CLINICAL LABORATORY MEDICINE

3rd Edition

Clinical Application of Laboratory Data

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Preface to Third Edition

CONTINUED CHANGE in the repertoire of the clinical laboratory necessitates a new edition. As in previous editions, the aim is to provide as much useful information as possible which may assist the understanding, selection and interpretation of laboratory tests. A new chapter has been added for serum level monitoring of therapeutic drugs. The chapters covering endocrine tests have been rewritten. Many other areas had to be revised. New tests have been added: for example, aminoglycoside serum level, amylase clearance/creatinine clearance ratio in pancreatitis, beta-subunit of hCG in tumor diagnosis, CPK isoenzymes, DNA antibody in rheumatoid-collagen disorders, estrogen receptor assay, ferritin assay, parathyroid hormone assay, prolactin assay, thyrotropin-releasing factor test, tumor-seeking radiopharmaceuticals, urine myoglobin and uroporphyrinogen-I-synthetase assay in acute intermittent porphyria. As far as possible, everything has been brought up to date. As in previous editions, information on nuclear medicine procedures has been incorporated; and now some data on ultrasound and on computerized axial tomography are included.

A major problem has been the fact that while new tests continue to be announced and new information about standard procedures must be recorded, out-of-date tests refuse to disappear from clinical use. Data on these tests have been retained in this edition, although frequently relocated. In the future it may be necessary simply to list the test and provide one or two references.

RICHARD RAVEL

Preface to First Edition

THE CLINICAL LABORATORY has a major role in modern medicine. A bewildering array of laboratory procedures is available, each of which has its special usefulness and its intrinsic problems, its advantages and its drawbacks. Advances in biochemistry and radioisotopes, to name only two conspicuous examples, are continually adding new tests or modifying older methods toward new usefulness. It seems strange, therefore, that medical education has too often failed to grant laboratory medicine the same prominence and concern that are allotted to other subjects. If ever a comprehensive, systematic and critical teaching system were needed, it is for this complex and heterogeneous topic. It would seem that if one were to consider ordering any laboratory procedure, several things should be known about that test, including:

1. In what situations is the test diagnostic, and in what situations does the test provide information without being diagnostic?
2. What commonly available tests give similar information, and when should one be used in preference to the others?
3. What are the disadvantages of the test and possibilities of error or false results?

The fact that this type of information is not adequately disseminated is quickly brought home to a clinical pathologist, who supervises the clinical laboratory and at the same time acts as liaison to clinicians on laboratory problems. It becomes quickly evident in two ways—the continually rising number of laboratory procedure requests and even a casual inspection of patients' hospital charts. Unnecessary tests represent severe financial and personal inconvenience to the patient; inappropriate tests or tests done under improper conditions mean wasted or misleading information, and often a loss of precious time.

In laboratory medicine, textbooks are available, as in all areas of general medicine considered detailed enough to warrant a specialty status. These fall into two groups: those mainly for the technician and those designed for clinicians. Technician-oriented books necessarily stress the technical aspects of individual tests, with emphasis on cookbook methodology. Textbooks for the clinician vary considerably in approach. Some excellent works concentrate almost exclusively on one subject or subspecialty, such as hematology. Many others combine technician methodology with discussion to varying degrees of the clinical aspects of tests. The latter aspect often suffers due to inevitable limitations imposed by mere length. Some texts which emphasize the clinical approach may be criticized on the grounds that they neglect either adequate attention to possible limitations

and sources of error in each particular laboratory procedure, or fail to delineate the background or the technical aspects of the tests enough to provide a clear picture as to just what information the test actually can provide.

This volume attempts to meet these criticisms. Its aim is to provide enough technical and clinical information about each laboratory procedure included so as to allow adequate understanding, selection and interpretation of these procedures. Many of the laboratory tests require varying amounts of individual discussion. Others are noted in the context of the diseases in which they may be useful. In addition, most of the common diseases in which laboratory tests render significant assistance are briefly outlined, and the role of the laboratory in each is explained. Also included are a considerable number of diseases or conditions which are uncommon or even rare, but which may be considered important from various points of view—either as well-known entities, diagnostic problems or cases which may benefit from early diagnosis and therapy.

There is a great temptation for a work of this type to become encyclopedic. Brevity and succinctness are preserved, therefore, at some cost, hopefully with more gain than loss. Probably the most striking examples are the chapters on infectious diseases and parasitology. In most cases, description of clinical syndromes and specific organisms has been eliminated or markedly reduced, because this book is not intended to be a treatise on internal medicine. Emphasis is on material which seems more directly concerned with selection and interpretation of laboratory tests. Nevertheless, a few diseases (such as leptospirosis) are important from the standpoint of laboratory diagnosis because their signs and symptoms mimic other conditions, so the clinical findings are included in some detail. On the other hand, syphilis serology has a chapter to itself due to confusion which surrounds the multiplicity of available tests. Likewise, certain subjects are discussed at unusual length. These are topics which, in my experience, seem to be common problem areas. The aim is to provide a reasonably thorough, yet compact, survey of laboratory medicine. This book is meant to provide some area of assistance to anyone who is engaged in clinical medicine, and to provide, in a sense, a reasonably comprehensive course in clinical pathology.

It is anticipated that the style and format of this book may be criticized; either because the uninitiated reader might gain an impression that laboratory medicine can be reduced to a relatively few rules or protocols, or that one approach to diagnosis is presented as though all others were invalid. Such inferences are not intended.

It should be obvious that no person could write a book covering clinical pathology entirely from his own experience. On the other hand, adequate citation of references would be a tremendous undertaking in itself. A compromise is therefore offered. At the ends of the chapters there are lists of suggested readings, composed of selected references which include textbooks with general or specific coverage, papers on certain specific subjects and occasionally an article selected because of an unusually inclusive bibliography. Due to spare considerations, those references with more than two authors have been listed in the first author's name only. This book is only a beginning; the reader is urged to consult these papers and others on

individual subjects in order to broaden the information presented here, and to evaluate contrasting points of view.

An Appendix is provided, in order to include certain information which is useful but which seemed better presented separately from the regular text. Much of this is in tabular form.

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Table of Contents

1 / Basic Hematologic Tests and Classification of Anemia

HEMATOLOGY is the study of the blood, the cellular elements of the blood and the metabolic processes by which the blood components are formed. The major emphasis in hematology is given to the three cellular elements of the blood—red cells, white cells and platelets—the plasma proteins, electrolytes, fluid and other constituents being covered elsewhere in this book. Each of the three cellular elements will be discussed separately for reasons of convenience.

MAJOR HEMATOLOGIC TESTS

There are several tests that form the backbone of laboratory diagnosis in hematology.

Hemoglobin (Hb)

This is the oxygen-carrying compound contained in red cells. Hemoglobin can be measured chemically, and the amount of Hb/100 ml of blood can be used as an index of the oxygen-carrying capacity of the blood. Total blood Hb depends mostly on the number of RBC (the Hb carriers) but also (to a much lesser extent) on the amount of Hb in each RBC. A low hemoglobin level thus indicates anemia. Depending on the method used and the care with which the laboratory checks its spectrophotometers, hemoglobin values are accurate to 2–3%. Older methods (Sahli) used a chemical technique in which the final compound was compared visually against a colored glass standard; at best, this gives 2–3 times the average error of methods using a good spectrophotometer.

Normal values are most frequently quoted as 14–18 gm/100 ml for males and 12–16 gm/100 ml for females (grams/100 ml is often abbreviated gm% or gm/dl). Some reports indicate lower values, expecially in women, so that it is probably better not to consider a patient anemic until Hb is less than 13 gm in males and 11 gm in females. Infants have different normal limits (p. 477). In addition, several investigators found a significant decrease in Hb (up to 1.0 gm) when one sample was obtained after some time in the upright position followed by another specimen after overnight bed rest. Finally, there is some evidence that heavy smokers have slightly increased Hb concentration (0.5 gm or more) compared to nonsmokers.

RBC Count

The number of RBC per cu mm gives an indirect estimate of the hemoglobin content of the blood. Blood cell counting chamber (hemocytometer)

methods give average errors of 4–8%, or even more—depending on the experience of the technician. Automatic counting machines reduce this error to about 2–4%. However, many smaller laboratories do not have these machines. Normal values are 4.5–6.0 million/cu mm for males and 4.0–5.5 million/cu mm for females.

Hematocrit (HCT)

After centrifugation, the height of the red cell column is measured and compared with the height of the original whole blood. The percentage of red cell mass to original blood volume is the hematocrit. Anticoagulated whole blood is centrifuged in a special tube. Since whole blood is made up essentially of RBC and plasma, after centrifugation the percentage of packed red cells gives an indirect estimate of the number of RBC/100 ml of whole blood (and thus, in turn, is an indirect estimate of the amount of hemoglobin). Hematocrit thus depends mostly on the number of RBC, but there is some effect (to a much lesser extent) from the average size of the RBC. Normal values are 40–54% for males and 37–47% for females. The HCT is usually about three times the hemoglobin value (assuming no marked hypochromia). The average error in HCT procedures is about 1–2%. Microhematocrits are generally as accurate as the older standard Wintrobe (macrohematocrit) technique. The HCT may be changed by position and heavy smoking in the same manner as Hb is changed.

Indices (Wintrobe Indices)

Wintrobe introduced a very useful method to demonstrate certain characteristics of red cells.

MEAN CORPUSCULAR VOLUME (MCV).—This concept utilizes the effect that the average size of the RBC has on the HCT. If the average RBC size is increased, the same number of RBC will have a slightly larger cell mass and thus a slightly increased HCT reading; the opposite happens if the average RBC size is smaller than normal. The MCV is therefore calculated from the HCT and RBC count as follows:

$$\frac{\text{HCT} \times 10}{\text{*RBC count}} = \text{MCV}$$ (HCT in %; RBC in millions/cu mm*; MCV in cubic microns [cu μ])

Normal values are 87 ± 5 cu μ (manual) and 90 ± 10 cu μ (Coulter Counter). Heavy smoking may increase MCV as much as 3 cu μ.

MEAN CORPUSCULAR HEMOGLOBIN (MCH).—This concept gives an estimate of the amount of hemoglobin in the average red cell; this is done by comparing the blood Hb level to the RBC count as follows:

$$\frac{\text{Hb} \times 10}{\text{*RBC count}} = \text{MCH}$$ (Hb in gm/100 ml; RBC in millions/cu mm*; MCH in micromicrograms [μ μg])

Normal values are 29 ± 2μ μ (manual) and 30 ± 4μ μ (Coulter Counter).

MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (MCHC).—This

*Use the number of millions rather than the actual count; e.g., 4,560,000 = 4.56 million.

concept estimates the average concentration of hemoglobin in the average RBC. It differs from MCH in that the average RBC concentration of Hb depends on RBC size as well as on the actual amount of Hb contained in the RBC. MCHC is calculated as follows:

$$\frac{\text{Hb} \times 100}{\text{HCT}} = \text{MCHC} \qquad \text{(Hb in gm/100 ml; HCT in \%; MCHC in \%)}$$

Normal values are 34 ± 2% (manual) and 34 ± 3% (Coulter Counter).

Several factors should be mentioned.

1. As an index of red cell hemoglobin, the MCHC is often more reliable than the MCH, since the MCHC does not incorporate the relatively inaccurate RBC count procedures. It is true that macrocytic or microcytic (larger or smaller than normal) RBC will alter the MCHC independently of the hemoglobin values. However, in those diseases where there is significant overall macrocytosis or microcytosis, the RBC counts of the blood are changed (decreased) in addition to the changes in average RBC size. Since HCT depends on the number of RBC more than on average RBC size, the decrease in RBC number more than compensates for the relatively small effect that RBC size has on the HCT. Therefore, in most clinical situations, alterations in average RBC size alone do not affect the MCHC significantly.

2. The various indices are affected only by *average* cell measurements, either of size or of quantity of hemoglobin. This is especially noticeable in the indices dependent on average RBC size (MCV and, to some extent, MCHC). There may be considerable variation in size between individual red cells (anisocytosis), but the indices do not show this, since they take into account only the average size.

3. Examination of a well-made peripheral blood smear will give most of the same information as the indices. Indices are not a substitute for examination of the peripheral blood smear, but they may be helpful in confirming equivocal cases. Indices are only as accurate as the various counts and procedures (plus calculation) that went into their preparation.

Examination of Wright-Stained Peripheral Blood Smear

This procedure gives a vast amount of information. It allows visual estimation of the amount of hemoglobin in RBC and the overall size of RBC. In addition, alterations in size, shape and structure of individual red cells (p. 37) or white cells are visible, which may have diagnostic significance in certain diseases. Pathologic early forms of the blood cells are also visible. Finally, a good estimate of the platelet count can be made in most cases from the peripheral smear alone.

The peripheral smear is the most useful laboratory procedure in hematology. There obviously are many limitations; for example, a peripheral smear cannot demonstrate the presence of anemia per se, which must be detected by means of either the Hb, HCT or RBC count. Also, many etiologies of anemia show peripheral blood changes that are nonspecific. In some cases in which the peripheral smear is highly suggestive, it may not be so in early stages of the disease. Even if characteristic cell changes are present, there may be different underlying causes for the same morphologic type of anemia, different causes that call for different treatment. Finally,

there are some conditions that produce anemia without any demonstrable morphologic changes in the RBC of the peripheral smear. The same comments about RBC may, in general, also be applied to the white cells of the peripheral smear. However, it is often possible to predict leukocytosis by comparing the overall visual ratio of WBC to RBC. A differential count of the various WBC forms is done from the peripheral smear.

Reticulocyte Count

Reticulocytes occupy an intermediate position between nucleated RBC in the bone marrow and mature (nonnucleated fully hemoglobinated) RBC. After the normoblast (metarubricyte) nucleus is extruded from the cell, some remnants of nuclear material remain for a short time. It is possible to stain this material using vital staining techniques and dyes such as methylene blue or cresyl blue. The material then is seen microscopically in the form of dark blue dots arranged in loose aggregates or reticulum. The reticulocyte count is an index of the production of mature red cells by the blood-forming organs, mostly the bone marrow. Increased reticulocyte counts mean an increased number of RBC being put into the peripheral blood in response to some stimulus. In exceptionally great reticulocyte responses, there may even be nucleated RBC pushed out into the peripheral blood due to massive red cell production activity of the bone marrow. Except in a very few diseases, such as erythroblastosis, peripheral blood nucleated RBC are usually few in number and of later maturity stage when they do appear. Reticulocytes are not completely mature RBC; therefore, when reticulocytes appear in the peripheral blood they may be slightly larger than normal RBC. This may give a slightly macrocytic MCV and show macrocytes on peripheral smear. Also, reticulocytes may sometimes have a slightly bluish (basophilic) tinge with Wright's stain (although this often does not occur); this phenomenon is called polychromatophilia, and results because the reticulocyte is not yet a mature RBC, and therefore does not have a full complement of (reddish-staining) hemoglobin.

Some authorities advocate correcting the reticulocyte count by the number of RBC to differentiate a true increase in reticulocyte production from a situation in which reticulocyte quantity is unchanged but RBC number is decreased. This may be done by multiplying the reticulocyte (%) count by the quotient of patient HCT divided by average normal HCT (47 for men and 42 for women). Alternatively, one can obtain the absolute number of reticulocytes by multiplying the reticulocyte count (%) by the RBC count.

WBC Count

This may be done using either a hemocytometer or machine (such as the Coulter Counter). The error produced by hemocytometer counts is about 4–8%, but may be higher with inexperienced personnel. Coulter counts have approximately 2–4% error. The machine has the disadvantage that WBC counts over 100,000/cu mm become increasingly inaccurate unless a dilution is used. In addition, some of the abnormal lymphocytes of lymphocytic leukemia are unusually fragile and may be destroyed when the specimen is prepared for a machine count, thus giving a false low value. With either hemocytometer or machine, nucleated RBC are counted as WBC, so a correction has to be made on the basis of the percentage of nucleated RBC (to 100 WBC) found on the peripheral smear.

Normal values are most often quoted as 5,000–10,000/cu mm. Several studies suggest that 4,500–11,000 would be more correct. However, there is a significant overlap of normal and abnormal between 4,500–5,000 and 10,000–11,000, especially the latter area. There is some evidence that normal range for blacks may be at least 500/cu mm lower than normal range for whites. Alterations in WBC levels are discussed in Chapter 5.

Platelet Count

The usual procedure that employs a hemocytometer counting chamber and a standard microscope has approximately a 10–20% error. A somewhat similar method that makes use of a phase contrast microscope has a reported error of about 8%. Platelet counting machines can reduce the error even further. Normal values are 150,000–300,000/cu mm for direct counts.

Bone Marrow Aspiration

Bone marrow aspiration is of help in several situations: (1) to demonstrate the diagnosis of megaloblastic anemia; (2) to establish the diagnosis of leukemia or multiple myeloma; (3) to show whether deficiency of one or more of the peripheral blood cellular elements is due to a deficiency in the bone marrow precursors (bone marrow hypoplasia); (4) to document a deficiency in body iron stores in certain cases of suspected iron-deficiency anemia; (5) in certain selected cases, to demonstrate metastatic neoplasm or some types of infectious disease (culture or histologic sections may be preferred to routine Wright-stained smears).

These nine procedures are the basic tests of hematology. Intelligent selection and interpretation of these procedures usually can go far toward solving the vast majority of hematologic problems. Other tests may be ordered to confirm or rule out a diagnosis suggested by the results of preliminary study. These other tests will be discussed in association with the diseases in which they are useful.

However, once again, certain points should be made. Laboratory tests in hematology are no different from any other laboratory tests. Two or more tests that give essentially the same information in any particular situation should not be ordered. For example, it is rarely necessary to order Hb, HCT and RBC count all together unless indices are needed. As a matter of fact, either the Hb or the HCT is usually sufficient, although, initially, the two are often ordered together as a check on each other. (As it is the least accurate, the RBC count is rarely helpful.) Both the WBC count and differential are usually done initially. If both are normal, there usually is no need to repeat the differential count if the (total) WBC count remains normal and there are no morphologic abnormalities of the RBC and WBC.

Another point to be stressed is the proper collection of specimens. The timing of collection is sometimes extremely important. Transfusion therapy may cause a megaloblastic bone marrow to lose its diagnostic megaloblastic features, sometimes in as little as 12 hours. On the other hand, transfusion will not affect a bone marrow that has no iron. Capillary blood (finger puncture) is best for making peripheral blood smears, because oxalate anticoagulant causes marked artifacts in WBC morphology and even will slightly alter the RBC. EDTA anticoagulant will cause a false decrease in HCT (Hb is not affected) if the amount of blood collected is less than

half the proper volume (for the amount of EDTA in the tube). When capillary (fingerstick) blood is used to make HCT, Hb or cell counts, too much squeezing of the finger or other poor technique may result in dilution of the blood by tissue juice and give falsely low values. On the other hand, dehydration may result in hemoconcentration and produce falsely high values. This may mask an anemia actually present, or, when the patient is properly hydrated, a repeat determination may give the false impression of a sudden drop in values, such as might otherwise come from an acute bleeding episode. If very severe, hemoconcentration may simulate polycythemia.

ANEMIA—CLASSIFICATION

Although anemia may be defined as a decrease in hemoglobin concentration, it may result from a pathologic decrease in the red cell count. Since mature RBC are fully saturated with hemoglobin, such a decrease means that total blood hemoglobin will also be affected. Anemia is a symptom of some underlying disease, and is not a diagnosis. There always is a cause, and most of the causes may be discovered by a relatively few simple procedures. The greatest help in finding the underlying disease responsible comes from knowing the common causes of anemia, getting a good history, doing a thorough physical examination, and ordering a logical sequence of laboratory tests based on what the situation and other findings suggest.

Classification of anemia is helpful because it provides a handy reference for differential diagnosis. There are several possible classifications; each is helpful in some respects.

Anemia may be classified according to pathogenesis. Using this concept, three mechanisms may be responsible.

1. *Deficiency of vital hematopoietic raw material—"factor deficiency anemia."*—The most common causes of deficiency anemia are iron deficiency and deficiency of vitamin B_{12} and/or folic acid.

2. *Failure of the blood-forming organs to produce or to deliver mature RBC to the peripheral blood—"production-defect anemia."*—This may be due to *(a)* replacement of marrow by fibrosis or by neoplasm (primary or metastatic); *(b)* hypoplasia of the bone marrow, most commonly produced by certain chemicals; or *(c)* toxic suppression of marrow production or delivery without actual marrow hypoplasia, found to variable extent in some patients with certain systemic diseases. The most common of these are severe infection, chronic renal disease, widespread malignancy (without extensive marrow replacement), rheumatoid-collagen diseases and hypothyroidism. (These conditions may sometimes be associated with an element of hemolytic anemia.)

3. *RBC loss from the peripheral blood—"depletion anemia."*—This is commonly due to *(a)* hemorrhage, acute or chronic (causing escape of RBC from the vascular system); *(b)* hemolytic anemia (RBC destroyed or RBC survival shortened within the vascular system), or *(c)* hypersplenism (splenic sequestration).

A second classification is based on a morphologic approach. Depending

on the appearance of the RBC on a peripheral blood smear and/or Wintrobe indices, anemias may be characterized as microcytic, normocytic or macrocytic. They may be further subdivided according to the average amount of RBC hemoglobin, resulting in hypochromia or normochromia. (Macrocytic RBC may appear hyperchromic on peripheral smear, but this is an artifact due to an enlarged and, therefore, thicker cell that, being thicker, does not transmit light through the central portion as it would normally.)

I. Microcytic
   A. Hypochromic
      1. Chronic iron deficiency (most frequent cause)
      2. Thalassemia
      3. Occasionally in certain chronic systemic diseases
   B. Normochromic
      Very uncommon; may be simulated by spherocytosis; present to mild degree in some cases of infection

II. Normocytic
   A. Hypochromic
      1. Some cases of anemia due to systemic diseases
      2. Many cases of lead poisoning
   B. Normochromic
      1. Acute blood loss
      2. Hemolytic anemia
      3. Bone marrow replacement or hypoplasia
      4. Hypersplenism
      5. Many cases of anemia due to systemic diseases
      6. Some cases of lead poisoning

III. Macrocytic
   A. Hypochromic
      Some cases of macrocytic anemia with superimposed iron deficiency
   B. Normochromic
      1. Pernicious anemia
      2. Malabsorption (vitamin B_{12} and/or folic acid)
      3. Folic acid deficiency
      4. Reticulocytosis
      5. Some cases of chronic liver disease and hypothyroidism
      6. Some cases of aplastic anemia

The more common causes of anemia will be discussed in greater detail in the following chapters. Only the more common hematologic diseases will be covered. No attempt will be made to list every known entity or every disease that either produces or is associated with anemia. For a more complete coverage, several excellent textbooks on hematology are available.

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