecting and monitoring germ cell tumors (non-seminomatous type). Presence of hCG in a seminoma indicates the co-existence of a choriocarcinoma.

Normal CSF : serum hCG = 1:60. Elevated levels in CSF indicate brain metastases.

hCG is used as a serum marker for detection of prenatal conditions such as Trisomy 21 (increased levels) and Trisomy 18 (decreased levels) along with \( \alpha \)-FP and unconjugated estriol.

**CA-125**

| NORMAL VALUE | < 35 kU/L |

A glycoprotein, expressed by normal tissues of Müllerian origin; function is unknown.

It is a carbohydrate marker for tumors of Mullerian origin eg. non-mucinous ovarian carcinomas (serous, endometrioid, clear cell etc.).

**Levels may be elevated in:**
1. cancers of lung, breast, GI tract, pancreas, cervix, fallopian tubes,
2. Cirrhosis, hepatitis
3. endometriosis
4. pericarditis, peritonitis, pleuritis

**NOTE:**
1. CA-125 is useful in evaluating patients with advanced endometriosis. 2. It is not useful in screening for ovarian cancer in asymptomatic women (low sensitivity and specificity). 3. Levels correlate well with tumor size and staging. 4. Useful in detecting residual disease, recurrence and metastases. 5. Levels of >35 kU/L usually indicate malignancy (as opposed to lower values for benign diseases). 6. Levels < 65 kU/L preoperatively, have a better prognosis than those with > 65 kU/L.
OTHER MUCIN TUMOR MARKERS

A. **CA 15-3**  
   **NORMAL VALUE**  < 25 kU/L  
   – Glycoprotein, non specific  
   – Useful in detecting, monitoring therapy and disease progression in metastatic **BREAST CANCERS**. Correlates well with tumor volume and stage  
   – May be elevated in cancers of pancreas, lung, ovary, liver, colon, and rectum  
   – Also benign breast and liver diseases (hepatitis, cirrhosis), SLE, sarcoidosis, TB.

B. **BLOOD GROUP ANTIGENS**

   **CA 19-9**  
   **NORMAL VALUE**  < 37 kU/L  
   – A glycolipid derivative of Lewis’ blood group. It is produced by normal pancreatic and biliary ductal cells; also by salivary, gastric, colonic and endometrial cells.  
   **USE:**
   1. It is a marker for colorectal (8% cases) and **PANCREATIC CARCINOMA** (70-80% cases).  
   2. Elevated levels are also seen in hepatobiliary, gastric (22% cases), hepatic and breast cancer.  
   3. Benign diseases of the pancreas, liver and GI tract (∆ levels seen).

ONCOFETAL TUMOR MARKERS

I. **α-FETOPROTEIN**  
   **NORMAL VALUE**  < 10 ng/ml  
   It is a glycoprotein produced by the fetal liver, GI tract, kidney, and yolk sac. The exact function is not known; it may act like serum albumin and help in the transport or binding of proteins.

   - Levels at birth: 10,000 ng/ml  
   - At the end of 1 year: < 10 ng/ml  

   α-Fetoprotein exists in an IgM complexed form and a free form. It is **NON-SPECIFIC. And has a half-life of 5 days.**

   **Elevated levels occur in tumors:**
   1. Germ cell tumor of gonads  75%  
   2. Hepatocellular Ca  >50%  
   3. Pancreatic cancer  23%  
   4. Gastric carcinoma  18%  
   5. Lung cancers  7%  
   6. Colon cancer  5%  

   **Benign causes of elevation:**
   1. Acute liver injury  
   2. Chronic active hepatitis  
   3. Cirrhosis  
   4. Neonatal hepatitis  
   5. Fetal and placental diseases in gestation

**NOTE:**  
1. AFP is useful in screening and diagnosis of hepatocellular carcinoma. AFP produced by hepatocellular carcinoma has an extra fucose moiety due to an abnormal fucosyl transferase (detected by lectin binding) whereas AFP produced in benign hepatic diseases **DOES NOT HAVE** this fucose. Cholangiocarcinoma does NOT produce AFP.  
2. Monitoring course of
disease, i.e., germ-cell tumors of gonads. 3. Detecting fetal abnormalities, e.g., anencephaly, spina-bifida (NTD –neural tube defects) Trisomy 18, Trisomy 13, Down’s syndrome..

Maternal serum or amniotic fluid analysis with elevated AFP is helpful. Amniotic fluid analysis of acetyl cholinesterase, along with AFP is a sensitive and specific marker for open neural tube defects.

II. **CARCINOEMBRYONIC ANTIGEN**

It is a cell surface glycoprotein, part of the 36 different glycoprotein CEA family, coded by 10 different genes on chromosome 19. It is NON-SPECIFIC. It may function as a cell adhesion molecule enhancing invasiveness/metastases.

**Elevated in**
1. lung, colon, liver, pancreas, stomach, ovary, breast cancers, etc.
2. non-tumor conditions such as IBD, peptic ulcer, pancreatitis, diverticulitis, benign liver diseases such as cirrhosis, etc.
3. smoking
4. renal failure

**NOTE:**
1. CEA is useful in monitoring the course of a disease (tumor). Levels of CEA (after cancer surgery or treatment) should return to normal within 6-12 weeks. Any subsequent rise would indicate tumor recurrence or metastases. 2. Prognostic marker. High CEA levels usually mean poor prognosis. Response to treatment will cause a fall in CEA. 3. 20-30% of tumors may not produce CEA. 4. Change in CEA levels by + 25% from the previous value is significant. 5. Liver damage impairs CEA metabolism and can hence cause increased CEA levels.

**CEA IS NOT USED FOR SCREENING (FOR TUMORS) DUE TO LOW SENSITIVITY AND SPECIFICITY.**

**NOTE:** For colon cancers the following methods are useful. 1. Fecal occult blood test sensitivity = 15-30%. 2. Virtual colonoscopy. 3. Shed cancer cells in feces are tested for gene mutations (RAS, p53, etc.).

**ESTROGEN AND PROGESTERONE RECEPTORS**

ER and PR are proteins present in the nuclei of cells of the mammary gland and other organs. They belong to a super family of genes that code for receptors for steroid and thyroid hormones, VitD₃, and retinoic acid. ER and PR also bind to DNA and modulate the expression of specific genes. 

**THEY ARE USEFUL BIOMARKERS FOR BREAST CANCER.**

- 80% of breast cancers are ER positive
- 80% of tumors that are ER and PR positive respond to hormonal therapy and also correlate with longer “disease free” intervals after surgery.
- ER negative tumors are treated by chemotherapy

Documentation of these receptors is done by immunohistochemical means. False positive and false negative results occur and may be attributed to tumor heterogeneity.

**NOTE:**

- | Tumors response to hormone R₂ |
  - | ER + PR + | 80% |
  - | ER + PR - | 26% |
  - | ER - PR + | 50% |
  - | ER - PR - | 5% |

NORMAL VALUE < 4 ng/ml
GENETIC MARKERS
Cancers may be due to genetic changes/mutations involving
A. Oncogenes
B. Tumor suppressor gene

A. ONCOGENES
Protooncogenes code for products involved in normal cellular processes such as proliferation and differentiation. Mutations of oncogenes lead to abnormal cell growth and tumor formation.

1. ras genes e.g., neuroblastoma p21 (abnormal gene product)
2. c-myc gene e.g., small cell lung cancer p62 (tumor maker)
3. c-erb B-2 gene – occurs on chromosome 17
   Also known as HER-2/neu, (neu = neural), the gene product, p185 is a glycoprotein associated with tyrosine protein kinase family and similar to human epidermal growth factor receptor. Amplification of this gene is found in breast, colon, ovary, prostate, urothelial, stomach, oral cavity squamous cell carcinoma, non-small cell lung cancer etc.
   Serum Her-2/neu, an extra cellular domain (ECD) p105 component is also a helpful marker.

Over expression of HER-2/neu protein is associated with poor prognosis, recurrence and short survival in breast cancer. Herceptin, a monoclonal antibody to HER-2/neu is useful in treating breast cancers with over expression of this protein (25-35% of cases of metastatic breast cancer).

Detection methods:
- FISH (gene amplification)
- IHC (protein overexpression).

B. TUMOR SUPPRESSOR GENES
These are found in solid tumors.

P-53
This active or wild type p53, a nuclear phosphoprotein, controls cell division by regulating entry into “S” phase of cell cycle. It binds to and inactivates a cellular protein needed for DNA replication or represses gene transcription. Cell cycle arrest, apoptosis and senescence follow.

Mutation in p53 allows cells to move freely into the cell cycle, replication and autonomous growth (tumor). Antibodies to mutant forms (p53 antibodies) occur and correlate with gene mutation.

Wild type p53 protein has a short half-life (about 20 minutes) and hence cannot be detected in blood. Mutant p53 over expression can be detected in tissue and serum, and hence can be used as a tumor marker. Loss of p53 alleles, mutations in p53 gene etc., may be seen in cancers of the colon, liver, etc.

BRCA1 and BRCA2
These are tumour suppressed genes. Mutations in BRCA1 are seen in 50% of all inherited breast cancers; also noted in cancers of ovary, colon and prostate. Mutations in BRCA2 occur in 35% of remaining inherited breast cancers; also increase risk of male breast cancer.
Asymptomatic women (with family history of breast cancer) may elect to screen for gene mutations; if present, they may opt for prophylactic mastectomies.

Ref. Chapter on “Diagnosis and management of carcinoma using serologic tumour markers