

LEARNING GUIDE

PROTEIN

ACKNOWLEDGEMENTS



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HOW TO USE THIS LEARNING GUIDE

This Learning Guide is an overview of the pathophysiological principles and the major disease states related to important serum proteins. It provides an overview of clinically important serum proteins and their use in diagnosing and managing common clinical situations. It reviews the different important analytical techniques used to identify and quantify these proteins. It also describes approaches to standardizing or harmonizing protein assays. The guide is intended to be used as a reference for the application of clinical laboratory tests and interpretation of laboratory results. Much of the material is drawn from the textbook *Proteins: Laboratory Testing and Clinical Use*, coauthored by Dr. Francesco Dati and Dr. Erwin Metzmann. We are grateful to DiaSys Diagnostic Systems and Dr. Guenther Gorka for granting copyright to use portions of the book.

This guide is organized into 13 sections and an appendix with a Summary of Specific Proteins and Their Characteristics, References (including general references, which can be used to expand upon the introductory discussions given here) and Correct Responses to Review Questions.

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FOREWORD

Measurement of proteins in blood and other body fluids provides a direct view of the function and dysfunction of the body's organs due to the close contact of the microvasculature with all tissues. This quality has been recognized for well over a century and has led to the evolution of methods for quantitating proteins, ranging from simple fraction by chemical precipitation to sophisticated techniques that assay even small concentrations of individual chemical species. This Protein Learning Guide reviews modern applications of protein analysis for assisting clinical decision-making in medical practice.

Diagnosis and monitoring by protein measurements is routine now for diseases of many different organ systems, with special value placed on patterns of protein abnormalities detected through use of panels or profiles of multiple proteins. One of the most important questions to be asked is whether the liver has impaired synthetic capacity through either hepatic disease or nutritional basis. The answer lies in measuring serum albumin and, more recently, serum prealbumin. These protein values complement clinical observations of malnutrition and responses of the body to stress. Renal function has long been estimated by quantitation of serum creatinine, but a potentially more reliable monitor has recently been proposed: the small protein cystatin, which is convenient to measure by immunoassay. Proteins in the urine also provide unique diagnostic information regarding glomerular versus renal tubular disease. Other newly minted protein markers have been identified for diseases and deficiencies affecting erythrocyte production, the gastrointestinal tract, the pancreas, the cardiovascular system and the lungs.

Another major area of the body's physiology coming under greater laboratory surveillance is the immune system and its participation in inflammation. The response of the body to physical or physiological stress consists of sequences of reactions between proteins that can destroy invading organisms (bacteria, viruses, fungi, parasites) through the interaction of complement proteins with antibodies targeted to those pathogens. A bonus of this response is that antibody titers can be measured afterward to confirm diagnosis and to predict future immunity. The entire inflammatory process involves not only these proteins, but also various classes of leukocytes that respond to foreign invaders to complete their elimination from the body. Alas, sometimes these proteins and cells are turned against the body itself in autoimmune disorders. Detection and quantitation of autoantibodies is a standard of care for diagnosis and monitoring of autoimmunity. Complement proteins C3 and C4 are extremely useful to monitor systemic autoimmune disease activity along with antibody titers.

A series of other proteins termed "acute-phase reactants" rise and fall in the stress response, most notably C-reactive protein (CRP), which is often used as an early warning sign of infection as an acute complication following surgery, a marker for serious bacterial infection and an indicator of autoimmune disease activity. Although these instances can cause skyrocketing values of CRP, even very slight elevations in baseline CRP are predictive of cardiovascular risk. More newly discovered protein markers of inflammation are now making their way into clinical practice.

The expansion of automated techniques for measuring individual proteins has facilitated their use in clinical management of a multitude of disorders, as this Protein Learning Guide describes. The choice of which methods to set up in-house for any particular laboratory will depend on the needs of the patient population being served. Almost certainly the diagnostic pace will demand measurement of more proteins more often and at smaller concentrations than could even have been imagined only a few decades earlier.

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SECTION 1

INTRODUCTION TO PLASMA PROTEINS

CLASSIFICATION AND GENERAL FUNCTIONS OF PLASMA PROTEINS 7

GENERAL FUNCTIONS OF PLASMA PROTEINS 10



LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the distribution of plasma proteins in the body.
- 2 List the classification of proteins.
- 3 Name the general functions of proteins.
- 4 Explain the structure of proteins.

CLASSIFICATION AND GENERAL FUNCTIONS OF PLASMA PROTEINS

A **plasma protein** is a protein with its highest concentration in the blood plasma that performs its main function in the plasma rather than at a target organ.

The term **protein** (from the Greek word proteios, of the first order) was first used by Jöns Jacob Berzelius in 1838. Berzelius assumed that there was only one protein in all living matter, including both plants and animals. In 1862, Anton Schmidt coined the term **globulin** for those proteins insoluble in pure water, and in 1894, Max von Gruber crystallized horse serum albumin.¹

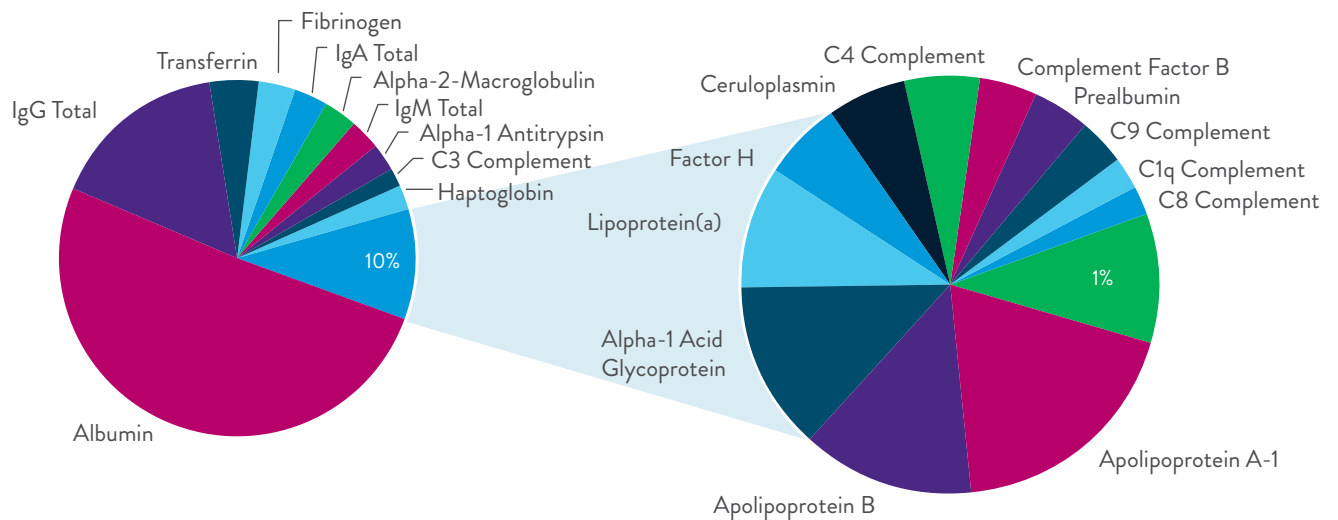


Figure 1-1: Major plasma proteins (99% of protein mass – according to Leigh Anderson, with permission).

The distribution of the plasma proteins in the body fluid compartments is under steady-state physiological conditions. However, the plasma protein concentration is dynamic and depends upon synthetic and catabolic rates and its distribution between intravascular and extravascular fluid compartments. Most plasma proteins are not present within cells, unless they have been synthesized within the cells or taken up by specific receptors. Although several hundred or even thousands of proteins are present in human plasma, the biological function of only a minor portion of proteins is known. They are highly heterogeneous in function and structure and occur in very different concentrations. The majority of plasma proteins are glycoproteins, usually with a carbohydrate content of 10%–25%, the most important exceptions being albumin and C-reactive protein, which do not contain any carbohydrate moieties. The relative abundance of the most important proteins in human plasma is shown in **Figure 1-1**.

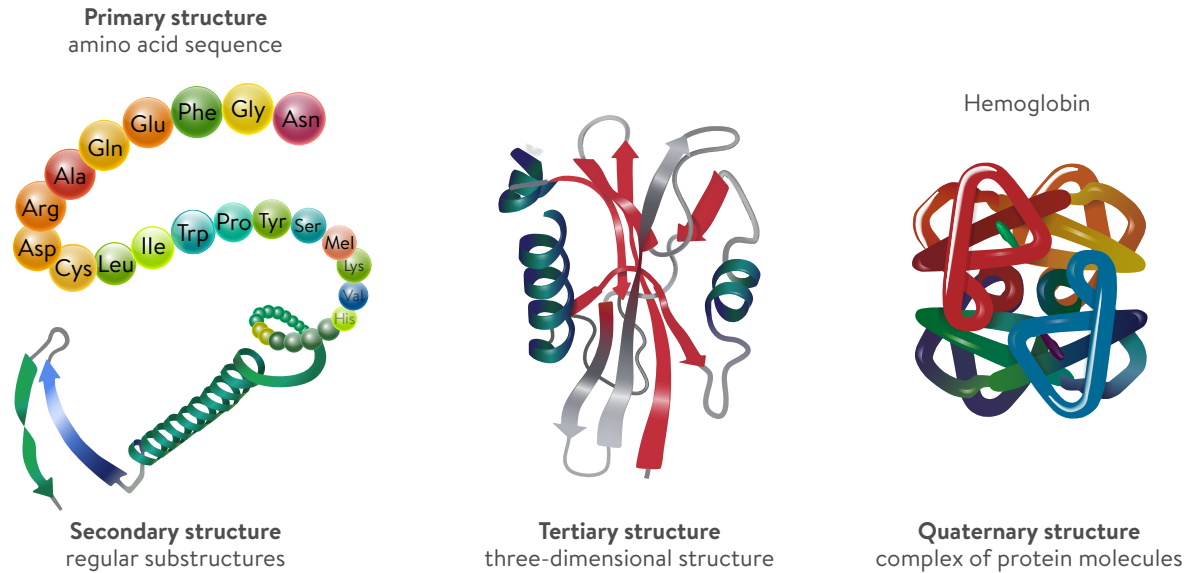


Figure 1-2: The building blocks of protein structure.

Structure: All proteins have a common primary structure (i.e., a linear peptide backbone constituted of amino acid subunits). Most proteins also possess secondary structures (e.g., longitudinal coiling). All proteins display tertiary structures resulting from folding, coiling (α -helix) and looping (β -pleated sheets) within chains, frequently due to the formation of disulfide bonds between cysteine residues. Quaternary structures refer to the conformations produced by the arrangement of more than one polypeptide chain (**Figure 1-2**). Many proteins have additional side chains made up of carbohydrates. Others contain or enfold lipids, cardiolipins or phospholipids. Still others are constructed so that various proteins, vitamins, drugs, elements or simple chemicals constitute an integral portion of the molecule (e.g., metallic ions).

Physiology: Plasma proteins are required to maintain physiological colloidal osmotic pressure (mainly albumin) and to transport lipids, metabolic products, hormones, drugs and trace elements. Immunoglobulins are an integral part of the immune defense. Their specific action is supported (“complemented”) by the complement proteins and CRP, which constitute the oldest proteins in the evolution of the immune system.

Some plasma proteins have enzymatic activity, while others are enzyme inhibitors. The dynamic equilibrium between enzymatic activation and inhibition is especially important in maintaining the balance between coagulation and fibrinolysis. Fibrinogen is the main substrate for coagulation, normally ensuring the fluidity of blood, and provides a rapid and lasting hemostasis in the case of injury to blood vessels.

Function	Specific protein(s)
Transport/binding	Albumin, Apo A-I, Apo B, transferrin (TRF), haptoglobin (HPT), hemopexin (HPX), retinol-binding protein (RBP), thyroxine-binding globulin (TBG), prealbumin (transthyretin) (PAL), transcortin, sex hormone-binding globulin (SHBG), histidine-rich- α -2-glycoprotein, transcobalamin
Oncotic pressure	Albumin mainly
Enzyme inhibitors	Alpha-1 Antitrypsin (AAT), alpha-1-antichymotrypsin (ACT), antithrombin (AT), alpha-2-macroglobulin, C1-esterase inhibitor, cystatin C, inter- α -trypsin inhibitor, plasminogen activator inhibitor-1 (PAI-1)
Immune defense	Immunoglobulins, complement components, C-reactive protein (CRP)
Acute-phase response	CRP, transferrin, alpha-1-acid-glycoprotein (AAG)
Coagulation/fibrinolysis	CRP, serum amyloid A (SAA), alpha-1-acid-glycoprotein, fibrinogen (FIB)
Enzymatic activity	Alpha-1 Antitrypsin, ceruloplasmin (CER), alpha-1-antichymotrypsin, haptoglobin, complement C3, complement C4
Tissue-derived proteins and oncofetal proteins	Fibrinogen, antithrombin, coagulation factors, proteins of the fibrinolysis process, fibronectin (FN)
Other functions (e.g., structure, protein reservoir, buffering, immunosuppression, adhesion)	Various proteins, structure: alpha-2-HS-glycoprotein (bone matrix); protein reservoir: albumin, prealbumin; immunosuppression: alpha-2-pregnancy associated glycoprotein; cell-to-cell adhesion: fibronectin

Table 1-1: Protein functions.

Synthesis: Plasma proteins are synthesized, predominantly, by hepatic, endothelial and immune cells such as lymphocytes and plasma cells. Protein synthesis is influenced significantly by nutritional status, various feedback mechanisms and hormonal factors, but genetic factors also play a key role. The importance of proteins in nutrition becomes obvious after considering the half-life of albumin, which is 19 days, and translates into a daily synthetic rate of about 10 g of albumin per day.

Degradation: Degradation of proteins occurs mainly in the liver, although some proteins like albumin are also eliminated renally (catabolism). After they have been taken up by the hepatocytes (endocytosis), the plasma proteins are deglycosylated and then cleaved into amino acids by proteinases and peptidases. This occurs both in the lysosomes and the cytosol of cells.

GENERAL FUNCTIONS OF PLASMA PROTEINS

A functional classification of plasma proteins is useful in understanding the changes that occur in diseases, as proteins with similar functions often form interacting systems (e.g., immunoglobulins, complement). Thus, a specific individual protein may occur in more than one of the functional classifications (**Table 1-1**).

The vascular system connects all parts and cells in the body with its plasma, and therefore contains many proteins that are at low concentration or do not have their main function in the plasma. The measurement of these proteins in human plasma/serum, however, may reveal important information about health and disease and about the function and status of our organs.

The blood itself is an organ, and its different cells are immersed in the plasma. The blood enables transport of signal molecules like the cytokines/interleukins and hormones. Depending on their concentration, other small molecules, which are also transported by the blood, can affect proteins. For example, high levels of blood glucose affect the glycation of proteins, and urea affects the carbamylation of proteins.

SECTION 2

IMMUNE SYSTEM AND COMPLEMENT SYSTEM

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the organization of the immune system.
- 2 Identify the different domains of the antibodies.
- 3 Specify the function of the major immunoglobulins.
- 4 Explain the mechanisms of activation of the complement cascade.

IMMUNE SYSTEM

DEFINITIONS

The term **immunology** describes the discipline that covers those natural processes that take place in every organism as an endogenous defense of the body against foreign substances.

The term **immunity** means protection against infectious disease and resistance to reinfection. This term is derived from the Latin word **immunitas** (“freedom from”) and refers to freedom from the burden of taxes or military conscription in Roman times.

The **immune system** is constituted by the cells and molecules of the body that are responsible for immunity. Their collective and coordinated response to the introduction of foreign entities is called the immune response and acts as the body’s immune defense.

However, since noninfectious extraneous components can also stimulate immune responses, a more comprehensive definition of immunity is a reaction to foreign substances, including microbes, as well as macromolecules, such as proteins and polysaccharides.

Historical annotations: The history of immunology starts with the formation of the primordial cells on Earth. It continues with the first observations by our ancestors of those vital processes that give protection against diseases and finds its splendor in the 19th and 20th centuries with the discovery of immunity by Emil von Behring, Paul Ehrlich, Robert Koch and Louis Pasteur, and its application in human medicine with the industrial production of vaccines (**Figure 2-1**).



Robert Koch
1843–1910



Emil von Behring
1854–1917



Louis Pasteur
1822–1895



Paul Ehrlich
1854–1915

Figure 2-1: Key investigators of immunology.

The 20th century experienced an explosion of fundamental research work and brilliant achievements in all branches of medical science, from the discovery that all chemical reactions in living cells are catalyzed by enzymes and that all enzymes are proteins, to the identification of the structure of numerous proteins, as well as the finding that all proteins responsible for immunity are made by specific genes.

The era of molecular biology started in 1953 when James Watson and Francis Crick described the mechanism of replication of DNA, and Matthew Meselson and Franklin Stahl proved that each of two DNA daughter double helices is made up of one parent strand and one newly synthesized strand. And finally, in 1959, Arthur Kornberg showed that the base sequence of the newly synthesized DNA strand is complementary to that of the parent strand. These discoveries opened the new era of molecular biological techniques and synthesis of proteins by means of recombinant technology.

The understanding that the response of the immune system to potent stimuli by different factors/agents as described by Emil von Behring, the first Nobel Prize laureate for Medicine in 1901, has resulted in the characterization of numerous plasmatic and cell-bound antigens, as well as numerous antibodies, and the development of new methods for their production. The availability of these technologies and the introduction of computer-supported analyzers has led to the development of modern immunochemical methods for the measurement of specific proteins.

THE ORGANIZATION OF THE IMMUNE SYSTEM

The immune system (i.e., the organization of cells and molecules with specialized defense roles) represents the evolutionary answer to how to protect an individual organism against pathogenic viruses, microorganisms and parasites. Immunity involves both specific and nonspecific components. The nonspecific components act either as barriers or as eliminators of a variety of pathogens/molecules, irrespective of antigenic specificity.

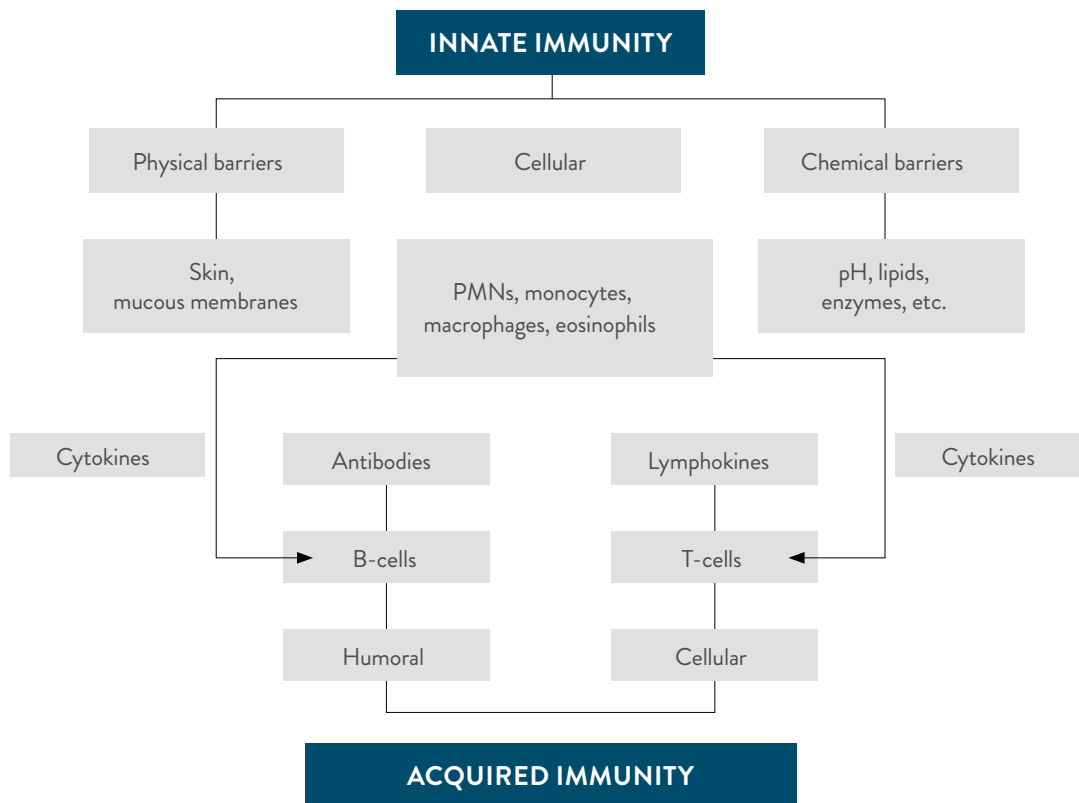


Figure 2-2: Pathways of acquired and innate immunity.

There are two different types of immunity determined by the speed and specificity of the reaction. The first is given by **innate (natural) immunity** and the second by **acquired (adaptive) immunity**, with the principal differences between the two relating to specificity and immunologic memory. The immune response is made up of a complex sequence of events that depends on the ability of the immune system to recognize foreign molecules (antigens) as potential pathogens and then to mount an appropriate reaction to eliminate the antigen. If the initial natural immune response is insufficient, a disease will result and the adaptive immune system is activated (**Figure 2-2**).

The innate (natural) immunity represents the body’s first line of defense to prevent the intrusion of a pathogen, and innate (natural) responses occur to the same extent as many times as the foreign substance is encountered.

The acquired (adaptive) immunity is more specific and becomes stronger and more rapid on successive exposure to a certain pathogen/antigen.

The innate immunity involves the elements of the immune system, which provide immediate host defense. These are phagocytic cells (neutrophils – polymorphonuclear neutrophils [PMNs], monocytes and macrophages), cells that release inflammatory mediators (basophils, mast cells and eosinophils) and natural killer (NK) cells. The molecular components of the innate response include complement, acute-phase proteins and cytokines, such as the interleukins and interferons.

Cell type	Main characteristics
Macrophage	Phagocyte; acts in nonspecific and specific responses; presents antigen to T-cells; cleans up and helps repair tissue damage
Neutrophil	Granulocyte; also called polymorphonuclear leukocytes (PMN); fast-acting phagocyte; takes part in inflammation, not in sustained responses; most effective against bacteria
Eosinophil	Secretes enzymes that attack certain parasitic worms; increased number in allergic reaction
Basophil and mast cell	Secrete histamine, other substances that act on small blood vessels to produce inflammation; contribute to allergies
Lymphocyte	Take part in most immune responses following antigen recognition, form clonal populations of effector and memory cells
B-cell	Effectors secrete four types of antibodies (IgA, IgE, IgG and IgM) that protect the host in specialized ways
Helper T-cell	Effectors secrete interleukins that stimulate rapid divisions and differentiation of both B-cells and T-cells
Cytotoxic T-cell	Effectors kill infected cells, tumor cells and foreign cells by a touch-kill mechanism
Natural killer (NK) cell	Cytotoxic cells of undetermined affiliation; kill virus-infected cells and tumor cells by a touch-kill mechanism

Table 2-1: The cell types of the immune system.

The function of adaptive immunity is to recognize previously encountered antigens on pathogenic microorganisms and mount an appropriate immune response resulting in elimination of the source of the antigen. The acquired (adaptive) immune response involves the proliferation of antigen-specific B and T lymphocytes, which occurs when the surface receptors of these cells bind to antigen.

ANTIBODIES

An **antibody**, also known as an **immunoglobulin**, is a large protein molecule produced by the immune system to identify and neutralize a foreign entity (antigen) that has entered the system. Each antibody recognizes a specific antigen (epitope) unique to its target. They have two important roles, namely to bind antigens, which may be free in solution, associated with microorganisms or expressed on the surface of infected cells, and to interact with host tissues and effector systems that remove the antigen.

Therefore, the antibodies are effectively bifunctional molecules. One part, which is extremely variable between different antibodies and known as the **variable domain** (Fab), is responsible for binding specifically to the many different infectious agents the body may encounter. The second region, the **constant domain** (Fc), is capable of interacting with different cells of the immune system, some phagocytic cells and the first component (C1q) of the classical complement system (**Figure 2-3**). One final important role of antibodies is to control the triggering of the inflammatory reaction and release of inflammatory mediators.

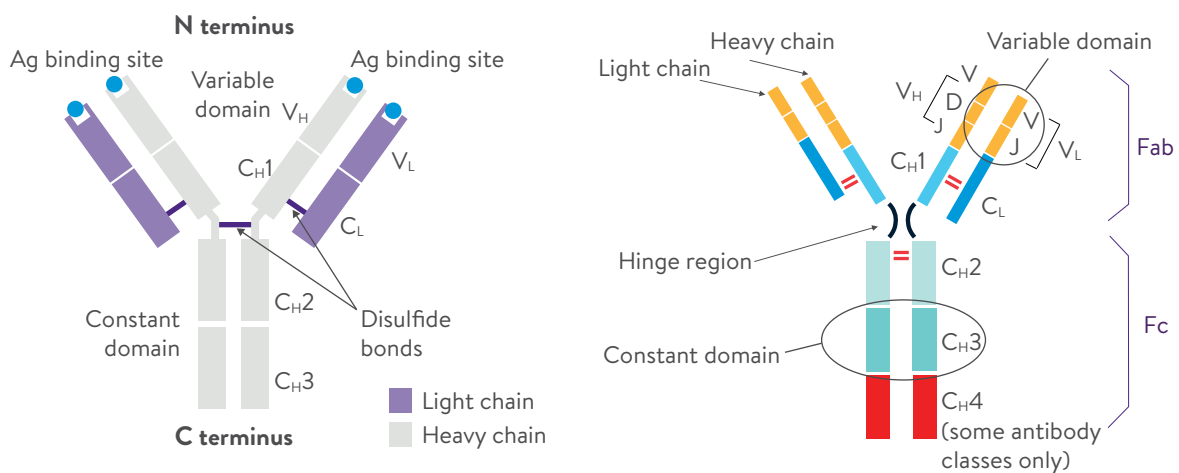


Figure 2-3: Antibody structure.

Antibody affinity is a measure of the strength of the bond between an antibody combining site and a single antigenic epitope. The functional affinity or avidity of the interaction additionally depends on the number of binding sites on the antibody and their ability to react with multiple epitopes on the antigen.

IMMUNOGENS AND ANTIGENS

An **immunogen** is a molecule that can induce an immune response in a particular host. The term “antigen,” which is frequently used interchangeably with immunogen, actually refers to the ability of a molecule to react with antibodies, not necessarily its ability to induce their formation. Thus, although all immunogens are also antigens, the converse is not true. Different antibodies bind to different antigens, as each antibody is specific for a particular antigen.

Each antibody binds to a particular part of the antigen called an antigenic determinant, or epitope. A particular antigen can have several different or identical epitopes. Antibodies are specific for the epitopes rather than for the whole antigen molecule, but since each antigen has its own particular set of epitopes, which are not usually shared with other antigens, the collection of antibodies in an antiserum is effectively specific for the antigen.

KINETICS OF THE IMMUNE RESPONSE

An individual's first encounter with a particular immunogen is called a priming event and leads to a relatively weak, short-lived antibody response designated the **primary immune response**, in which mainly IgM antibodies are first produced, followed by IgG antibodies (**Figure 2-4**). The response can be divided into different phases. The initial or latent phase is the time between the contact with the immunogen and the detection of antibodies in the circulation, which averages about 1 week. During this period (primary response), activation of T and B lymphocytes will take place. The exponential phase marks a rapid increase in the quantity of antibodies that are secreted by plasma cells in the circulation. After an interval during which the antibody level remains relatively constant because synthesis and degradation are occurring at approximately equal rates (steady-state phase), the antibody level gradually declines (declining phase) as synthesis of new antibodies wanes. A second or subsequent encounter with the same immunogen leads to a response (**secondary immune response**) that is qualitatively similar to the primary response, but manifests marked quantitative differences and is dominated with production of IgG antibodies.

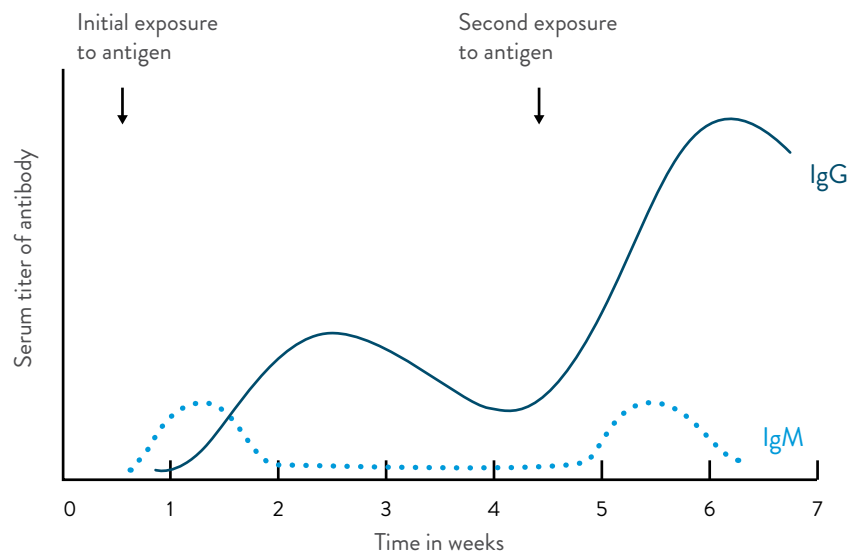


Figure 2-4: Typical immunoglobulin response.

In this secondary response, the lag period is shortened, and antibody levels rise more rapidly to a much higher steady-state level (steeper slope), remaining detectable in serum for much longer periods. Memory T- and B-cells generated during the primary response are responsible for the more rapid kinetics and greater intensity and duration of secondary responses. This explains why booster injections of vaccines are so effective and productive.

IMMUNOGLOBULINS – A FAMILY OF PROTEINS

First hypothesized by Rodney Porter in 1962, the basic immunoglobulin unit consists of a glycosylated protein of two identical heavy (“H”) and two identical light (“L”) polypeptide chains that are linked together by disulfide bonds. Amino acid sequences in both heavy and light chains are divided into regions that are either constant or variable. In addition, each variable region contains sequences that are hypervariable. There are five different classes (known as isotypes) of antibody in the human immune system: IgG, IgA, IgM, IgD and IgE. They differ in mass, charge, amino acid composition and carbohydrate content. The light chains of most vertebrates have been shown to exist in two distinct forms called kappa (κ) and lambda (λ). These isotypes are present in all individuals. Either of the light chain types may combine with any of the heavy chain types, but in any one molecule, both light chains and both heavy chains are of the same type. All immunoglobulins are glycoproteins, but the carbohydrate content ranges from 2%–3% for IgG to 12%–14% for IgM, IgD and IgE.

The immunoglobulins have different roles in the immune system, namely:

- IgG is the secondary immune response, can cross the placenta to pass immunity to a fetus and works efficiently to coat microbes, speeding their uptake by other cells.
- IgM is the primary immune response and is very effective at killing bacteria in concert with complement proteins.
- IgA concentrates in body fluids (tears, saliva, secretions of the respiratory tract and the digestive tract), protecting entrances to the body.
- IgE shields against parasitic infections and is involved in allergic diseases.
- IgD remains attached to B-cells and plays a key role in initiating early B-cell response.

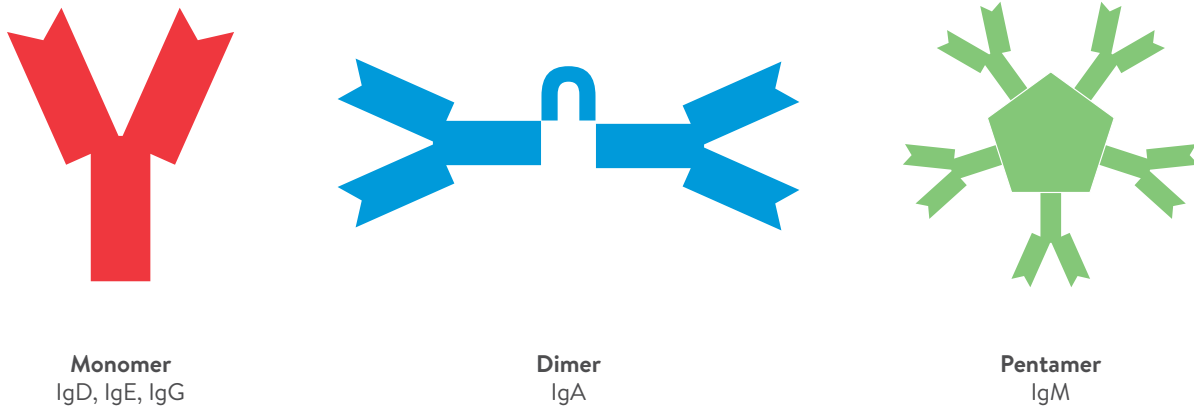


Figure 2-5: Immunoglobulin structures.

IMMUNOGLOBULIN/ANTIBODY STRUCTURE

The basic antibody unit (which is biochemically a tetramer) is bivalent, with two antigen-binding arms of identical specificity. The region that binds to a particular antigen is known as the Fab region, while the constant Fc region determines the isotype and is responsible for evoking effector systems. Each of these arms can be cleaved proteolytically in the laboratory to yield individual Fab monovalent antigen-binding fragments.

The typical IgG molecule has two intrachain disulfide bonds in the light chain, one in the variable region and one in the constant region. The secretory IgA at mucosal surfaces is a tetravalent **dimer** (Figure 2-5), whereas circulating IgM is a decaivalent **pentamer**. IgA and IgM polymers are stabilized by a polypeptide, the J (joining) chain. Secretory IgA also contains a molecule called secretory component, which may protect the IgA against proteolytic cleavage within the gastrointestinal tract.

Receptors for immunoglobulins are expressed on mononuclear cells (monocytes/macrophages), neutrophils, natural killer cells, eosinophils and mast cells. They interact with the Fc regions of different classes of immunoglobulins and promote activities such as phagocytosis, tumor cell killing and mast cell degranulation. Most of the Fc receptors are members of the immunoglobulin superfamily and have two or three extracellular immunoglobulin domains.

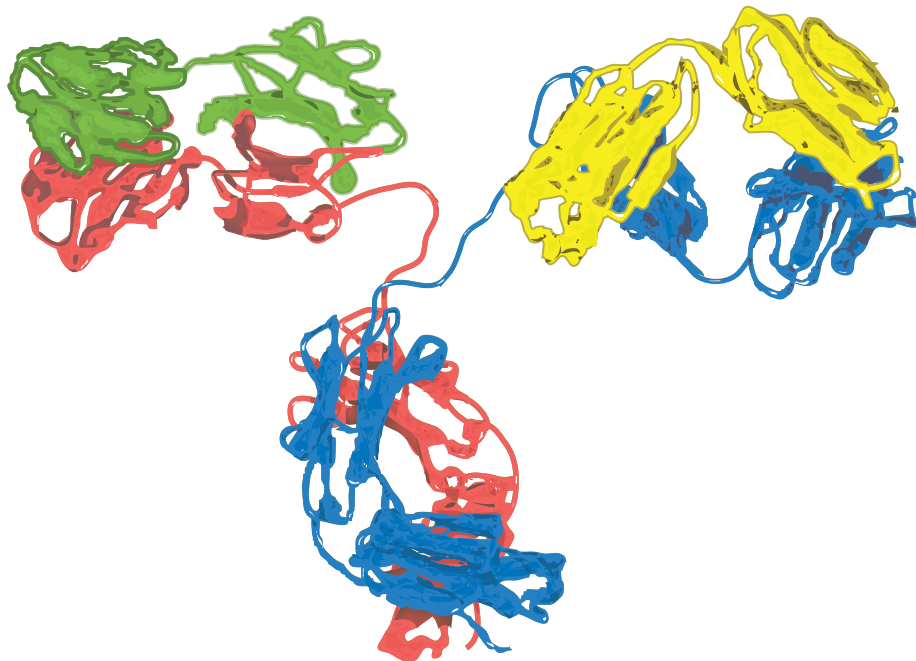


Figure 2-6: Three-dimensional illustration of a typical IgG molecule. Red and blue represent heavy chains; green and yellow represent light chains.

Immunoglobulin G (IgG) is the most important class of immunoglobulin in secondary immune responses and is equally distributed between the intravascular and extravascular pools. IgG has a half-life in the blood of approximately 23 days. In healthy adults, IgG constitutes approximately 80% of total serum immunoglobulins. It consists of one basic unit comprised of two identical light (L) chains (kappa or lambda, molecular mass 22 kDa) (**Figure 2-6**) and two identical heavy (H) chains (γ chain, molecular mass 50 kDa), joined together by a variable number of disulfide bonds. IgG is produced 5–7 days after the first contact with an antigen (primary immunization) when IgM titers begin to decrease. It is the major Ig to be produced immediately after repeat contact with the antigen (re-immunization) as a memory or secondary immune response. There are four IgG subclasses (i.e., IgG1, IgG2, IgG3 and IgG4), which are different in their structure of heavy chains and biologic properties. Their ability to bind to C1q complement varies in the order IgG3 > IgG1 > IgG2, with subclass IgG4 unable to bind C1q but capable of activating the alternative complement pathway. IgG1, IgG3 and their Fc fragments can also bind to the surface receptors of macrophages. IgG is the only immunoglobulin that crosses the placenta, conferring a high degree of passive immunity to the newborn. IgG has a half-life of 23 days, which becomes longer when its concentration decreases.

Elevated IgG levels can be polyclonal, oligoclonal or monoclonal. Elevated polyclonal IgG levels are associated with autoimmune diseases (systemic lupus erythematosus [SLE]), rheumatoid arthritis (RA), Sjögren's syndrome, sarcoidosis, chronic liver disease (infectious hepatitis and alcoholic cirrhosis), cystic fibrosis, some parasitic diseases, and chronic or recurrent infections. Increased oligoclonal IgG levels are associated with infections (especially in the elderly, HIV/AIDS and chronic active hepatitis), malignancies, some dysgammaglobulinemias and autoimmune disorders. Oligoclonal IgG only in cerebrospinal fluid but not serum indicates an immune response within the central nervous system, such as multiple sclerosis, but can also be due to infection such as viral encephalitis. Increased monoclonal IgG levels are associated with multiple myeloma (IgG type), lymphomas and leukemia. Monoclonal IgG is commonly seen in monoclonal gammopathy of undetermined significance (MGUS). Of these, about one-fifth transform into a malignant disease.

Immunoglobulin M (IgM) is the predominant antibody in primary immune responses, although it has the lowest concentration of the three major immunoglobulins, IgG, IgA and IgM (about 6% of total serum immunoglobulins). IgM normally consists of 10 heavy μ -chains and 10 κ - or λ -type light chains, which are identical within a molecule. A J-chain links the μ -chains together to form the pentameric IgM molecule (molecular mass 971 kDa). IgM, compared with IgG, is the first specific antibody to be detected in the blood of patients with an infectious disease. Its concentration decreases at a fairly rapid rate after the infection has receded. This circumstance gives an advantage in the differential diagnosis of acute and chronic infections by comparing specific IgM and IgG titers. If IgM is prevalent, the infection is acute; whereas, if IgG predominates, the infection is chronic (e.g., in infection by rubella or hepatitis viruses). As soon as IgM is bound to its target, it powerfully activates the complement through the classical pathway. As a pentamer, IgM is highly efficient, serving as both an opsonizer and agglutinator to assist the phagocytic system to eliminate many kinds of microorganisms. The half-life of IgM is 5 days.

Monoclonal IgM (M-component) is commonly seen in Waldenström's macroglobulinemia, a type of lymphoma.

Immunoglobulin A (IgA) accounts for 13% of the serum immunoglobulins. IgA may play an important role in local immunity by combining with viruses and bacteria, thus preventing their adherence to mucosal surfaces. It is the predominant immunoglobulin class at mucosal sites (saliva, tears, respiratory, GU and GI tracts, and colostrum), where it provides an early antibacterial and antiviral defense. Secretory IgA (sIgA) is synthesized in the subepithelial regions of the GI and respiratory tracts. It consists of two α H-chains, two κ or λ L-chains, and one molecule each of J-chain (molecular mass 380 kDa) and secretory component, which is attached in epithelial cells as it is secreted into exocrine fluids. There are two subclasses of IgA: IgA1 and IgA2. In serum, IgA1 is the predominant subclass and, in many secretions, such as nasal secretions, tears, saliva and milk, accounts for 70%–95% of total IgA. However, in the colon, IgA2 predominates (approximately 60% of the total IgA). Although IgA does not bind to the first component of complement, it induces activation of the alternative complement pathway.

Elevated IgA levels are associated with both polyclonal (more than IgA affected) and monoclonal increases. Polyclonal IgA increases include chronic liver disease (especially alcohol induced), chronic infections (especially of GI and respiratory tracts), recurrent otitis, neoplasia of the lower GI tract, inflammatory bowel disease and autoimmune diseases such as rheumatoid arthritis. Monoclonal increases include IgA multiple myeloma, MGUS and, occasionally, other lymphomas.

The **immunoglobulin D (IgD)** molecule is a monomer, and its molecular mass of approximately 180 kDa is slightly higher than that of IgG. It accounts for less than 1% of the total immunoglobulin in serum. IgD is the predominant immunoglobulin on the surface of developing B-cells and possibly acts as a triggering receptor for the growth and development of B-lymphocytes. Hyper IgD syndrome is a periodic fever syndrome. It is characterized by attacks of fever, arthralgia, skin lesions (including cyclical mouth ulcers) and diarrhea. Laboratory findings include an acute-phase response with elevated CRP and markedly elevated IgD level.

Immunoglobulin E (IgE) (reaginic, skin-sensitizing or anaphylactic antibody) is found primarily in respiratory and GI mucous secretions. In serum, IgE is present in very low concentrations. It has four domains of α H-chains with a molecular mass of 188 kDa. IgE attaches via its Fc region to receptors on mast cells. The bridging of two IgE molecules by allergen may induce the release of mediators from mast cell granules that cause the clinical features of acute allergy. IgE levels are elevated in atopic diseases (e.g., allergic rhinitis or extrinsic asthma, hay fever and atopic dermatitis, parasitic diseases, advanced Hodgkin's disease, and IgE monoclonal myeloma). IgE may have a beneficial role in the defense against parasites.

COMPLEMENT SYSTEM

The complement system has several important functions in innate immunity, including:

- Making bacteria and cell debris more susceptible to phagocytosis.
- Direct lysis of cells (through damage/rupture of plasma membranes).
- Producing chemotactic substances (for directed migration of immune cells).
- Increasing vascular permeability.
- Initiating inflammation via direct activation of mast cells.

The **complement** pathway is a complex, multicomponent system consisting of a series of approximately 26 proteins, some of these secreted by both hepatocytes (liver cells) and monocytes, and combining with antibodies or cell surfaces. Although these proteins may be activated by the adaptive immune system (classical pathway) or the innate immune system (alternative pathway, also called “alternate pathway”), the nomenclature is derived from the fact that the proteins help (complement) the work of antibodies in destroying bacteria. Indeed, the term “complement” was introduced by Paul Ehrlich in the late 1890s, as part of his larger theory of the immune system.

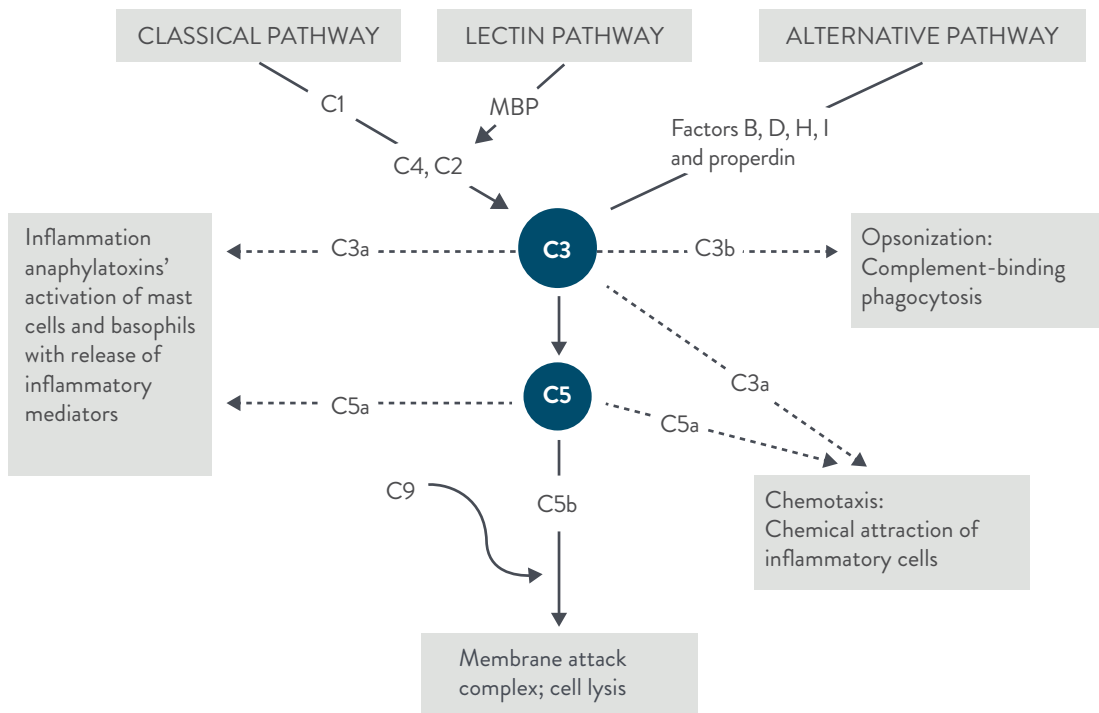


Figure 2-7: Mechanisms of activation of the complement cascade.

Complement proteins circulate in the blood in an inactive form. Some must be cleaved enzymatically, others must be activated either by cell receptors or by simply combining to form active complexes to exert their function. The so-called **complement cascade** (**Figure 2-7**) is set off when the first complement molecule, C1, encounters antibodies bound to antigens, resulting in induction of the inflammatory response, phagocyte chemotaxis and opsonization, and ultimately, cell lysis. Each of the complement proteins performs its specialized job in turn, acting on the molecule next in line.

The complement cascade occurs via two distinct pathways – the classical pathway and the alternative pathway – and consists of a series of amplification stages. This means that activation of a single molecule will lead to thousands of molecules being generated.

The recently identified mannose-binding lectin pathway feeds into the classical sequence by activating it independently of the C1rs complex and is stimulated by mannose-binding proteins (MBPs) and carbohydrates on microbes.

SECTION 3

INFLAMMATION, SEPSIS, ACUTE-PHASE REACTION AND ACUTE-PHASE PROTEINS

INFLAMMATION/SEPSIS.....	23
STEPS OF THE ACUTE-PHASE REACTION	24



LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the inflammatory reaction and its symptoms.
- 2 Identify the steps of an acute-phase reaction.
- 3 Explain the difference between positive acute-phase reactants and negative acute-phase reactants.
- 4 Specify the significance of CRP as a marker of inflammation.
- 5 List the important acute-phase proteins.

INFLAMMATION/SEPSIS

Inflammation is a protective reaction of the body and a nonspecific response to various damaging stimuli, such as bacterial infections, viruses or parasites, toxins, extreme temperatures and trauma (**Figure 3-1**). The aim of the inflammatory process is, first, to hinder the spread of an infection and eliminate the invading organism and, second, to reconstitute tissue function.

The classical, general symptoms of an inflammatory reaction include fever, tiredness, somnolence, loss of appetite, increased heart rate, hyperventilation, local swelling and pain. These are accompanied by a series of local and systemic clinical, biochemical and cellular changes referred to collectively as the **acute-phase reaction** (APR).

If the inflammatory response is not controlled properly, then the uncontrolled inflammation can lead to a circulatory collapse (shock) and, eventually, death.

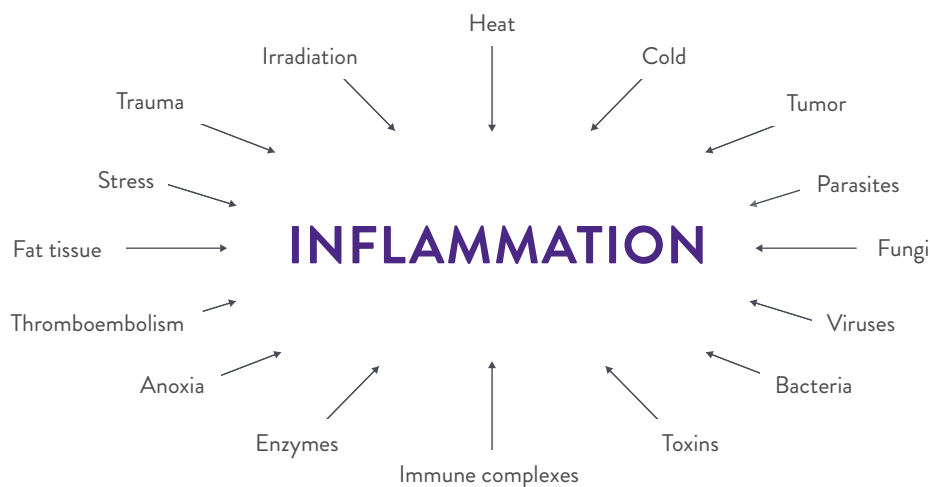


Figure 3-1: Causes of the inflammatory response.

Sepsis is life-threatening organ dysfunction caused by a dysregulated host response to infection. The immune system may develop this inflammatory response to an infection caused by microorganisms (e.g., bacteria) in a particular body region or widespread in the bloodstream, resulting in hemodynamic consequences and damage to organs. However, this inflammatory response can also occur in patients without the presence of infection (e.g., in those with burns, polytrauma or the initial state in pancreatitis and chemical pneumonitis).

The inflammatory reaction is an unspecific response caused by microbial agents (viruses, bacteria, fungi and parasites), noninfectious inflammatory stimuli (e.g., rheumatoid arthritis, graft-versus-host disease), tissue necrosis (e.g., cancer, anoxia, burns), toxins or irradiation.

STEPS OF THE ACUTE-PHASE REACTION

The activation of the local inflammatory cells at the site of the local inflammatory process releases a number of different substances with local and general effects. This systemic reaction is called **acute-phase reaction/acute-phase response (APR)**. The changes are caused by the activation of **inflammatory cells**, which release different types of mediators, proteases, bradykinin, histamine, prostaglandins, leukotrienes, cytokines/interleukins and growth factors, but also reactive species like nitrous oxide, O₂⁻, OH⁻ and lactic acid. The inflammatory response also affects the complement and coagulation systems (**Figure 3-2**).

The local inflammatory reaction can be divided into three steps:

First, there is a nervous reflex action to bring the body out of the danger zone.

Second, the coagulation system is activated to prevent loss of blood and spread of the infection.

Third, the complex APR is started, which activates unspecific defense processes and causes local and general symptoms like warmth, swelling, redness, fever, pain, loss of appetite, increased heart rate and hyperventilation.

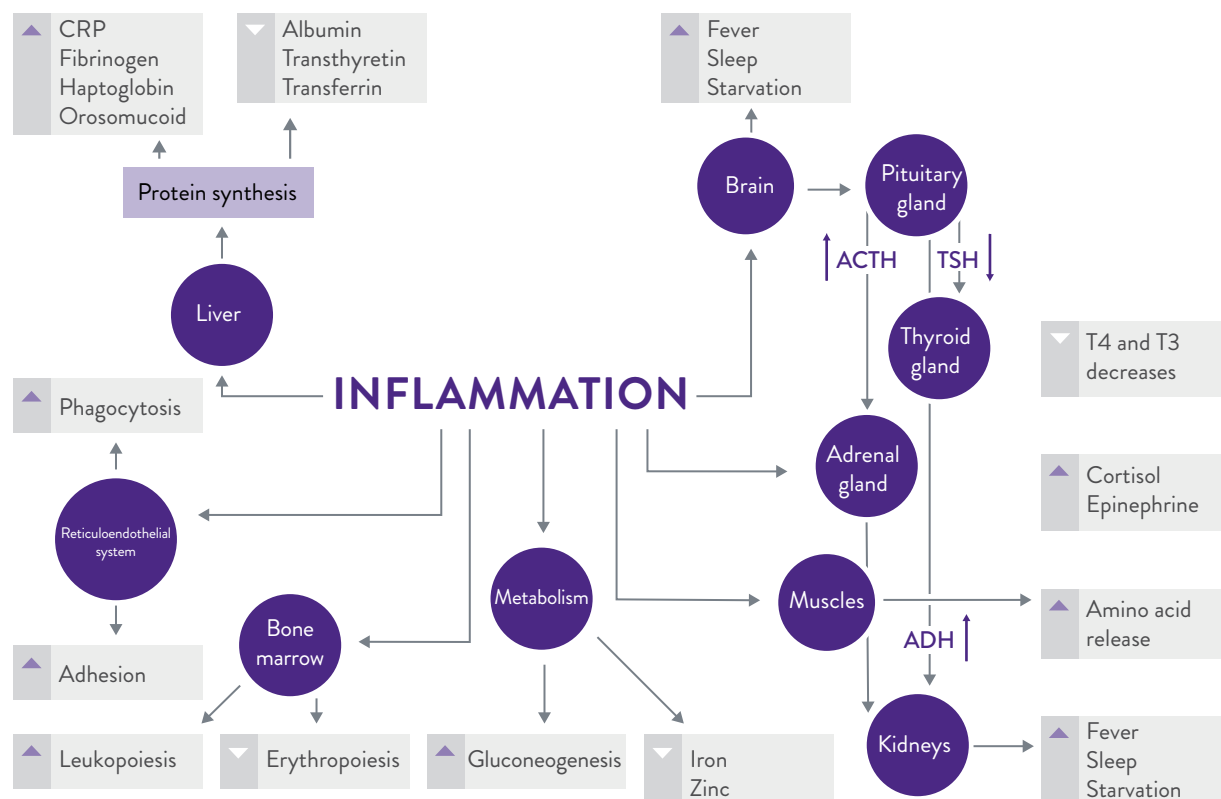


Figure 3-2: The local inflammatory process, depending on its severity, will also influence the function of most organ systems and their body functions.

The inflammatory process involves both specific cellular and immunological defense reactions. The release of the inflammatory mediators, tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), IL-6 and IL-8 will affect the hepatocytic protein synthesis with markedly decreased production of albumin, transferrin and prealbumin, while simultaneously the synthesis of the so-called acute-phase proteins (C-reactive protein [CRP], serum amyloid antigen [SAA], fibrinogen, antitrypsin, antichymotrypsin, haptoglobin and α 1-acid glycoprotein [AAG]) are significantly increased.

Ultimately, the APR helps to limit tissue damage by a series of reactions that neutralize and eliminate the inflammatory agent(s) and initiate repair mechanisms for restoring the normal body functions. Normally, the APR lasts only a few days or a week depending on the cause of the process, but in cases of chronic or recurring inflammation, some features of the inflammatory response may continue. This will further contribute to the tissue damage that accompanies the disease, thus causing further complications (e.g., protein deposition diseases). Depending on the severity and how often and how long inflammation is present, the inflammatory signals will also affect the vascular endothelial cells. This will ultimately lead to fatty streaks and then plaque formation in the vascular wall, with an increased risk of future cardiovascular events.

THE ROLE OF ACUTE-PHASE PROTEINS

The acute-phase proteins (APPs) have a variety of activities that maintain host defense. In fact, they can directly deactivate inflammatory agents, reduce local tissue damage, and participate in tissue repair and healing. Subsequent to a fast increase in blood concentration of many components of the complement and coagulation cascades and their activations, there is a local accumulation of neutrophils, macrophages and plasma proteins. These all participate in the elimination of microbial agents, the removal of foreign and toxic cellular debris, and the repair of damaged tissue. Coagulation factors, such as fibrinogen, not only play an important regulatory role in minimizing the spread of the infection by encapsulation, but also promote wound healing and tissue remodeling. Protease inhibitors counterbalance the release of lysosomal proteases – following activation of neutrophils and macrophages – into the extracellular space, thus regulating the activity of pro-inflammatory enzymes to the local site of inflammation.

General functions of acute-phase proteins:

- Activation of coagulation
- Activation of complement
- Enhancement of opsonization
- Antiproteolytic activity
- Scavenging of free oxygen radicals
- Participating in restoring tissue and tissue function

The decrease of transferrin in the serum during the inflammatory process reduces the level of iron available for uptake by microbial agents. Simultaneously, the serum level of zinc is reduced, which also has a negative impact on microbial growth.

DEFINITION OF THE TERM “ACUTE-PHASE PROTEINS”

APPs have been defined as those proteins whose concentration increases rapidly during the first 7 days following inflammation (**Table 3-1**). C-reactive protein (CRP) and serum amyloid A (SAA) can increase more than 100-fold. According to this definition, some 30 plasma proteins fall into the category. Most are controlled directly by cytokines (TNF- α , IL-6 and IL-8) and show changes fairly specific to inflammation and tissue damage. Other proteins such as ferritin and β 2-microglobulin, which may increase in inflammation, are mediated by different processes and are not generally referred to as APPs.

POSITIVE AND NEGATIVE ACUTE-PHASE PROTEINS

Several transport proteins, such as albumin, retinol-binding protein (RBP), transferrin and prealbumin, fall in concentration during the acute-phase response and are known as **negative acute-phase proteins**. The synthesis of these **negative APPs**, mainly produced in the liver, decreases during the APR to enable an increased production capacity of the **positive APPs**, and thus their serum concentration decreases.

Increase by factor 1.5 x	Increase by factor 2 to 4-6 x	Increase by factor 10 to > 100 x	Decrease
Slow response	Moderate response	Rapid response	
Antithrombin C3, C4 Ceruloplasmin C1-esterase inhibitor	Alpha-1 Antitrypsin (AAT) Alpha-1-acid glycoprotein (AAG) Alpha-1-antichymotrypsin (ACT) Haptoglobin (HP) Fibrinogen (FIB)	C-reactive protein (CRP) Serum amyloid A (SAA)	Albumin (ALB) Transferrin (TF) Retinol-binding protein (RBP) Prealbumin (PAL) Thyroxine-binding globulin (TBG)

Table 3-1: Classification of some acute-phase proteins.

During the initiation of an inflammatory process, commonly the concentration of the positive APPs changes following a specific pattern. The rate of increase or fall in serum concentration of the different APPs varies considerably, and there is a wide range of sensitivity among the APPs, with SAA and CRP being the most sensitive and responsive and thus the major APPs in humans. Significantly, individuals unable to synthesize these proteins have not been described so that these APPs are likely to be of considerable importance for the body (**Figure 3-3**).

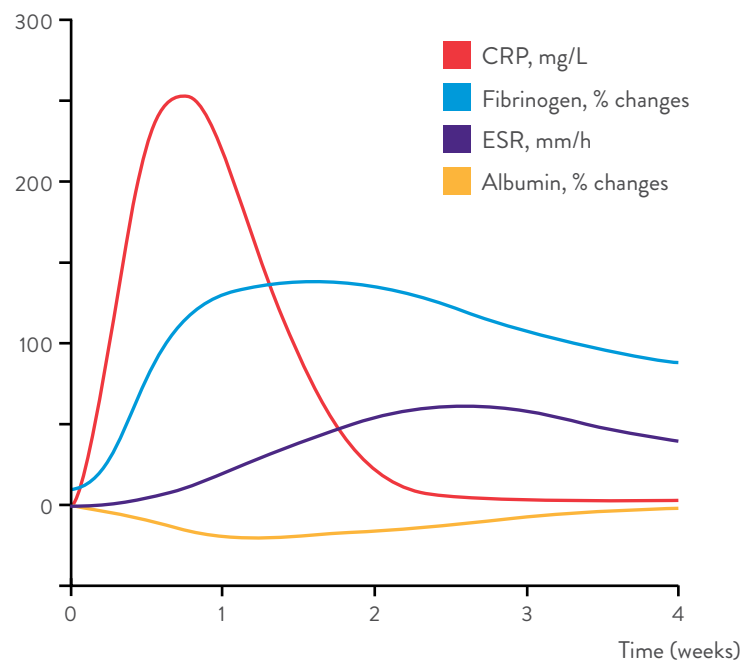


Figure 3-3: Typical responses of key analyses to the acute phase.

Changes in APPs occur in a wide range of pathological processes, from bacterial infection to autoimmune inflammation, myocardial infarction and postoperative situations. However, the changes are not specific for any one condition. A monitoring of APP changes is clinically useful and often a more sensitive indicator of disease activity than the erythrocyte sedimentation rate (ESR), viscosity or leukocyte count.

The measurement of acute-phase proteins is important clinically in the detection and therapeutic monitoring of patients with infections, trauma, burns, immune complex and autoimmune diseases, malignancy, and postoperative conditions. Although not specific for one particular pathological process, APP measurement is highly sensitive and most useful when interpreted in the light of the clinical setting, especially if sequential sampling is used (**Table 3-2**).

Detection of organic disease	Monitoring of the effectiveness of therapy
Bowel symptoms (IBD/IBS)	Antibiotic therapy
Arthralgia and back pain	Rheumatoid arthritis
Chest pain	Systemic vasculitis
Suspected venous thrombosis	Crohn's disease
Detection of bacterial infection	Assessment of severity and prognosis
Fever in children	Polymyalgia rheumatica
Pneumonia	Rheumatoid arthritis
Septicemia	Different infectious diseases
Neutropenic patients	Inflammatory bowel diseases
Complications following surgery	AA amyloidosis
Meningitis	Malignant disease
Connective tissue disease	Cardiovascular diseases

Table 3-2: Clinical use of measurements of acute-phase proteins.

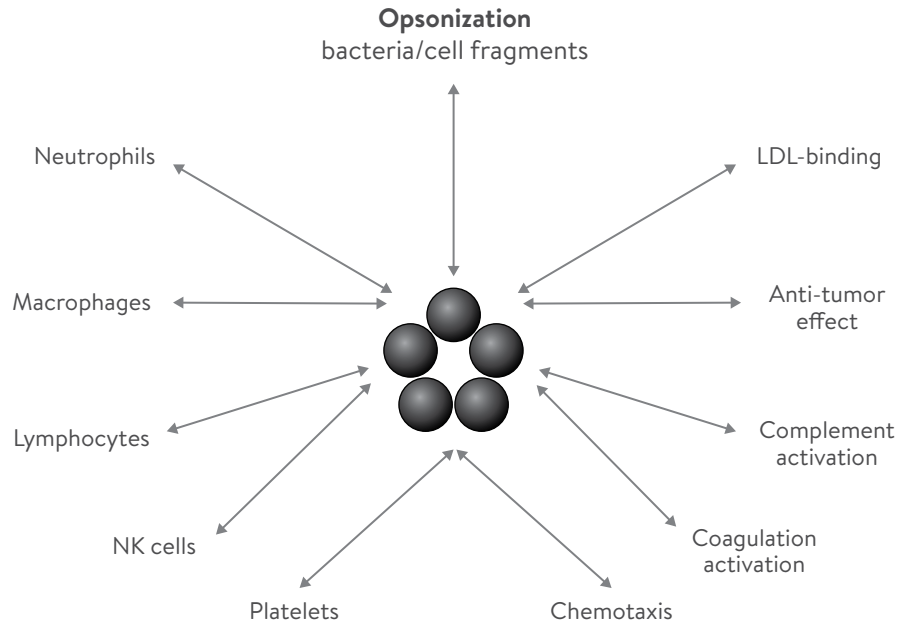


Figure 3-4: Interactions of CRP during inflammation and host defense.

C-REACTIVE PROTEIN (CRP)

The function of C-reactive protein (CRP, molecular mass 21.5 kDa) is to recognize foreign pathogens and damaged cells and cell fragments and to initiate their removal by the macrophages. Initially, CRP binds to bacterial polysaccharides and glycolipids, damaged membranes, and exposed nuclear antigens. CRP is known to bind to complement C1q, leading to the activation of the classical complement pathway, thus promoting macrophage phagocytosis. CRP also has opsonic properties by binding to Fc receptors to enhance phagocytosis of particulate antigens and microorganisms. It also binds to both native and oxidized low-density lipoprotein (ox-LDL) and may clear LDL from the site of atherosclerotic plaques by binding to cell surface receptors on macrophages, which thereby are transformed gradually into foam cells within the plaques (**Figure 3-4**).

CRP is a multifunctional protein that has important roles in inflammation and host defense.

The majority of healthy adults have serum CRP levels below 1 mg/L, the median of the normal CRP concentration being 0.8 mg/L, with approximately 90% < 3 mg/L and 99% < 10 mg/L.

CRP has been detected in plasma, serum and various body fluids, including cerebrospinal, synovial, pleural and ascitic fluids. It does not cross the placental barrier, so the small amounts present in umbilical cord blood are of fetal rather than maternal origin.

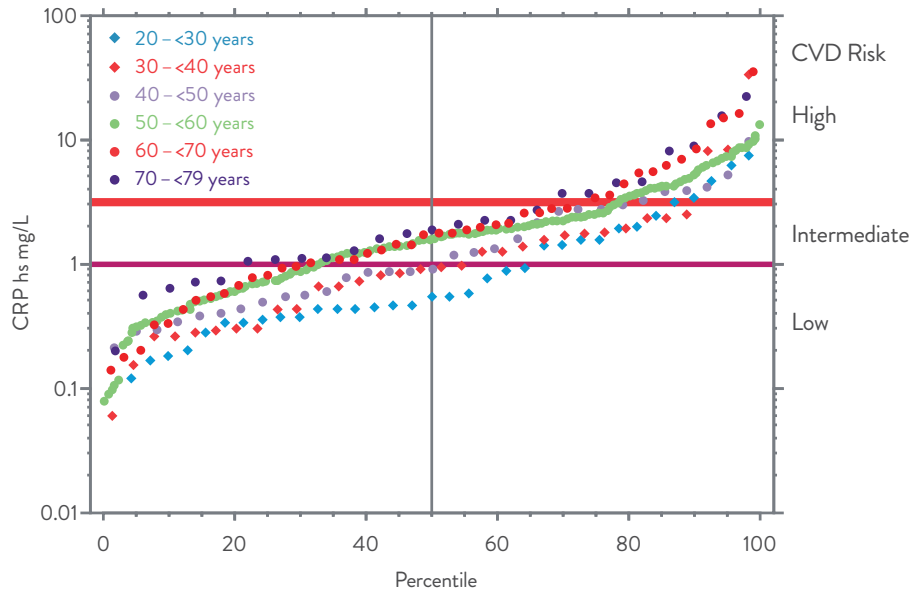


Figure 3-5: Serum CRP values in 472 apparently healthy subjects divided in age intervals of 10 years from 20 to 80 years.

Clinical significance of CRP as marker of inflammation: CRP is the most sensitive among the APPs, and its concentration increases rapidly during inflammatory processes. Whereas APP concentrations rarely increase by more than fourfold, CRP levels increase several hundredfold. The rise and clearance of CRP is exponential, with a half-life of 17 hours. Peak levels are usually reached within 48–72 hours. If tissue repair proceeds normally, the CRP level peaks on the second or third postoperative day and returns toward normal levels within about a week. A persistent elevation in CRP indicates continuation of the pathologic process or a complication (**Figure 3-5**).

An elevation of CRP is not diagnostic of any one specific disease as it occurs in all diseases involving tissue damage or inflammation. However, because of its extreme sensitivity, CRP can be used to screen apparently healthy persons, such as blood donors or outpatients, for the presence of disease. It provides a valuable adjunct to clinical assessment and is a very sensitive index of ongoing inflammation. One of the main uses of CRP is the monitoring of infectious diseases with the goal to minimize the use of antibiotics. Thus, a low CRP value in subjects with symptoms of an infection indicates that probably there will be no need for an antibiotics treatment.

In disease, the highest CRP concentrations are seen in bacterial infections, with values > 600 mg/L, with lower levels in fungal and parasitic infestations and some other diseases. Viral infections are less likely to cause substantial elevations. However, in some patients with influenza, CRP values > 120 mg/L can be seen without any sign of a concomitant bacterial superinfection. An elevated CRP value is highly specific for inflammatory diseases, but it must be taken into account that an increased CRP value is not specific for any particular disease. However, CRP measurements are sometimes useful in differentiating two diseases with similar clinical manifestations or to indicate the presence of complications (e.g., pyelonephritis) is regularly associated with elevated CRP, while cystitis is not. The great value of a CRP measurement is given when the results are interpreted by an experienced clinician (**Figure 3-6**).

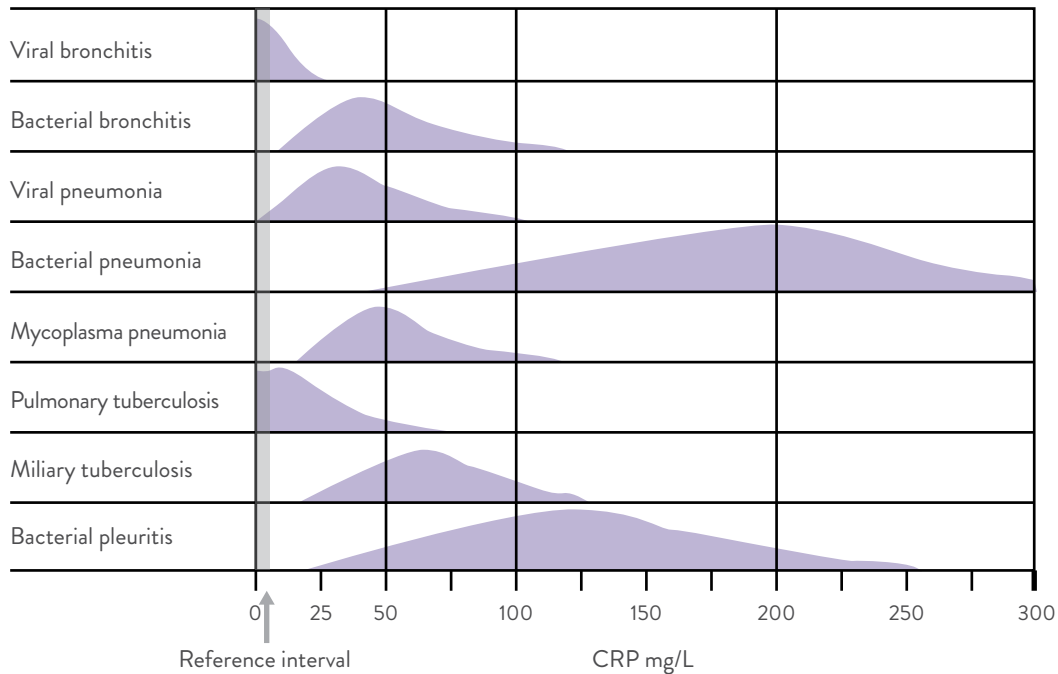


Figure 3-6: Typical CRP values in serum of subjects with different types of respiratory tract infections.

When assessing cardiovascular disease (CVD) risk using hsCRP (high sensitivity CRP), measurement of the following should be considered:

- The CVD risk is related to both the sum of inflammation (duration of CRP increase) and the intensity of the inflammatory episodes (the amount of CRP increase) during life, independent of the location of the inflammation in the body.
- Non-acutely threatening active, smoldering chronic infections, such as gingivitis and periodontitis, can cause CRP elevations and an increased CVD risk.
- Obesity and smoking cause CRP elevations. This CRP increase is due to a rise of cytokines.
- Significant weight reduction decreases CRP levels in serum due to decreased IL-6 production in adipose tissue.
- Although estrogen and hormone replacement therapies may induce a slight increase of CRP levels in serum, probably due to a direct effect of the CRP production in the liver, this might not be related to an increase in CVD risk.

When using hsCRP for CVD risk assessment, the following risk categories are often used:

CVD risk	hsCRP mg/L
Low	< 1.0
Intermediate	1.0–3.0
High	> 3.0

CRP values > 10 mg/L suggest that the assessment of CVD risk might be confounded by a recent or acute inflammatory disease (e.g., trauma, infection), and thus the CRP value using a sensitive assay should be checked again at a later time after the subject has been free of any clinical symptoms for about 2 weeks.

Reference value: < 3.0 mg/L

ALPHA-1 ANTITRYPSIN

Function: Alpha-1 Antitrypsin (α 1-antitrypsin, α 1-AT, AAT, also called alpha-1-proteinase inhibitor or simply antitrypsin — molecular mass: 52 kDa) is quantitatively the most important proteinase inhibitor (Pi) in serum. It is synthesized mainly by the hepatocytes in the liver. AAT specifically inactivates several serine proteases (e.g., leukocyte elastase, trypsin, chymotrypsin, collagenase, plasmin, thrombin) by irreversibly forming an enzyme-inhibitor complex. There are various AAT isoforms, the most common being PiMM. The isoform associated with severe deficiency of AAT is PiZZ.

Clinical significance: AAT is an important acute-phase reactant found in elevated concentrations in inflammatory processes (e.g., infectious and rheumatoid diseases, tissue necrosis, malignancy, traumas). Inflammation of the liver parenchymal cells often causes elevated AAT levels, with other acute-phase reactants relatively unaffected. AAT deficiency has a genetic cause. Hereditary AAT deficiency is suspected in cases of neonatal hepatitis accompanied by progressive yellow liver cirrhosis. AAT deficiency is also a major cause of severe pulmonary emphysema occurring in young people and adults.

Elevated AAT values are not caused by genetic defects, but in most cases, they are due to an inflammatory reaction (e.g., an infection). However, elevated AAT values also occur during pregnancy and following intake of some estrogen-containing oral contraceptives and hormone replacement drugs.

When measuring AAT in a subject and values are < 60 mg/dL (0.60 g/L), it is very important to clarify the Pi type.

Reference value: 90–200 mg/dL (0.9–2.0 g/L)

TRANSFERRIN (SEE IRON METABOLISM)

Transferrin (TF) is a glycoprotein with molecular mass 79.6 kDa, which transports iron in plasma, and its rate of synthesis in the liver can be altered in accordance with the body's iron requirements and iron reserves, and by estrogen (e.g., during pregnancy). Serum transferrin is increased in iron deficiency. Transferrin is also a negative acute-phase protein, with low concentrations present in inflammatory diseases as well as in protein-losing enteropathy, malnutrition and nephrotic syndrome and in disorders of hemoglobin synthesis (e.g., porphyria, thalassemia).

Reference value: 200–360 mg/dL (2.0–3.6 g/L)

ALPHA-1-ACID GLYCOPROTEIN

Function: Alpha-1-acid glycoprotein (α 1-acid glycoprotein, also called “orosomuroid,” AAG — molecular mass: 40 kDa) is a major constituent of the alpha-1 globulins. It is an APP synthesized mainly in the liver in response to inflammation and tissue damage and inhibits lymphocyte function by down regulation of the immune response. Furthermore, it inhibits platelet aggregation. AGP is one of the major acute-phase proteins.

Clinical significance: Elevated AAG levels are observed in a number of conditions, such as inflammation, myocardial infarction, trauma and surgery, with concentrations rising rapidly after tissue injury and reaching peak concentrations at 3–5 days. AAG is helpful in differentiating elevated levels due to inflammatory conditions from those due to estrogen effects in which AAG levels are normal or decreased. In monitoring various malignant diseases, AAG is indicated together with AAT. Low serum AAG concentrations are due to increased excretion of the protein (e.g., in nephrotic syndrome). However, in early reduction of glomerular function, a slight increase in AAG values is often observed. Also, corticosteroids increase the AAG concentration in serum.

Reference value: 50–120 mg/dL (0.5–1.2 g/L)

HAPTOGLOBIN

Function: Haptoglobin (HP) is a polymorphic protein with three different major phenotypes, namely, HP 1-1, HP type 2-1 and HP 2-2 (molecular mass: 85–400 kDa). HP binds hemoglobin that is released during lysis of erythrocytes in the circulation as well as during erythropoiesis in the bone marrow (ineffective erythropoiesis). HP carries the hemoglobin to the reticuloendothelial system in the liver, where the haptoglobin/hemoglobin complex is rapidly taken up, degraded and reused in the form of amino acids and iron. In this way, haptoglobin inhibits free-hemoglobin-induced lipid peroxidation and protects the kidneys from damage.

Clinical significance: Normally, about half of the amount of HP produced daily is consumed in the bone marrow in relation to the ineffective erythropoiesis. If this is increased, for example, as in pernicious anemia (megaloblastic anemia, vitamin B₁₂ deficiency), the serum HP level becomes almost undetectable. Low or undetectable HP concentrations occur more often in intravascular hemolysis than in extravascular hemolysis, and during severe hemolysis, there is a complete consumption of haptoglobin. Therefore, HP is useful in the workup of hemolytic disease states. HP is also decreased in liver disease. A congenital absence can occur in people of African and Asian descent. As an acute-phase protein, haptoglobin can develop very high serum levels during inflammatory conditions (e.g., in collagen diseases, infections, tissue destruction, advanced malignant neoplasms). In nephrotic syndrome, HP type 1-1 is eliminated renally.

Reference value: 30–200 mg/dL (0.30–2.00 g/L)

CERULOPLASMIN

Function: Ceruloplasmin (CP) is a multifunctional protein (molecular mass: 151 kDa) synthesized in the liver. CP has a very high copper content: 8 mol Cu/mol CP. However, CP does not transport copper via circulation. CP is essential in the regulation of redox potential and utilization of iron. Ferrous ion released by ferritin is oxidized to ferric ion in the presence of ceruloplasmin and immediately taken up by transferrin for reuse, thus preventing the generation of reactive oxygen species, such as superoxide and hydrogen peroxide. CP also has an antioxidative action, which prevents the oxidation of lipids in the cell membrane through its ferroxidase activity. CP can also modulate the function of endothelial nitric oxide synthase and thus controls NO-dependent relaxation of the vessels.

Clinical significance: Ceruloplasmin is decreased in most cases of Wilson's disease, in which there is a decreased ability to incorporate copper into apoceruloplasmin. As a result, free copper levels in serum and tissue, especially liver, pancreas and brain, are greatly increased. Ceruloplasmin levels are also low in Menkes kinky hair syndrome, in which the defect is secondary to poor absorption and utilization of dietary copper, from protein loss in the nephrotic syndrome, protein-losing enteropathy and malabsorption, and from decreased synthesis in advanced liver disease. Elevated serum levels of ceruloplasmin occur in a variety of neoplastic diseases (e.g., lymphoma) and inflammatory states due to the acute-phase reaction, although levels rise more slowly than for other APPs. It can be elevated also in cholestasis, primary biliary cirrhosis, SLE, rheumatoid arthritis and pregnancy. Ceruloplasmin synthesis is increased during pregnancy, with estrogens and oral contraceptive use, including hormone replacement therapy.

Reference value: Adults: 20–60 mg/dL (0.20–0.60 g/L)

COMPLEMENT FACTOR C3

Complement C3 (C3c) is an acute-phase protein (molecular mass: 185 kDa), and increased concentrations can occur during inflammatory processes (e.g., systemic infections and noninfectious chronic inflammatory conditions, primarily chronic polyarthritis). C3c is also elevated in physiological states (e.g., pregnancy). Elevations rarely exceed twice the upper reference limit of healthy subjects and can mask a reduction in C3c consumption.

Activation of the complement system takes place largely via the classical and alternative pathways. As complement C3c is common to both pathways, the C3c concentration can be used as a measure of the activation of the complement system. Activation is indicated by decreased concentrations of C3c. Additional differentiation between activation of the classical or alternative pathways can be made by determining C4. If the C4 level is normal, activation of the alternative route is likely.

Decreased C3c values are observed in a number of inflammatory and infectious diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis, subacute bacterial endocarditis, viremia, parasitic infections and bacterial sepsis. C3c deficiency can be found in patients with membranoproliferative glomerulonephritis when the C3c nephritic factor is present. C3c measurement is mainly used in the diagnosis and treatment of glomerulonephritis, rheumatoid arthritis, SLE and bacterial infections.

Concerning the analytical process, it has to be considered that complement activation will be started at the moment of the venipuncture and continues to activate the proteolytic enzymes, especially in serum samples where calcium is available. This means that decreasing amounts of intact C3 are present in stored specimens, as long as no special precaution has been applied. Therefore, in order to minimize these effects, blood specimens for complement measurement should preferentially be collected in EDTA-containing sample tubes, and serum samples should not be used. The most stable degradation product of C3 is the C3c fragment. This fragment also contains very immunogenic neopeptides delivering a very strong immunoprecipitation with polyclonal C3 antisera leading to a virtual increase in C3 in stored samples. Therefore, the C3c fragment is the “laboratory equivalent” of C3. As complement C3 is common to both complement activation pathways, the C3c concentration can be used as a measure of the activation of the complement system. Activation is indicated by decreased concentrations of C3c.

Reference range: 90–180 mg/dL (0.90–1.80 g/L) – C3 values are lower in fresh samples

COMPLEMENT FACTOR C4

Similar to C3, C4 is an acute-phase protein (molecular mass: 206 kDa), and an increase in its concentration occurs during inflammatory processes (e.g., systemic infections), during noninfectious chronic inflammatory conditions (primarily chronic polyarthritis) and in physiological states (e.g., pregnancy). Elevations rarely exceed twice the upper limit of its reference range and thus can mask a reduction in C4 consumption. The complement factor C4 participates in activation of the classical pathway only.

A decrease in C4 serum concentration is common, whereas a complete deficiency is rare but can occur in immune complex diseases such as SLE, autoimmune thyroiditis and juvenile dermatomyositis. Early SLE can often be detected in patients with C4 deficiency, and the course of the disease is milder than in patients with normal complement levels. Infections such as bacterial and viral meningitis, streptococcal and staphylococcal sepsis, and pneumonia are associated with decreased C4. Where C4 levels are normal but C3 levels are decreased, this suggests activation of the alternate pathway.

A total congenital C4 deficiency is rare, but a partial C4 deficiency is rather common. Partial and complete congenital C4 deficiencies have been associated with immune complex diseases, SLE, autoimmune thyroiditis and juvenile dermatomyositis. Infections associated with C4 deficiency include bacterial or viral meningitis, streptococcus and staphylococcus sepsis, and pneumonia.

Reference range: 10–40 mg/dL (0.10–0.40 g/L)

PROCALCITONIN

Function: The active calcitonin in normal conditions is formed and secreted by the C-cells of the thyroid gland after proteolytic splitting of the prohormone procalcitonin (PCT; molecular mass: 12.6 kDa). Because PCT is degraded by proteolysis in healthy individuals, PCT is usually present in healthy persons only at very low concentrations (< 0.05 ng/mL [$\mu\text{g/L}$]). In severe infections due to bacteria, fungi and parasites, as well as in sepsis, intact PCT is found in blood in concentrations which may increase to > 500 ng/mL ($\mu\text{g/L}$). There is indication that macrophages and monocytic cells of various organs (e.g., the liver) are involved in the synthesis and release of PCT under the conditions of a systemic inflammatory response.

Clinical significance: High levels of PCT (> 2.0 ng/mL [$\mu\text{g/L}$]) indicate severe infection, sepsis or multiple organ dysfunction syndrome (MODS); PCT levels > 10 ng/mL ($\mu\text{g/L}$) occur almost exclusively in patients with severe sepsis and/or shock. Various other stimuli, including major surgical procedures, polytrauma or burns, and prolonged circulatory failure, also induce serum elevations of PCT, but usually lower than those observed in patients with severe sepsis (generally 0.5–2.0 ng/mL [$\mu\text{g/L}$]).

The fact that PCT increases earlier than all other acute-phase proteins, but after IL-6 and IL-8 in plasma (rapid kinetics with a maximum at 12–24 hours), and due to its short half-life, makes PCT suitable for monitoring the evolution of the underlying disease and the success of the therapy applied. Therefore, PCT measurement, especially if performed together with CRP, seems to be useful for:

- Early diagnosis of bacterial and mycotic infection, as well as septic conditions.
- Assessment of the degree of severity and thus to make a prognosis on the outcome of systemic infection, sepsis and multiple organ failure.
- Monitoring patients after multiple trauma, extensive surgery or organ transplantation, subject to immunosuppression, or in acute pancreatitis for a possible development of infections.
- Differential diagnosis between systemic infection and acute inflammatory disease for differential diagnosis between bacterial and viral infection.
- Algorithms using PCT to guide antibiotic use enables antibiotic stewardship that can decrease antibiotic duration, reduce Length of Stay (LOS) in the ICU and hospital, decrease incidence of C-difficile infections and decrease development of antibiotic resistance.

Reference range: Healthy subjects: < 0.5 ng/mL ($\mu\text{g/L}$)

SECTION 4

LIPOPROTEINS AND LIPID TRANSPORT, PROTEIN LOSS, NUTRITIONAL STATUS AND NUTRITION MARKERS

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 List the major human apolipoproteins and describe their functions.
- 2 Explain the difference between selective and nonselective protein loss.
- 3 Identify the proteins used as markers of nutritional status.
- 4 Explain the function of albumin as the most abundant plasma protein.
- 5 Describe the use of prealbumin to reflect the recent dietary intake.

LIPOPROTEINS AND LIPID TRANSPORT

LIPOPROTEIN FUNCTION

Lipoprotein physiology is the study of fat transport through the aqueous environment of the body. Fats serve two main functions in the body. First, phospholipids and cholesterol are the major structural components of all cell membranes, and second, triglycerides and free fatty acids are the major sources of energy for the body. Initially, triglycerides are absorbed from the gut or manufactured by the liver prior to transport either to the muscle for use as energy or to adipose tissue for storage. Cholesterol is formed by all tissues and is used for the synthesis of bile acids in the liver, the manufacture and repair of cell membranes, and the synthesis of steroid hormones. Excess cholesterol is transported back from the different tissues to the liver. As the body cannot degrade cholesterol, the major problem that most tissues face is an excess of cholesterol, not a deficiency. Therefore, apart from being the end product of very low-density lipoprotein (VLDL) metabolism, the physiological function of low-density lipoprotein (LDL) is unclear. Nevertheless, failure to remove LDL cholesterol from plasma can lead to serious damage at the vascular wall with atherosclerotic plaque formation.

LIPOPROTEIN COMPOSITION

The lipoproteins have traditionally been classified into six major classes based on size, density, electrophoretic mobility, and lipid and protein composition (**Table 4-1**).

	Chylomicrons	HDL	LDL
Protein portion (%)	2	50	25
Apolipoproteins	A, B, C	A	B
Lipid portion (%)	98	50	75
Cholesterol ester (%)	2	20	40
Cholesterol (%)	2	6	11
Triglycerides (%)	90	4	4
Phospholipids (%)	4	20	20

Table 4-1: Composition of lipoproteins.

Chylomicrons and chylomicron remnants originate from the intestine; VLDL, intermediate-density lipoprotein (IDL) and LDL originate from the liver; and HDL originates from both hepatic and nonhepatic sources. Phospholipids, cholesterol and triglycerides are carried through the blood in lipoprotein particles,

which allow these lipids to be dispersed into small droplets for easy circulation through the vascular system. The reference method for separation of blood lipoproteins is the analytical ultracentrifugal method based on buoyant density (**Figure 4-1**). Frederickson's electrophoretic technique led to the phenotypic classification of the dyslipoproteinemias, based on biological patient values (i.e., hypercholesterolemia, hypertriglyceridemia and mixed hyperlipidemia), but did not differentiate patients according to genotypic variation. Genotypic methods are required to classify the primary hyperlipidemias according to biochemical differences.

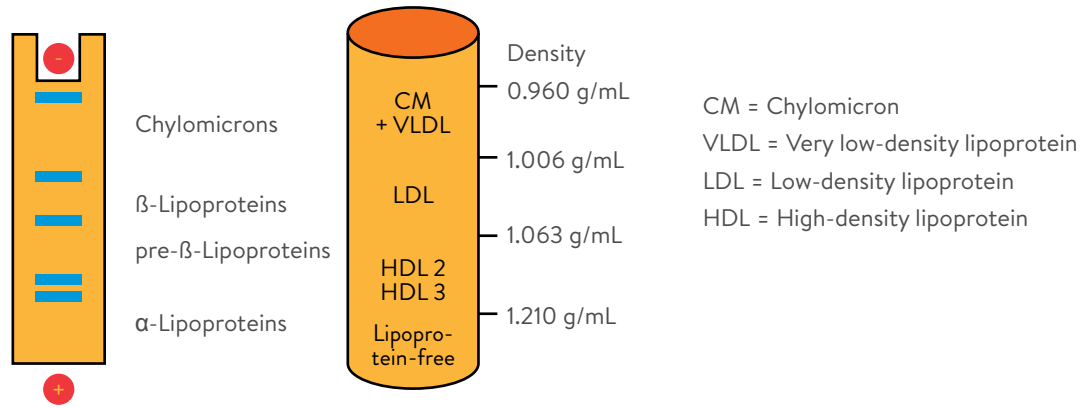


Figure 4-1: Classification of lipoproteins.

LIPOPROTEIN STRUCTURE

The plasma lipoproteins are water-soluble macromolecules composed of spherical pseudo-micellar particles with hydrophobic core regions containing cholesteryl esters and triglycerides (**Figure 4-2**). A monolayer of amphiphilic lipids, mainly unesterified cholesterol and phospholipids, surrounds the core. Specific proteins (apolipoproteins) that bind noncovalently to the lipids are located on the surface. In 1974, it was proposed that the α -helices of the apolipoproteins displayed a high density of polar residues on one face and nonpolar residues on the opposite face — the so-called **amphipathic helix**. The helices bind to lipids beneath the lipoprotein surface, via the nonpolar face, and to the polar head groups of the phospholipids and the surrounding aqueous phase, via the polar face, which encompass the nonpolar lipid core.

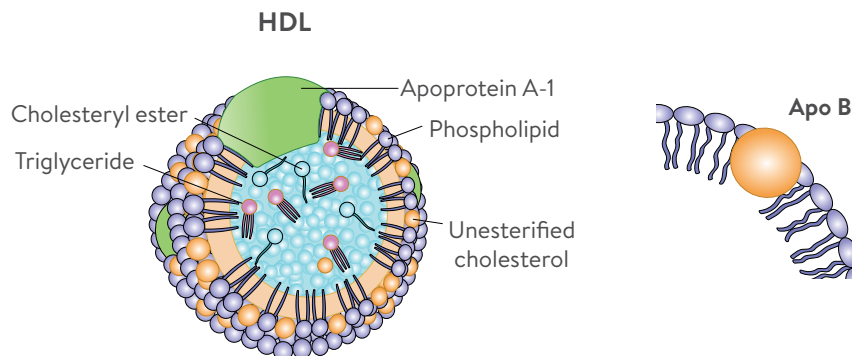


Figure 4-2: Illustration of lipoprotein structure.

LIPOPROTEIN METABOLISM

The lipoprotein metabolism handles lipids of both exogenous (dietary lipid) and endogenous (hepatic lipid) origins. Circulating lipoproteins are not static entities but are constantly modified, both by their intravascular exchange between apolipoprotein and lipid, and by enzymatic and receptor-mediated interactions with target tissues. Lipoproteins are removed from the circulation directly by receptor-mediated endocytosis (LDL, chylomicron remnants, VLDL remnants), or indirectly, by exchange of the lipid contents between lipoproteins or by specific lipid uptake at cell surface receptors (HDL) (**Figure 4-3**).

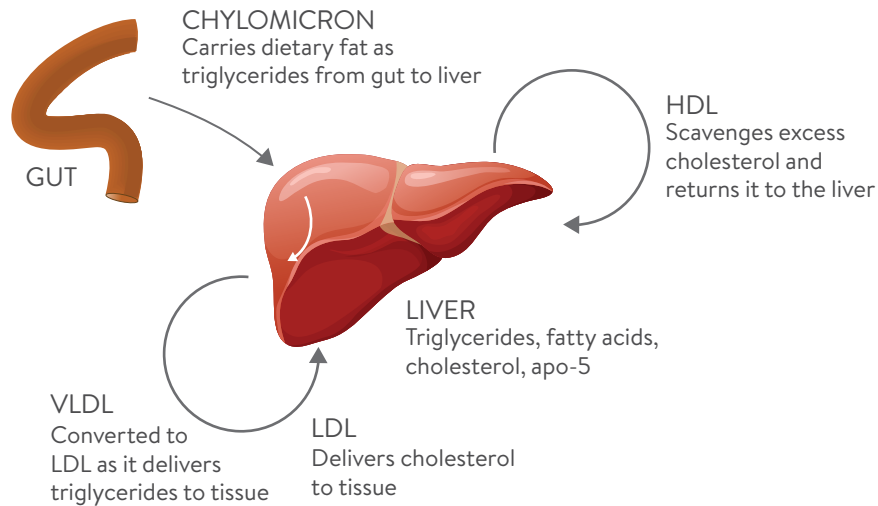


Figure 4-3: Lipoprotein metabolism.

APOLIPOPROTEINS: CLASSES AND FUNCTION

There are several major classes of apolipoproteins associated with lipoproteins and various subclasses, namely:

- Apolipoprotein A (Apo A-1, Apo A-2, Apo A-4 and Apo A-5)
- Apolipoprotein B (Apo B-48 and Apo B-100)
- Apolipoprotein C (Apo C-1, Apo C-2, Apo C-3 and Apo C-4)
- Apolipoprotein D
- Apolipoprotein E
- Apolipoprotein H
- Apolipoprotein J
- Apolipoprotein (a)

Apolipoproteins act as cofactors for specific enzymes or are ligands for specific receptors that are involved in lipoprotein metabolism. Certain lipoproteins contain very high molecular weight apolipoproteins that do not migrate from one particle to another, as do the smaller apolipoproteins. These species (apolipoproteins B) vary in their relative apparent molecular weight and are designated accordingly. Apo B-48, found in chylomicrons and their remnants, is formed in the intestine, whereas Apo B-100, present in VLDL, VLDL remnants and LDL, is synthesized in the liver. Smaller apolipoproteins distribute variably among the lipoproteins. Apo A-1 is a cofactor for lecithin-cholesterol acyltransferase (LCAT), and Apo C-2 is a cofactor for lipoprotein lipase (LPL). Apo D catalyzes the transfer of cholesteryl ester from HDL subspecies containing LCAT to other lipoproteins. Several isoforms of Apo E, based on single amino acid substitutions, are recognized.

APOLIPOPROTEIN A-1

Apolipoprotein A-1 (Apo A-I, Apo A-1) is the main component of the high-density lipoprotein class. It accounts for approximately 65% of the HDL particle. Apo A-1 contains various repeats in its secondary structure, which are highly conserved between species. It has a physiological role due to its cofactor activity in the lecithin-cholesterol acyltransferase (LCAT) reaction and its ability to take up free cholesterol from cells. These processes are important for the reverse cholesterol transport to the liver. In addition, Apo A-1 seems to play a role in prostacyclin stabilization, thus improving vasodilation and inhibiting platelet aggregation. These functions are important in protection against the development of atherosclerosis. Many different mutations have been described for Apo A-1, which are not directly associated with increased or decreased levels, etc. Tangier disease — a familial disorder — is an alpha-lipoproteinemia with very low Apo A-1 and cholesterol levels. Very interesting is a mutation called Apo A-1 Milano with low levels of Apo A-1 and HDL. The carriers of this mutation rarely have CVD and live very long.

Reference ranges: Men: 110–170 mg/dL (1.10–1.70 g/L); Women: 120–190 mg/dL (1.20–1.90 g/L)

APOLIPOPROTEIN B

Apolipoprotein B (Apo B) is the main major protein moiety component of all lipoproteins other than HDL (i.e., low-density lipoprotein [LDL] and intermediate-density lipoprotein [IDL] classes, and also very low-density lipoprotein [VLDL] and chylomicrons). Apo B accounts for approximately 95% of the total protein content of LDL. It occurs in two forms: Apo B-100 in the VLDL/LDL and Apo B-48 in the chylomicrons. Apo B-100 originates in the liver, whereas Apo B-48 (the N-terminal half of Apo B-100) is mainly formed in the small intestine. Its synthesis is regulated by steroid and thyroid hormones and can be regulated by lipid-lowering drugs, such as HMG-CoA reductase inhibitors, “the statins.” Apo B is essential to the formation and release of lipoproteins into the plasma and interacts with the LDL receptors of peripheral cells, thereby functioning in the recognition of cellular receptors for the cellular uptake of LDL. After the action of lipases on Apo B-containing lipoproteins and removal of triglycerides, the lipoproteins become enriched in cholesterol, and the remnants are removed from circulation by hepatic receptors. The removal and degradation of LDL takes place through the LDL receptor.

Reference ranges: Men: 80–155 mg/dL (0.80–1.55 g/L); Women: 75–150 mg/dL (0.75–1.50 g/L)

LIPOPROTEIN (a)

Function: Lipoprotein (a) [Lp(a)] is a spherical lipid particle found, upon ultracentrifugation, predominantly in the 1.055–1.110 g/mL density range. It is larger and denser than LDL, with a diameter of approximately 30 nm. Similar to low-density lipoprotein (LDL) in core lipid composition and having Apo B-100 as a surface apolipoprotein, Lp(a) differs from LDL by containing an additional glycoprotein, apolipoprotein(a) (Apo[a]). Apo(a) is covalently bound to Apo B-100 through a disulfide bridge. It is a highly glycosylated hydrophilic protein, with a size varying from 300 to 700 kDa. Studies have shown that the Apo(A) portion of Lp(a) is heterogeneous, resulting from a variation in the number of protein domains known as kringles. Both the cDNA sequence and the immunochemical structure of Apo(a) have been shown to be strikingly similar to the kringle domains of plasminogen. Because of this similarity, Lp(a) may inhibit fibrinolysis by competition with plasminogen for fibrin. Lp(a) may contribute to the growth of arterial lesions of atherosclerosis by promoting proliferation of vascular smooth muscle cells.

PROTEIN LOSS

Protein loss may be divided into selective and nonselective loss.

Selective protein loss is generally through a semipermeable membrane or tight intracellular channels. Minimal change nephrotic syndrome, a type of chronic kidney disease, is a classic example of selective protein loss. Proteins are lost in the urine in concentrations inversely proportional to their molecular size, as through a sieve. Small proteins pass through the kidney and into the urine in high concentrations, while large proteins pass through the kidneys minimally, if at all. As a result, serum concentration of small proteins declines, while those of larger proteins remains the same or increases.

Nonselective protein loss is due to either whole blood or serum loss, and all serum proteins are lost equally. Whole blood loss may be acute, as with trauma, or chronic, as with types of gastrointestinal or uterine bleeding. In acute whole blood loss, there is a rapid influx of extracellular fluid into the vascular space in an attempt by the body to maintain blood pressure. This dilutes the remaining plasma and further reduces the concentration of most proteins. The extracellular fluid itself contains fairly high concentrations of the smaller serum proteins, including albumin and transferrin.

Concentration of these may, therefore, not drop as dramatically as those of larger proteins, such as IgM and alpha-2-macroglobulin. In chronic, low-grade blood loss, the liver can often replace the lost proteins as long as nutrition is adequate for new protein synthesis, so changes may be minor, if seen at all. Nonselective loss of serum proteins also occurs as a result of burns or severe glomerular disease.

NUTRITIONAL STATUS/PROTEIN ENERGY MALNUTRITION

Measurement of serum proteins is perhaps the most useful biochemical assessment tool for assessing nutritional status. Unlike carbohydrates and fats, the body does not store protein in a nonfunctional form. Therefore, a gain or loss of function reflects gain or loss of protein. Declining lean body mass can have a negative effect on organ function (decreased cardiac contractility and a corresponding reduction in blood flow and peripheral oxygen delivery), immune response to infections and healing. Serum protein values, when measured serially, provide an index of the rate of protein synthesis. A change from catabolism to anabolism reflects the degree of stress in the hospitalized patient.

PROTEINS USED AS MARKERS FOR NUTRITIONAL STATUS

ALBUMIN

Function: Albumin is the most abundant protein in human plasma (molecular mass: 66.3 kDa), accounting for about 60% of all plasma proteins. It is very important as a buffer to maintain pH at its physiological level and to maintain the normal viscosity and oncotic pressure of plasma.

Because of its high solubility, albumin is an important carrier of many hydrophobic molecules, such as free fatty acids, bilirubin, thyroxine, hemin and xenobiotics (e.g., phenytoin, valproic acid). Albumin is also present in the extracellular space, where its total amount exceeds the intravascular amount by 30%. Albumin is synthesized by the liver at a rate of about 12 g/day and has a half-life in blood of 18–20 days.

Albumin is not catabolized during starvation, but on a low-protein diet, the synthetic and catabolic rates of albumin decrease, and the mass of extravascular albumin is reduced. Decreased serum albumin concentrations occur in severe impairment (about 50% decrease) of hepatic synthetic capacity (e.g., in hepatic cirrhosis, severe hepatitis or chronic malnutrition) and in marked protein loss (e.g., nephrotic syndrome, gastroenteropathy, severe burns). The range of diseases causing decreased serum albumin is so large that albumin concentration is considered to be a nonspecific indicator of illness. Increased serum albumin concentration is observed only in hemoconcentration, either as an artifact or due to dehydration.

Clinical significance: Historically, albumin levels have been used as a determinant of nutritional status. Serum albumin levels have been linked to nutritional assessment and long-standing malnutrition, based on the fact that albumin has a long half-life of 19 days, and there is reduced albumin synthesis when the supply of amino acids is limited. However, serum albumin concentrations are affected by the patient's state of hydration and renal function, and levels typically take 14 days to return to normal when the pool has been depleted. On a low-protein diet, the synthetic and catabolic rates of albumin decrease, and the mass of extravascular albumin is reduced. The synthetic rate rapidly returns to normal when the dietary protein is replaced. However, the strongest factor causing decreased serum concentration of albumin is inflammation, and therefore it is recommended to assay an acute-phase protein like CRP when using albumin as a nutritional marker. APR also leads to an increase in vascular permeability and a significant reduction in serum albumin levels by this mechanism.

Decreased serum albumin concentrations also occur in severe impairment of hepatic synthesis capacity, as well as in marked protein loss (e.g., nephrotic syndrome, gastroenteropathy, severe burns). Posture also has a marked effect on serum albumin concentration and may fall by up to 15% after only 30 minutes in the recumbent position, especially in diseased elderly patients.

Another good use of serum albumin measurement is as a prognostic marker of mortality in nursing home patients or in elderly, hospitalized patients. People with low serum albumin levels (< 3 g/dL [30 g/L]) have an increased incidence of complications and mortality because of the serious or chronic nature of their illness or through malnourishment.

Albumin levels below 2.5 g/dL (25 g/L) are associated with a poor prognosis and accurately predict survival prognosis in 90% of critically ill patients.

Reference range: Serum: 3.5–5.2 g/dL (35–52 g/L)

PREALBUMIN

Function: Prealbumin (Transthyretin, PAL) — also referred to as thyroxine-binding prealbumin (molecular mass: 55 kDa) — is a transport protein for thyroxine and a carrier protein for retinol-binding protein (RBP). Due to its short half-life of 2 days, measurement of PAL can be useful as a sensitive indicator of protein-calorie malnutrition and of early response to enteral or parenteral (nutritional) treatment. PAL levels reflect recent dietary intake rather than overall nutritional status. In the absence of an acute-phase reaction, serial measurements of PAL provide a valuable indication of nutritional adequacy in premature infants. Furthermore, because of its synthesis in the liver, PAL is a reliable index of liver function in hepatobiliary diseases.

Determination of the PAL level may allow for earlier intervention in malnutrition. Adequate nutritional support should be established so that PAL levels rise by approximately 0.5 mg/dL (0.005 mg/L) per day. An increase of < 2 mg/dL (0.02 g/L) in 1 week indicates either inadequate support or inadequate response.

PAL will decrease to low levels (less than 8 mg/dL [0.08 g/L]) in severe protein-calorie malnutrition and will respond quickly to nutritional support with a daily increase of up to 1 mg/dL (0.01 g/L) (**Figure 4-4**). PAL is a better marker than RBP in patients with renal failure, where RBP concentration is more elevated than PAL in blood due to its decreased catabolism by the kidney.

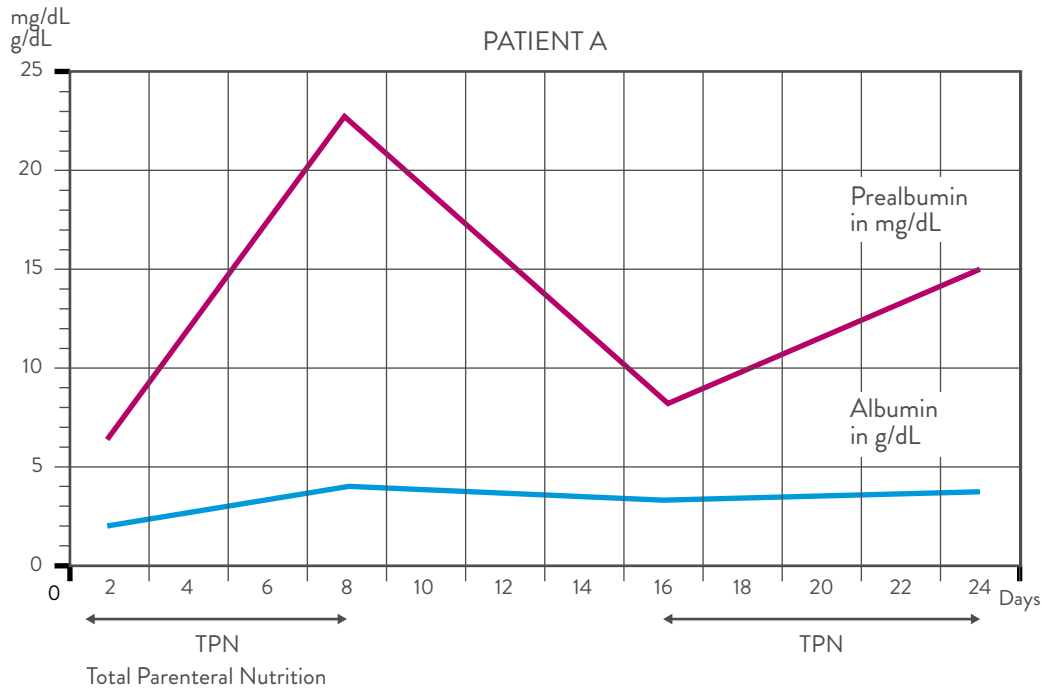


Figure 4-4: Changes in prealbumin and albumin with total parenteral nutrition.

There are some limitations to the use of PAL as a nutritional marker. PAL levels will transiently decrease in the presence of inflammation and the immediate postsurgical period. Levels also decrease in pregnancy, as well as in patients with protein malnutrition due to malignancy, cirrhosis, protein-losing enteropathy and zinc deficiency. Increased levels are observed when using oral contraceptives, with excessive alcohol intake, with corticosteroids (including prednisone therapy, anabolic steroids), in hyperthyroidism and in Hodgkin’s disease.

Interpretation of a low value also requires other laboratory tests, such as CRP, in order to rule out a concomitant acute-phase reaction due to inflammation.

Serum PAL concentrations of 20–40 mg/dL (0.2–0.4 g/L) are considered physiological, while levels of 10–20 mg/dL (0.1–0.2 g/L) indicate mild malnutrition, 5–10 mg/dL (0.05–0.1 g/L) moderate malnutrition, and 5 mg/dL (< 0.05 g/L) severe malnutrition, provided that no inflammation is present.

Reference range: 20–40 mg/dL (0.20–0.40 g/L)

RETINOL-BINDING PROTEIN

The physiological role of retinol-binding protein (RBP; molecular mass: 21 kDa) involves the transport of retinol (vitamin A aldehyde) from the liver to the various peripheral tissues. RBP-carrying retinol is bound to prealbumin (PAL). Therefore, in vitamin A deficiency, the RBP serum level decreases due to impaired secretion. Due to its short half-life of approximately 12 hours and its association with PAL, RBP reacts similarly to PAL. It responds to short-term changes in energy and, to a lesser extent, protein deficiency, and it decreases rapidly in response to starvation. RBP is therefore an indicator of recent dietary intake and energy restriction rather than body composition. Levels are increased by reduced glomerular filtration rate and alcoholism and decreased in patients with cystic fibrosis, hyperthyroidism, chronic liver disorders, and vitamin A and zinc deficiencies.

Reference range: 3–6 mg/dL (0.03–0.06 g/L)

TRANSFERRIN (NUTRITIONAL MARKER)

The main diagnostic use of transferrin (TF) is the assessment of iron status. Yet, as in malnutrition, the synthesis of transferrin is reduced. It has been proposed that transferrin be used as a measure of protein status because its half-life is intermediate to that of albumin and prealbumin.

Transferrin concentration is affected by protein status and not by energy intake. Total starvation in healthy subjects does not cause a significant change in transferrin concentrations until after 3–5 days. In obese subjects given a low-energy, high-protein diet for 24 days, transferrin concentration is maintained. However, when a low-energy diet was combined with low protein content, the concentration of transferrin decreased significantly over time. The usefulness of transferrin determination for protein-energy assessment is limited when transferrin concentration is decreased during an inflammatory process/disease, or if there is concurrent iron deficiency. Subnormal concentrations are also seen in liver disease and protein-losing states. Transferrin is less useful than prealbumin because of its longer half-life of about 10 days. Its measurement is used because of modern automated analytical techniques, but the results should be interpreted in the context of a total protein profile, especially taking into account the inflammatory state of the patient.

Serum transferrin levels of 200–360 mg/dL (2.0–3.6 g/L) are considered normal, with values of 150–200 mg/dL (1.5–2.0 g/L) indicating mild malnutrition, 100–150 mg/dL (1.0–1.5 g/L) moderate malnutrition and < 100 mg/dL (< 1.0 g/L) severe malnutrition if no inflammatory process is present.

REVIEW QUESTIONS: SECTIONS 1-4

Answers are provided at the end of this Learning Guide.

1. In which body fluids are proteins present?
 - A Blood
 - B Plasma
 - C Serum
 - D Cerebrospinal fluid
 - E Urine
 - F All of the above
2. The basic immunoglobulin (Ig) unit is composed of:
 - A Two identical heavy and two identical light chains
 - B Two identical heavy and two different light chains
 - C Two different heavy and two identical light chains
 - D Two different heavy and two different light chains
 - E Noncovalently bound polypeptide chains
3. Where are most plasma proteins synthesized?
 - A Kidney
 - B Liver
 - C Brain
 - D Not known at this time
4. Which group of proteins is synthesized in the lymphatic tissues (e.g., lymphocytes, plasma cells)?
 - A Positive acute-phase reactants
 - B Negative acute-phase reactants
 - C Immunoglobulins
5. Indications of an acute-phase response include:
 - A Changes in serum protein levels
 - B Fever
 - C Increased or decreased white blood cell count
 - D Elevated ESR
 - E All of the above

6. Which is a sign of infection?
- A Muscle cramp
 - B Nosebleed
 - C Bruise
 - D Fever
7. Positive acute-phase reactants are proteins that increase in concentration in response to disease.
- A True
 - B False
8. Negative acute-phase reactants are proteins that increase in concentration in response to disease.
- A True
 - B False
9. Which serum protein is most useful in determining if an acute-phase response is due to bacterial or viral infection?
- A C3
 - B C-reactive protein
 - C IgG
10. The most common cause of low prealbumin concentration is:
- A Anti-inflammatory therapy
 - B Estrogens
 - C Malnutrition
 - D Inflammatory disease
11. A characteristic of IgD is:
- A Four subclasses
 - B < 1% of globulin concentration
 - C Responsible for the symptoms of allergy
 - D Pentameric structure
12. Which are the functions of proteins?
- A Antibodies
 - B Part of the endocrine system
 - C Carriers for other compounds
 - D Provide tissue nutrients
 - E Function as enzymes
 - F All of the above

SECTION 5

ATHEROSCLEROSIS,
MARKERS OF
CARDIOVASCULAR RISK,
LIPID PARAMETERS AND
APOLIPOPROTEINS

ATHEROSCLEROSIS AND ASSOCIATED CARDIOVASCULAR DISEASES..... 47
LIPID PARAMETERS: CLINICAL IMPORTANCE 53



LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe atherosclerosis and the associated cardiovascular diseases.
- 2 List the most important classical risk factors.
- 3 Identify the role of CRP as a marker of cardiovascular risk.
- 4 Specify the characteristics of hsCRP assays.
- 5 Explain how cystatin C as a marker of renal function can be useful in cardiovascular risk prediction.
- 6 Describe the functions and clinical significance of the apolipoproteins A-1 B and Lp(a).

ATHEROSCLEROSIS AND ASSOCIATED CARDIOVASCULAR DISEASES

DEFINITION

Atherosclerosis is the name for complex pathological changes affecting the large- and medium-sized arteries of the human body, which are the main causes of coronary heart disease and related death, as well as cerebrovascular disease. Most cardiovascular disease (CVD) is thrombotic in nature, meaning that pathologic mechanisms within the vascular wall trigger the formation of clots that occlude the vessel and cause death of the surrounding tissue. In many cases, a thrombus may travel from the heart or a peripheral vessel to the arteries of the brain, causing a transient ischemic attack or ischemic stroke. An acute event is typically also precipitated by thrombosis at the site of atherosclerotic plaque disruption and results ultimately in an occlusive disease of the coronary, carotid, cerebral or peripheral arteries. This manifests as unstable angina pectoris (UA), acute myocardial infarction (MI), ischemic stroke or intermittent claudication. Despite therapeutic advances, cardiovascular events are often fatal, and strokes account for the majority of rehabilitation needs in the Western world (**Figure 5-1**).

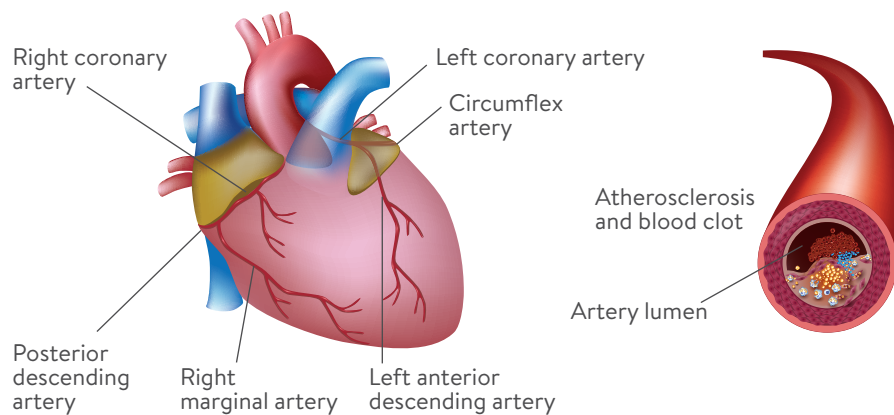


Figure 5-1: Changes in the vascular system with cardiovascular disease.

At present, there is strong evidence of an association between inflammation, low-grade but also more advanced (independent of its cause), and the slow but progressive development of atherosclerosis. It is also known that a link exists between acute systemic inflammatory response process (e.g., pneumonia, influenza) and transiently increased risk of an acute cardiovascular event.

CLASSICAL RISK FACTORS

The most important classical risk factors that predispose to severe atherosclerosis, particularly as it affects the coronary arteries, include the following:

- Tobacco smoking
- Hypertension
- Family history of atherosclerotic-related diseases
- Presence of a family history of hyperlipidemia
- Obesity
- Diabetes mellitus or a family history of this disease

Elevated LDL-cholesterol levels are atherogenic and are found in both acquired and familial hypercholesterolemia, whereas elevated HDL-cholesterol levels are protective. Hormonal factors play a role in atherosclerosis, with lesions progressing steadily in males, but not until after menopause in females, who are protected during their reproductive life.

ATHEROSCLEROSIS – AN INFLAMMATORY PROCESS

Recently, a new perspective on atherosclerosis has been developed based upon evidence showing that the entry of inflammatory cells, such as monocytes, into the arterial wall plays a pivotal role in the progression of this disease. This new paradigm might be collectively called the “inflammation hypothesis,” in which atherosclerosis and the evolution of plaque instability underlying atherothrombotic events are considered inflammatory processes. Inflammatory stimuli arising from within atheromatous lesions, either infectious or noninfectious, are also postulated to play an important role in the etiology of atherosclerosis. However, it must be remembered that all inflammatory processes in the body will induce a state with increased levels of both pro-inflammatory cytokines and CRP, which could penetrate the vascular endothelium, especially at already vulnerable sites, and contribute to the atherosclerotic process of plaque formation.

CRP AND ITS ROLE IN INFLAMMATION²

It has been very clear, during the last decade, that inflammation is largely involved in the propagation of arteriosclerotic plaque formation through the action of cytokines on the vascular endothelium and through CRP. In fact, within the plaque, CRP participates in the uptake of native and ox-LDL into macrophages via the CD32 receptor. This process will ultimately lead to the transformation of the macrophages into foam cells, which are pathognomonic for the arteriosclerotic plaques.³ The longer and more often inflammation persists in a subject, the higher the tendency for plaque formation and the higher the future risk of CVD will be.

C-reactive protein and pathophysiological implications for plaque instability and rupture:

- CRP is localized in atherosclerotic lesions, but not in normal intima.
- CRP is chemotactic for monocyte recruitment in the arterial wall.
- CRP induces complement activation.
- CRP is associated with impaired endothelial function.
- CRP mediates LDL uptake by macrophages.
- CRP acts as a procoagulant.

Several studies have brought evidence that CRP may contribute directly to the pro-inflammatory state. Opsonization of low-density lipoprotein (LDL) by CRP mediates LDL uptake by macrophages. CRP, localized directly within the atheromatous plaque, is an activator of complement and has been shown to colocalize with the membrane attack complex in early atherosclerotic lesions. Although the principal source of CRP production is the liver, recent data have shown that arterial tissue (smooth muscle cells) can produce CRP as well as complement proteins. This supports the concept that CRP may be an endogenous activator of complement in atheromatous lesions.

CRP AS A MARKER OF CARDIOVASCULAR RISK: CLINICAL EVIDENCE⁴

CRP is an indirect risk factor for CVD, and elevated plasma CRP levels (**Figure 5-2**) may reflect one or more of the following:

- The amount and activity of circulating pro-inflammatory cytokines
- A coronary artery inflammation in response to infectious agents
- The severity of the inflammatory response in atherosclerotic vessels
- The extent of inflammation related to myocardial ischemia
- The extent of inflammation related to myocardial necrosis
- The uptake of LDL by macrophages within the plaque

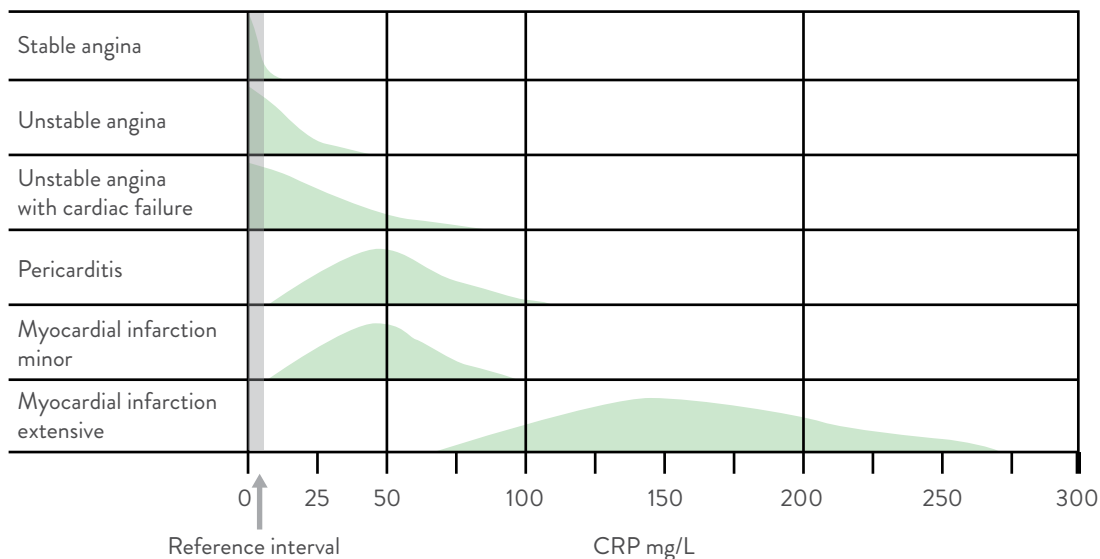


Figure 5-2: CRP in various types of CVD.

A review of the available evidence from prospective studies in which CRP testing was used to predict the short- and long-term risks of a recurrent CVD event or death due to cardiac causes, in patients who were followed from 90 days to 9 years, revealed the following major findings:

- Mean serum CRP levels are generally elevated in CVD cases compared with CVD free controls, before and after adjustment for other CVD risk factors.
- Serum CRP levels are correlated with established cardiovascular risk factors, such as smoking, age, body mass index and diabetes, as well as clinical variables such as prior MI and severity of the coronary artery stenosis.
- Serum CRP levels increase in women taking hormone replacement therapy.
- Elevated baseline CRP levels are independently predictive of the short- and long-term risks of recurrent MI or death in patients with UA or a history of MI.
- One study of hypercholesterolemic men with severe CVD found that the link between CRP and CAD prognosis was attenuated by other risk factors for the disease.

Related to these theories that hsCRP is a surrogate marker of part of the pathogenesis of CVD, the following CVD risk categories are often used:

CVD-risk	hsCRP mg/L
Low	< 1.0
Intermediate	1.0–3.0
High	> 3.0

Table 5-1: Classification.

Values for high sensitivity CRP (hsCRP) assays greater than 10 mg/L suggest that the screening effort might be confounded by recent or acute inflammatory disease (e.g., trauma, infection), and thus the hsCRP value should be rechecked later when the subject has been free of any clinical symptoms for about 2 weeks (**Table 5-1**).

hsCRP seems to be one of the best predictors of future CVD risk. In fact, those subjects with hsCRP levels in the upper 25th percentile are three times as likely to suffer a heart attack. Although epidemiological studies demonstrate an association between low-grade inflammation and vascular risk, application of hsCRP testing in clinical practice requires estimates of risk across a spectrum of hsCRP levels. For each quintile increase in hsCRP, the adjusted relative risk of suffering a future cardiovascular event has been reported to increase 26% for men and 33% for women.

The potential use of the hsCRP test as a means to improve the cost-benefit ratio in lipid-lowering therapy with statins has been suggested. Statin therapy leads to the greatest reductions in serum hsCRP levels in CVD patients with the highest levels of hsCRP. Consequently, the increased risk of recurrent disease or death is reduced. Statins also affect the hsCRP level by decreasing it even in subjects with normal levels of LDL. However, the exact mechanism for this anti-inflammatory effect is unclear. Study data have shown that the decrease in hsCRP by statins is associated with a decrease of the CVD risk, independent of the effect of statins on the lipid levels.

Other study results indicate that if hsCRP levels are elevated, a patient must exercise, stop smoking, lower blood pressure, lose weight and eat a heart-healthy diet, irrespective of cholesterol levels. Both statins and acetylsalicylic acid (aspirin) are useful in these situations.

CRP ASSAYS: TEST CHARACTERISTICS⁵

Standard clinical assays for CRP as inflammation markers typically were set to have a detection limit of 3–8 mg/L. However, these assays do not have good sensitivity and precision within the low-to-normal range, and they cannot be used for CVD risk prediction. The present commercially available “high-sensitivity” or “ultra-sensitive” assays for CRP (hsCRP or us-CRP) detect concentrations ≤ 0.1 mg/L, and formal standardization programs have been undertaken and completed to ensure comparability of results across CRP assays.

hsCRP MEASUREMENTS: APPLICATIONS⁶

In the low-to-normal range needed for cardiovascular risk detection, the variability and classification accuracy of CRP is similar to that of total cholesterol. CRP results may be confounded, however, from CRP elevations due to acute infection and trauma. Thus, testing should be avoided within a 2- to 3-week period for people who have had an upper respiratory infection or other acute illness. In such cases, the CRP measurement should be repeated to exclude the possibility of recent infection. If a second clinically elevated level is observed, evaluation for a previously unsuspected inflammatory condition may be needed.

Many conditions and diseases may cause significant inflammation, which will result in increased CRP levels. These conditions include myocardial infarction, unstable angina, obesity, diabetes mellitus, rheumatic fever, rheumatoid arthritis (RA), SLE, inflammatory bowel disease (IBD), tuberculosis, pneumococcal pneumonia, cancer, postoperative infection, trauma (e.g., injuries, burns) and heat stroke. Individuals with clinical inflammatory conditions such as RA, IBD or SLE are likely to have elevations of CRP even during subjectively optimal treatment. This might indicate that for these subjects it might be of value for the clinician to reconsider their treatment in view of their cardiovascular risk.

However, for most individuals, CRP levels appear to be very stable over long periods of time. These data support the possibility that enhanced inflammatory response, and hence, increased propensity to plaque development and rupture, may involve important genetic determinants.

In a survey of several thousand American men and women, < 2% of all CRP values have been > 15 mg/L, a level considered to be indicative of a clinically relevant inflammatory condition.

CYSTATIN C

Several studies have recently shown a risk association between high cystatin C and CVD mortality. Cystatin C (molecular mass: 13 kDa) as a marker of renal function can support cardiovascular risk prediction in addition to classical risk factors and CRP, across the whole spectrum of patients with CVD and mild chronic kidney disease. Among elderly persons without chronic kidney disease, cystatin C is not only a prognostic marker for death risk, CVD and chronic kidney disease, but for residual physical functional capacity. In this setting, cystatin C seems to identify a “pre-clinical” state of kidney dysfunction that is not detected with serum creatinine or estimated glomerular filtration rate (eGFR) with the Modification of Diet in Renal Disease (MDRD) study equation.

Elevated serum cystatin C concentrations are associated with all-cause mortality, cardiovascular events and chronic heart failure incidents among ambulatory persons with CVD. Moreover, increased cystatin C concentrations can predict increased risk of these adverse clinical outcomes, even among persons without elevated urine albumin (microalbuminuria) or low-MDRD estimated GFR.

The background for this is general atherosclerosis throughout the vascular system. Simultaneously, this inflammatory process activates macrophages within the glomeruli, leading to destruction and thickening of the glomerular membrane and a loss of renal function, and thus increased cystatin C concentration in blood.

A chain of events for the development of atherosclerosis can be depicted starting early in life and culminating in cardiovascular disease and CVD-related mortality. The progress of the atherosclerotic process depends on the amount of inflammatory burden throughout life in combination with various metabolic factors, such as increased LDL-C levels.

Other factors might also contribute, either directly or indirectly. The background for the inflammatory burden might be smoking, infectious or chronic inflammatory diseases, obesity or diabetes mellitus. Indeed, increased cystatin C concentration seems to be associated with increased risk for development of metabolic syndrome and progression to diabetes mellitus and, later on, strongly related to increased risk of chronic kidney disease and CVD.

FIBRINOGEN

Fibrinogen (molecular mass: 340 kDa) is one of the major acute-phase reactants and rises rapidly during tissue inflammation or injury. Numerous prospective epidemiological studies and clinical observations have shown elevated levels of fibrinogen to be strongly and independently related to CVD risk.

The pathophysiologic mechanisms by which elevated fibrinogen levels mediate coronary risk are as follows:

- It is a marker of the amount and activity of circulating pro-inflammatory cytokines.
- It forms the substrate for thrombin, thus being the final step in the coagulation cascade.
- It is essential for platelet aggregation, modulates endothelial function and promotes smooth muscle cell proliferation and migration.
- It interacts with the binding of plasminogen and its receptor.

Fibrinogen has emerged as an important additional marker of coronary risk and is a marker of long-term pathophysiological changes. High plasma fibrinogen concentration in adulthood is associated with elevated risk of CVD and stroke. Prospective studies in healthy men and women have shown that a single fibrinogen measurement predicts fatal and nonfatal cardiovascular events as much as 16 years later, and levels predict restenosis after angioplasty. Fibrinogen may promote, together with other hemostatic factors, atherosclerotic changes and thrombosis through effects shown *in vitro* on platelet aggregability, blood viscosity and foam

cell formation. Such processes are compatible with a causal role for fibrinogen. An alternative view is that the prospective fibrinogen-cardiovascular disease association may be a consequence, rather than a cause, of the disease process, perhaps due to an inflammatory response to progressive endothelial damage. Both perspectives, which are certainly not mutually exclusive, support the use of fibrinogen as a cardiovascular risk factor.

LIPID PARAMETERS: CLINICAL IMPORTANCE

LOW-DENSITY LIPOPROTEIN (LDL)

Lipoproteins play a central role in atherogenesis. Countries with high dietary fat intake have a high rate of CVD, and within a given society, those individuals with elevated LDL-cholesterol levels are at higher risk for atherosclerosis. Multiple lines of evidence, including epidemiological, autopsy, animal studies and clinical trials, have established that LDL is atherogenic, and that the higher the LDL-cholesterol (LDL-C) level, the greater risk of atherosclerosis and its clinical manifestations. A certain level of LDL-C elevation appears to be a necessary factor in the development of atherosclerosis, although the process is modified by other factors (e.g., blood pressure, tobacco use, blood glucose level, antioxidant level, inflammation, clotting factors). In mammals that do not develop atherosclerosis, the serum LDL-C level is < 80 mg/dL (0.80 g/L), similar to that of humans who follow a very low fat diet similar to the diet of our distant ancestors. In contrast, the average adult male in Northern Europe has an LDL-C level of 125 mg/dL, which is sufficient to cause developing atherosclerotic plaques. Most individuals with CVD do not have a specific genetic disorder of lipid metabolism, so the appropriate approach to controlling the atherosclerosis epidemic is through public health means, including health education, agricultural policy-making, food labeling and similar measures, but also aiming at lowering the inflammatory burden during life.

Atherosclerotic disease of both coronary and peripheral arteries is a dynamic process. Evidence from studies in both animals and humans indicates that progression of atheroma formation can be slowed or even reversed if elevated serum concentrations of the atherogenic lipoproteins can be reduced. An understanding of the biology of the lipoproteins and the pathophysiology of the various hyperlipidemic states is essential to the rational choice of treatment regimen.

HIGH-DENSITY LIPOPROTEIN (HDL)

Certain inherited low HDL syndromes are associated with premature coronary artery disease, whereas others are not. Epidemiological study has shown that within a population group, HDL-cholesterol (HDL-C) level is strongly and inversely correlated with the risk of CVD, independent of LDL-C level. The mechanism for this potentially protective effect of HDL is unknown but may be through reverse cholesterol transport, in which HDL removes cholesterol from extrahepatic sites, including the arterial wall. It is recommended that treatment to increase HDL be considered in CVD patients with a low HDL level, even if the total cholesterol level is low. Treatment should begin with such nonpharmacological measures as smoking cessation, weight loss and increased exercise, but should advance to pharmacological therapy if necessary.

TRIGLYCERIDES

The evidence is insufficient to conclude that elevated levels of triglycerides (hypertriglyceridemia) causes CVD. Observational studies have shown that a triglyceride level > 200 mg/dL (2.25 mmol/L) significantly increased the risk of CVD in individuals with elevated LDL-C and low HDL-C levels. It is likely that triglyceride elevation does not per se cause atherosclerosis, but that the metabolic derangements associated with hypertriglyceridemia do.

APOLIPOPROTEIN A-1 AND APOLIPOPROTEIN B: CLINICAL SIGNIFICANCE

Since apolipoproteins are unique markers for the identification and differentiation of lipoproteins, they are the most probable determinants of the structural integrity and functional specificity of lipoproteins. Increasing evidence links apolipoproteins to atherosclerosis, indicating that their measurement is as reliable (or even more so) as measurements of lipoproteins (LDL and HDL) for CVD risk assessment and response to lipid-lowering therapy. Furthermore, they also enable identification of patients with certain inheritable abnormalities of lipoprotein metabolism not detectable using other methods. Another advantage of using Apo A-1 and Apo B is the availability of international WHO reference materials, which are nonexistent for LDL-C and HDL-C.

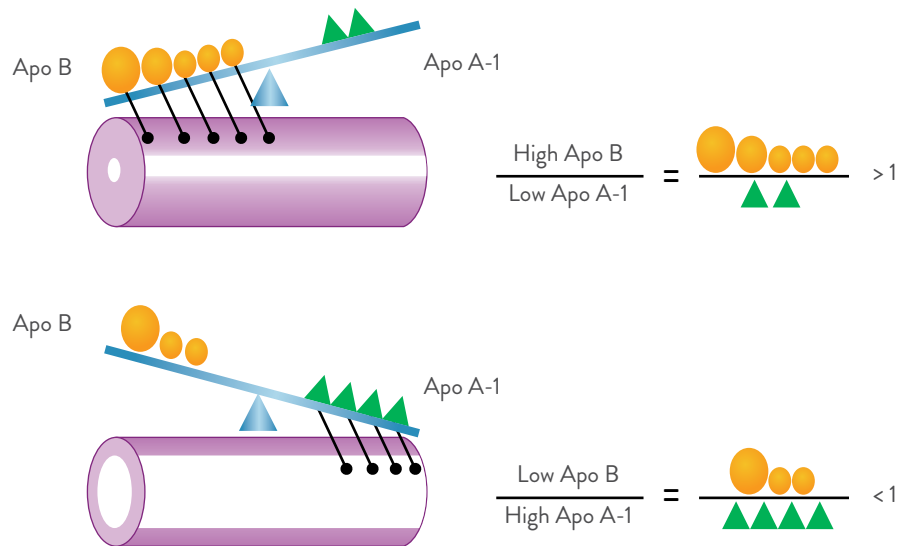


Figure 5-3: The atherogenic balance maintained by Apo A-1 and Apo B, where Apo B promotes atherogenesis, and Apo A-1 counteracts it. With permission from AstraZeneca™, G. Walldius and I. Jungner.

The ratio of Apo B/Apo A-1 allows distinguishing those subjects with and without risk of coronary artery disease (CAD) and seems to be useful for assessment of the effectiveness of pharmacological treatment with lipid-lowering drugs (**Figure 5-3**).

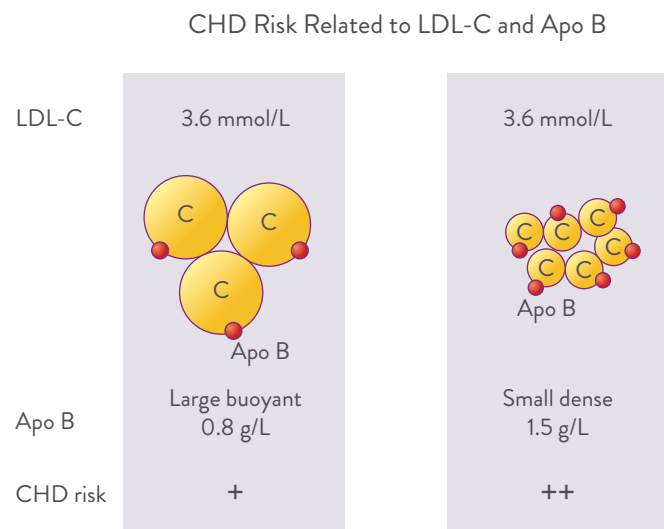


Figure 5-4: For individuals with the same LDL-cholesterol level in serum, the cardiovascular risk is dependent on the level of Apo B in their serum. With permission from AstraZeneca, G. Walldius and I. Jungner.

Elevated levels of Apo B are also found in normolipidemic patients with early CVD, even when levels of total and LDL-cholesterol are normal. Apo B measurement may be more relevant to CVD risk assessment because the amount of Apo B per LDL particle is constant, whereas that of cholesterol is variable. In addition, the measurement of Apo B provides information regarding the number of LDL particles and the relative density of this lipoprotein. Such information is useful since small and dense LDLs are more atherogenic than buoyant ones. Apo B can differentiate small, dense LDL enriched with cholesterol ester from less atherogenic particles. Apo A-1 serum levels correlate better with peripheral vascular disease than other lipid parameters (**Figure 5-4**).

According to Tietz, the apolipoproteins are better indicators of the risk of cardiovascular diseases than other parameters of the lipid status (cholesterol, triglycerides, HDL-C, LDL-C).

Clinical relevance of the apolipoproteins:

- The apolipoprotein content in lipoproteins is more stable than the lipid concentration, which may vary according to the metabolic disease.
- HDL and LDL (and thus the usual lipid parameters) are not uniform lipoproteins, but very heterogeneous macromolecules.
- By determining HDL-C and LDL-C, it is not possible to make assumptions regarding the total HDL/LDL mass or the total concentration of HDL/LDL particles.
- By determining HDL-C and LDL-C, there are substantial differences between fasting and nonfasting patients. This is not the case for Apo A-1 and Apo B.

In routine clinical laboratories, the immunochemical measurement of Apo A-1, Apo B and Lp(a) is a state-of-the-art procedure using commercially available immunoassays. Since the assays for measuring Apo A-1 and Apo B are now internationally standardized through the work of IFCC, clinically meaningful cutpoints for risk stratification have been established.

LIPOPROTEIN(a)

Clinical significance: Several studies reported an association of elevated Lp(a) levels with increased risk of coronary vascular disease (CVD). The association between elevated Lp(a) levels and risk of myocardial infarction (MI) has been documented and has substantiated Lp(a) to be an independent risk factor.

Lp(a) serum levels seem to be almost entirely determined by genetic factors, and elevated levels of Lp(a) are commonly seen in patients and families with premature CVD. Thus, Lp(a) may be an important initiator and promoter of, as well as an early marker for, the atherosclerotic process. Lp(a) concentration should be determined in patients with dyslipoproteinemia, diabetes, renal failure and CVD or cerebrovascular disorders, as well as in premature onset of atherosclerosis. Lp(a) should be monitored also in patients with a strong family history of CVD and patients who are candidates for lipid-lowering. Most of the lifestyle modifications (diet, exercise) and pharmaceutical agents known to reduce cholesterol (statins, bile acid sequestrants and probucol) have little or no effect on Lp(a) concentration. Specific drugs are now needed to modify Lp(a) levels or otherwise prevent the inhibition of plasminogen activation and vascular smooth muscle cell proliferation.

An elevated Lp(a) level is considered to be the most sensitive parameter for the development of coronary heart disease, irrespective of other serum lipoproteins.

Lp(a) should be determined together with TC, Apo A-1 (HDL-C), Apo B (LDL-C) and triglycerides when assessing the total arteriosclerotic risk.

Reference range: < 30 mg/dL (< 0.3 g/L)

SECTION 6

AUTOIMMUNE DISORDERS, RHEUMATIC DISEASE, DIABETES AND RELATED DISORDERS

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the autoimmune diseases and their origin by action of autoantibodies.
- 2 List the different rheumatic diseases and the associated laboratory findings.
- 3 Give examples of important laboratory tests for autoantibodies.
- 4 Identify the general tests that can detect inflammation in rheumatic diseases.
- 5 Specify the utility of HbA1c and urine albumin testing in diabetes.

AUTOIMMUNE DISORDERS/RHEUMATIC DISEASE

The term **autoimmune disease** refers to a varied group of more than 80 serious chronic illnesses that involve almost every human organ. It includes diseases of the nervous, endocrine and gastrointestinal systems, as well as joints, skin and other connective tissues, the kidneys and blood vessels. In all of these diseases, the underlying problem is similar to the body's immune system becoming misdirected and attacking those organs it was designed to protect. Individually, autoimmune diseases are not very common, with the exception of thyroid disease, diabetes, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). However, taken as a whole, they represent the fourth-largest cause of illnesses in Europe and the USA.

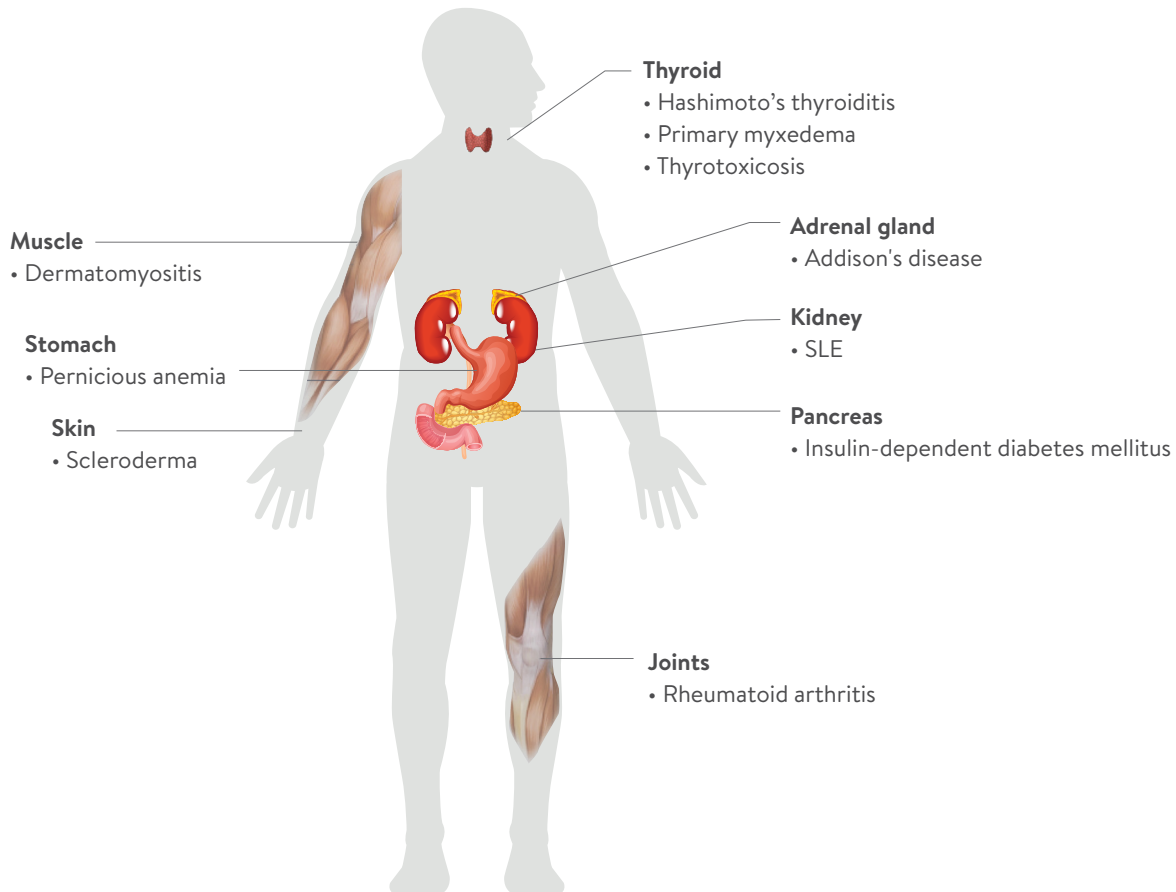


Figure 6-1: Organs affected by autoimmune disease.

The immune system has the central function to detect a variety of foreign agents, such as pathogens, and to distinguish them from the body's own components in order to function. Normally, self-tolerance mechanisms prevent autoimmune disease. In fact, several mechanisms exist to prevent lymphocytes from reacting against self-antigens and thus acquire self-tolerance. As T-cells develop in the thymus in the perinatal period and express antigen-specific receptors, those with high affinity to self-components existing within the thymus are deleted. At later stages of development, a functional suppression of autoreactive T- and B-cells will follow. However, occasionally there is a failure of the organism to discriminate between self- and nonself-antigens, which causes an autoimmune response directed against autologous antigens of normal body tissues. An autoimmune response resembles a normal immune response to pathogens in that it is specifically activated by antigens, in this case self-antigens or autoantigens. This is characterized by activation and clonal expansion of autoreactive T-cells and/or B-cells and by the production of autoantibodies.

Such autoantibodies can be directed against cell surface antigens, intracellular antigens or extracellular molecules. The tissue that is attacked responds to the injury with a series of events counteracting or amplifying tissue destruction.

ORGAN-SPECIFIC AND NONORGAN-SPECIFIC AUTOIMMUNE DISEASE

Autoimmune diseases, mainly those in which autoimmunity contributes to or has an association with the pathogenesis of the disease, can be classified into two broad but overlapping groups, namely organ-specific and nonorgan-specific (or systemic) autoimmune diseases.

In organ-specific disease, the autoantibodies are produced against a specific target antigen in a specialized cell, tissue or organ or against a very specific protein (e.g., a coagulation factor) in response to injury, inflammation, dysfunction or other stimulus (**Figure 6-1**).

Clinical examples include autoimmune hemolytic anemia (erythrocyte autoantibodies), Hashimoto's thyroiditis (thyroid autoantibodies and autoreactive T-cells), Graves' disease (thyrotropin receptor autoantibodies), myasthenia gravis (acetylcholine receptor autoantibodies), type 1 (insulin-dependent) diabetes (pancreatic beta-cell autoreactive T-cells and autoantibodies) and Goodpasture's syndrome (anti-GBM autoantibodies).

By contrast, in nonorgan-specific (systemic) autoimmune disease, the tissue injury and inflammation are usually initiated by the vascular leakage and tissue deposition of circulating autologous immune complexes (ICs) in multiple organs (e.g., muscles, kidneys, skin, joints) (**Figure 6-1**). Such reactions are formed by autoantibody responses to ubiquitous soluble cellular antigens of nuclear, or less commonly cytoplasmic origin. SLE, which is characterized by the production of multiple autoantibodies, particularly antinuclear and anti-DNA antibodies, is the classical example of an IC-mediated systemic autoimmune disease.

RHEUMATIC DISEASES

Rheumatic diseases are a group of autoimmune diseases of the musculoskeletal system, which can be classified as:

- Inflammatory rheumatic disorders.
- Destructive joints and spine diseases.
- Extra-articular rheumatic diseases.
- Para-rheumatic disorders (inflammation of joints and vertebrae).

SYSTEMIC LUPUS ERYTHEMATOSUS

PATHOPHYSIOLOGY

William Osler described the systemic manifestations of SLE in 1895. It presumably got its name (literally “red wolf”) from a reddish rash on the cheeks, which is a frequent early symptom. SLE is now known to be a chronic systemic inflammatory disease that follows a course of alternating exacerbations and remissions. Multiple organ system involvement (central nervous system, heart and kidneys) characteristically occurs during periods of disease activity. Renal involvement occurs as a glomerulonephritis with deposition of immune complexes in glomerular capillaries and subsequent development of renal failure. The kidney lesions are a frequent and serious cause of mortality in SLE. Hypercoagulation, leading to arterial and venous occlusive disease, is seen in SLE patients with antiphospholipid antibodies called lupus anticoagulants. SLE predominantly affects females of childbearing age (ratio of 4:1 to males). However, the age of SLE onset ranges from 2 to 90 years.

The major immunologic features of SLE include:

- Lupus erythematosus (LE) cell phenomenon.
- High-titer antinuclear antibodies (diffuse or outline pattern on immunofluorescence).
- Presence of anti-double-stranded (ds) DNA and anti-Smith (Sm) antibodies.
- High serum IgG levels, together with reduction of serum complement concentrations.

LABORATORY FINDINGS

Anemia is the most common hematologic finding in SLE. Eighty percent of patients present with a normochromic, normocytic anemia due to marrow suppression (secondary anemia). Most patients with SLE (80%) present with elevated levels of immunoglobulins, especially IgG. Hypoalbuminemia is occasionally observed, especially in patients with albuminuria. SLE patients normally have low CRP levels, even in active disease. An elevated CRP level in an SLE patient most often indicates a concomitant bacterial infection. However, CRP may be normal in the presence of an infection, as corticosteroids, often administered in SLE, may suppress CRP expression. Complements C3 and C4 and total hemolytic complement activity are frequently reduced in the presence of active disease because of increased utilization due to immune complex formation, reduced synthesis or a combination of both factors. Therefore, C3 and C4 measurements are indicated to assess disease activity. The activity of the membrane attack complex of complement, C5-9, is increased during disease activity. The plasma of people with active SLE occasionally contains circulating cryoglobulin, consisting of IgM/IgG aggregates and complement.

RHEUMATOID ARTHRITIS

PATHOPHYSIOLOGY

RA is a chronic, recurrent, systemic inflammatory disease primarily involving the joints. It affects 1%–3% of people in the Western world, with a female to male ratio of 3:1. Clinical symptoms include malaise, fever and weight loss. The disease characteristically begins in the small joints of the hands and feet and progresses in a centripetal and symmetric fashion. Deformities are common. Extra-articular manifestations include vasculitis, atrophy of the skin and muscle, subcutaneous nodules, lymphadenopathy, splenomegaly and leukopenia. The disease can occur at any age, but it is most common among females aged 40–70 years, and its incidence increases with age. The geographic distribution of RA is worldwide, with a notably low prevalence in rural Africa and a high prevalence in certain American tribes. Unlike other types of rheumatism, RA was not described or evidenced before the 19th century. However, osteoarticular signs compatible with RA have been discovered in pre-Columbian mummies, suggesting that the disease came from the New World and swept through Europe. All of these facts support the concept of a recent environmental origin of the disease.

The major immunologic features of rheumatoid arthritis are:

- Presence of rheumatoid factors in serum and synovial fluid.
- Increase of the serum IgA concentration.
- Decreased complement in synovial fluid.
- Presence of vasculitis and synovitis.
- Presence of anti-CCP antibodies. (See below.)

Whatever the primary stimulus, synovial lymphocytes produce IgG that is recognized as foreign and stimulates an immune response within the joint, with production of IgG, monomeric IgM and pentameric IgM anti-immunoglobulins (i.e., the rheumatoid factors [RFs]). The presence of IgG aggregates or IgG-rheumatoid factor complexes results in activation of the complement system via the classic pathway. Breakdown products of complement accumulate within the joint and amplify the activation of complement by stimulation of the alternative system. Complement activation results in a number of inflammatory phenomena, including histamine release, the production of factors chemotactic for polymorphonuclear and mononuclear cells, and membrane damage with cell lysis.

LABORATORY FINDINGS

The erythrocyte sedimentation rate (ESR) is elevated as well in various acute-phase proteins (e.g., CRP, fibrinogen, SAA, AAG, HP), and the degree of elevation correlates roughly with disease activity. Complements C3 and C4 can be increased but are frequently reduced in the presence of active disease because of increased utilization due to immune complex formation. It is very common to find increased serum IgA levels in people with RA. The synovial fluid shows signs of more inflammation than is seen in degenerative osteoarthritis or SLE. Rheumatoid factor can be found in synovial fluid, and complement is often depressed.

IMPORTANT LABORATORY TESTS IN AUTOIMMUNE DISEASES

LABORATORY TESTS FOR AUTOANTIBODIES

Anti-CCP (autoantibodies specific to cyclic citrullinated peptide)⁷

Several years ago, it was recognized that antibodies to anti-perinuclear factor (APF) and anti-keratin antibodies (AKA) are highly specific for rheumatoid arthritis. It was subsequently reported that both of these antibodies reacted with native filaggrin, and they are now referred to as anti-filaggrin antibodies (AFA). More recently, it was shown that all of these antibodies are directed to citrulline-containing epitopes. Citrulline is a nonstandard amino acid, as it is not incorporated into proteins during protein synthesis. It can, however, be generated via post-translational modification of arginine residues by the enzyme peptidylarginine deiminase (PAD). Linear peptides containing citrulline (CP) have been reported to be very specific for RA antibodies (96%). It was subsequently demonstrated that cyclic variants of these peptides, termed cyclic citrullinated peptides (CCP), were equally specific for RA but with a higher sensitivity than linear peptides. To further improve the sensitivity of the CCP test, several dedicated libraries of citrulline-containing peptides were screened with RA sera, and a new set of peptides (CCP2) was discovered that gave superior performance compared to the CCP1 test. In the meantime, many independent studies have confirmed the diagnostic performance of the CCP2 test. RA-associated antibodies, and anti-CCP antibodies in particular, may be detected in serum of patients years before RA symptoms develop. Looking back at patients with RA who were blood donors before the onset of disease symptoms, it was suggested that these autoantibodies may assist in the early detection of RA in high-risk populations.

In prediction models to forecast persistence, disability or erosive disease, both RF and anti-CCP are important and significant complementary covariates. The anti-CCP test seems to be superior to RF to predict a more aggressive disease course and more severe radiological damage.

In 2007, the European League Against Rheumatism (now, the European Alliance of Associations for Rheumatology [EULAR[®]]) published guidelines for the diagnosis of early RA, and the measurement of antibodies to anti-CCP was included as a serology marker for RA.

Reference values: A cutoff of 5.0 U/mL has been set, whereby a result of ≥ 5.0 U/mL is considered positive and a result of < 5.0 U/mL is considered negative.

RHEUMATOID FACTORS

In the 1940s, it was discovered that the majority of sera of people with RA agglutinated sheep red blood cells sensitized with rabbit anti-erythrocyte antibodies. Rheumatoid factor forms IgG-anti-IgG complexes in the circulation or joint fluid. RF may play a direct role in the causation of extra-articular disease. RFs do not initiate the inflammatory process that causes rheumatoid disease, but they probably perpetuate and amplify that process.

Most laboratory techniques detect pentameric IgM RF, but rheumatoid factor properties are also seen in monomeric IgM, IgG and IgA. Pentameric IgM RF may combine with IgG molecules to form a soluble circulating high-molecular-mass immunoglobulin complex in the serum. However, RFs are not specific for RA and occur in other connective tissue diseases, in chronic infectious diseases (e.g., endocarditis, tuberculosis, hepatitis B) and in up to 20% of overtly normal elderly individuals, although usually at low titer. Rheumatoid factor is also present in 30% of patients with SLE, in 90% of patients with Sjögren's syndrome, and less often in patients with scleroderma or polymyositis. The transient appearance of rheumatoid factor has been noted following vaccinations in military recruits. It should be emphasized that a negative RF by laboratory procedures does not exclude the diagnosis of rheumatoid arthritis.

The so-called seronegative patient may have IgG or IgM RF or circulating IgG-anti-IgG complexes. In RA serum protein, electrophoresis may show increased APP (AAG, AAT, HP, fibrinogen, CRP), polyclonal hypergammaglobulinemia (especially IgA and IgG) and hypoalbuminemia. Cryoprecipitates composed of

immunoglobulins are often seen in rheumatoid vasculitis. Many patients have antinuclear antibodies (ANA).

The most commonly measured RF is IgM antibody to IgG by latex agglutination or by immunoturbidimetric or immunonephelometric methods based on the immunochemical agglutination principle, with enhancement of the reaction by latex particles. IgM RFs are multivalent and are efficient agglutinators of antigen-coated particles.

The prevalence of RF in healthy elderly patients may be as high as 10%, although the titer is usually low (1:40 or lower).

The pathologic role of RF in RA remains unclear. The RF titer does not correlate with disease activity, but high-titer RFs are generally considered to be associated with more severe disease, including the presence of rheumatoid nodules and extra-articular features. The RF test subclassifies RA patients into seropositive and seronegative disease. RF-positive (seropositive) RA patients are more likely to have progressive, erosive arthritis with loss of joint function and are also more likely to have extra-articular complications, such as rheumatoid nodules, rheumatoid pulmonary disease, vasculitis, Felty's syndrome (neutropenia and splenomegaly associated with rheumatoid arthritis) and secondary Sjögren's syndrome than those with low-titer RF. The tendency toward earlier and more aggressive treatment of severe RA makes identifying people who are likely to develop severe disease a major goal.

RF, although not a perfect test, is one of the best prognostic indicators available. People with RA but who are negative for RF need to be monitored more closely for the development of a different disorder, such as psoriatic arthritis or one of the spondyloarthropathies.

The diagnostic specificity of RF for RA ranges from 80%–95%, depending on the age and health of the population studied. The diagnostic sensitivity of RF ranges from approximately 10% in patients with polymyositis to more than 90% in those with Sjögren's syndrome or cryoglobulinemia. RF testing is a useful screening tool when Sjögren's syndrome or cryoglobulinemia is suspected. Serial RF measurements can be helpful in patients with Sjögren's syndrome because the disappearance of RF may signal the onset of lymphoma. The detection of RFs is one of the diagnostic criteria of the American College of Rheumatology® for classifying rheumatoid arthritis.

Reference range: < 10 IU/mL

ANTISTREPTOLYSIN O

In 1928, it was demonstrated that Lancefield group A β -hemolytic streptococci produce lysin for red blood cells and that, following an infection, antibodies against this particular antigen can be found in the serum. The streptolysin was differentiated into two serologically identified lysins: streptolysin O and streptolysin S. The anti-streptolysins (ASL) represent specific antibodies to the extracellular products of *Streptococcus pyogenes* (Group A streptococcus, GAS), among which antistreptolysin O (ASO) is the test most used for clinical laboratory evaluation.

A significantly increased titer of ASO has been linked to rheumatic fever (major symptoms: carditis, polyarthritis, chorea minor, subcutaneous nodules, erythema annulare), poststreptococcal acute glomerulonephritis, rheumatoid arthritis and ankylosing spondylitis.

The ASO reaction provides useful information for diagnosis and monitoring of human streptococcal infections, such as in tonsillitis, otitis, erysipelas and scarlet fever, as well as in related diseases like rheumatic fever or glomerulonephritis. A rise in ASO titer begins approximately 1 week after infection, with the highest titers measurable 2–5 weeks later. If no complications or reinfection occur, the ASO titer will usually fall to preinfection levels within 6–12 months. However, the ASO titer is also known to increase after Group C or G streptococcal infections.

Various methods are available for assaying antistreptolysin, the modern ones based on the principle of immunochemical agglutination using latex particles for reaction enhancement.

Reference ranges: Adults: \leq 200 IU/mL; Children: \leq 150 IU/mL

GENERAL TESTS OF INFLAMMATION

C-reactive protein (CRP)

The C-reactive protein concentration is another marker of systemic inflammation used to evaluate systemic rheumatic diseases, especially SLE, rheumatoid arthritis or other arthritic diseases. CRP levels increase rapidly, and when the condition subsides, CRP falls, reaching normal levels very quickly. The degree of elevation of CRP can be a clue to diagnosis, given that CRP can be quite elevated (> 100 mg/L) in patients with RA or bacterial infections (**Figure 6-2**). Such elevations are not seen in patients with SLE or viral infections. Monitoring of CRP in RA is especially useful and shows significant correlation with the RA disease activity indices. Among the other acute-phase reactants and immunoglobulins, only fibrinogen, serum amyloid A and haptoglobin are useful indicators of disease activity in RA. However, the ESR and acute-phase proteins are nonspecific measures of inflammation and are not diagnostic of any condition, but they are important indicators of disease activity.

Reference value: < 3.0 mg/L

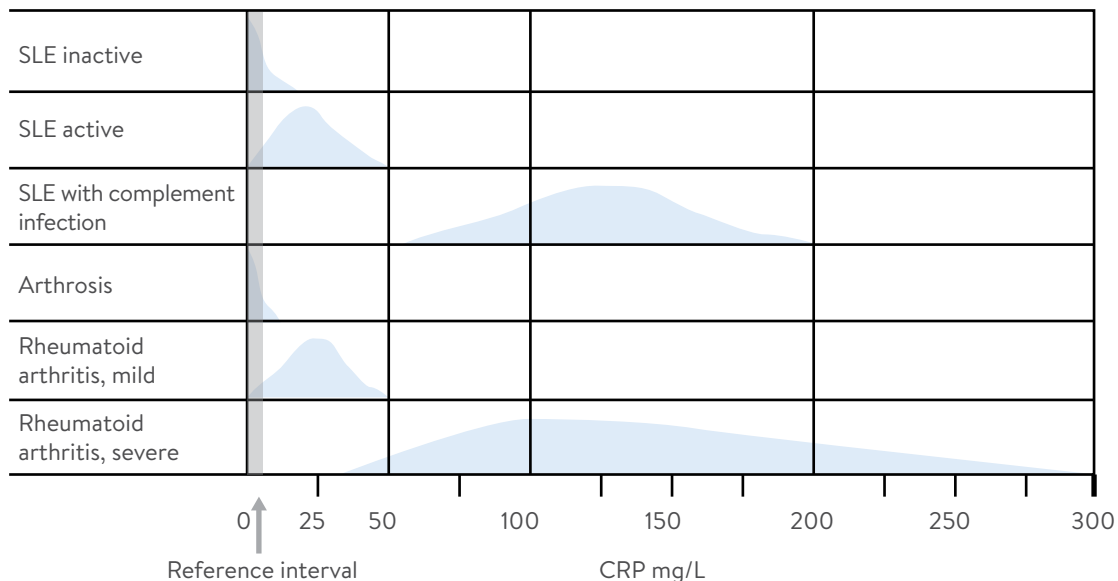


Figure 6-2: CRP concentrations in various autoimmune diseases.

COMPLEMENT

During activation of the classical pathway, which occurs in immune complex diseases (e.g., active SLE, especially with nephritis and mixed cryoglobulinemia), the components of the classical complement pathway are consumed, leading to a decrease in the serum levels of C3, C4 and CH50 (i.e., total complement activity). During activation of the alternative complement pathway, concentrations of the alternative pathway components decrease, while the serum level of C4 is normal.

People with SLE frequently show decreased serum levels of C3 and C4. Lowered serum complement levels may indicate both consumption of components and a decrease in complement synthesis, as in lupus nephritis. C3 is the most useful initial measure of the complement system. C3 is the most stable complement component, and its decrease indicates activity in either or both classical and alternative pathways. C4 is also a measure of the classic complement pathway and may be low in active SLE. In SLE individuals, hereditary complement deficiency tends to be more common than in the general population. In such subjects, the age of onset is earlier, and there is a higher incidence of males compared to those with no complement deficiency. In SLE, the incidence of hereditary complement deficiency may be as high as 6%, and there is milder renal involvement in SLE individuals with complement deficiency.

DIABETES AND RELATED DISORDERS

DEFINITION OF DIABETES MELLITUS

Diabetes mellitus is a disease caused by a failure of glucose homeostasis. The term diabetes mellitus describes a metabolic disorder of multiple etiology, which is characterized by hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The WHO diagnostic criteria for diabetes are fasting plasma glucose > 126 mg/dL (> 7.0 mmol/L) or 2 hours (post 75 g oral glucose load) plasma glucose > 200 mg/dL (> 11.1 mmol/L) on two or more occasions. HbA1c can be used as a diagnostic test for diabetes, providing that stringent quality assurance tests are in place, assays are standardized to criteria aligned to the international reference values, and there are no conditions present that preclude its accurate measurement. A HbA1c level of 6.5% (48 mmol/mol) is regarded as the cut off point for diagnosing diabetes. A HbA1c value of less than 6.5% (48 mmol/mol) does not exclude diabetes diagnosed using glucose tests.

ETIOLOGY

Several pathogenetic processes are involved in the development of diabetes. Diabetes results from deficient insulin secretion, decreased insulin action or both. Many processes can be involved, ranging from autoimmune destruction of the beta-cells of the pancreas, with consequent insulin deficiency, to incompletely understood abnormalities that result in resistance to insulin action. Genetic factors are involved in both mechanisms. In type 1 diabetes, there is a beta-cell destruction and, thus, an absolute deficiency of insulin, whereas in type 2 diabetes, insulin resistance and an inability of the pancreas to compensate are involved. The abnormalities of carbohydrate, fat and protein metabolism are due to the deficient action of insulin on target tissues resulting from the insensitivity to or lack of insulin. Hyperglycemia sufficient to cause tissue damage can be present without clinical symptoms for many years before diagnosis.

The classification of diabetes mellitus includes four clinical classes:

1. Type 1 diabetes (immune mediated or idiopathic), characterized by deficient insulin production and requiring daily administration of insulin
2. Type 2 diabetes, resulting from a progressive insulin secretory defect and a background of insulin resistance; largely the result of excess body weight and physical inactivity
3. Other specific types of diabetes, due to other causes (e.g., genetic defects in β -cell function, genetic defects in insulin action, diseases of the exocrine pancreas, endocrinopathies [drug or chemical-induced] infections)
4. Gestational diabetes mellitus (GDM), hyperglycemia with onset or first recognition during pregnancy

METABOLIC SYNDROME

A major diagnostic and therapeutic challenge is the person with hypertension, central (upper body) obesity, and dyslipidemia, with or without hyperglycemia. This group of people is at high risk of both macro- and microvascular disease. Often a person with impaired glucose tolerance (IGT) will be found to have at least one or more of the other CVD risk components. This clustering has been labeled variously as syndrome X, the insulin resistance syndrome or the metabolic syndrome.

THE INTERNATIONAL DIABETES FEDERATION (IDF) CONSENSUS WORLDWIDE DEFINITION OF THE METABOLIC SYNDROME (2006)

According to the IDF definition, for a person to be defined as having metabolic syndrome, they must have central obesity (defined as waist circumference* with ethnicity-specific values) **and** any two of the following:

- **Raised triglycerides** – > 150 mg/dL (1.7 mmol/L), or specific treatment for this lipid abnormality
- **Reduced HDL cholesterol** – < 40 mg/dL (1.03 mmol/L) in males, < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality
- **Raised blood pressure** – Systolic BP > 130 or diastolic BP > 85 mmHg, or treatment of previously diagnosed hypertension
- **Raised fasting plasma glucose (FPG)** – > 100 mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes; if FPG > 5.6 mmol/L or 100 mg/dL, OGTT glucose tolerance test is strongly recommended but not necessary to define presence of the metabolic syndrome

Evidence is accumulating that insulin resistance may be the common etiological factor for the individual components of metabolic syndrome, although there appears to be heterogeneity in the strength of the insulin resistance relationship with different components between and within populations. Alone, each component of the cluster conveys increased CVD risk, but as a combination, they are more powerful. It is well documented that the features of metabolic syndrome can be present for up to 10 years before detection of the glycemic disorders. This is important in relation to the etiology of the hyperglycemia and the associated CVD risk, and the potential to reduce morbidity and mortality from CVD in persons with glucose intolerance.

It is also well documented that increased levels of both CRP and cystatin C are early markers of risk for developing metabolic syndrome. Therefore, the use of these two markers might be advocated for early identification of risk for developing metabolic syndrome, future CVD and premature mortality.

GLYCATED HEMOGLOBIN/HbA1c

Glycated hemoglobin (glycohemoglobin, GHb, hemoglobin A1c, HbA1c, A1C or Hb1c) is formed in a nonenzymatic glycation pathway by exposure of hemoglobin to plasma glucose. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) has defined HbA1c as the stable adduct of glucose to the N-terminal valine of the β -chain of hemoglobin.

There are several types of normal hemoglobin, but the predominant form – about 95%–98% – is hemoglobin A. Hemoglobin A can be further subdivided, with one of the subcomponents known as hemoglobin A1c. As glucose circulates in the blood, some of it spontaneously binds to hemoglobin A, the glucose-hemoglobin molecules formed being glycated. The higher the concentration of glucose in the blood, the more glycated hemoglobin is formed. Once the glucose binds to the hemoglobin, it remains there for the life of the red blood cell, on average about 120 days. HbA1c is produced on a daily basis and slowly cleared from the blood as older RBCs are degraded and younger RBCs (with nonglycated hemoglobin) substitute them.

*If BMI is > 30 kg/m², central obesity can be assumed and waist circumference does not need to be measured.

CLINICAL USEFULNESS OF HbA1c MEASUREMENT

Determination of HbA1c is used as a retrospective estimate of the average plasma glucose level over a period of 120 days. The HbA1c assay is an accurate and precise measure of chronic glycemic levels and correlates well with the risk of diabetes complications.

Clinical trials in type 1 diabetes and type 2 diabetes have shown that intensive treatment of hyperglycemia resulting in improved glycemic control is beneficial for patients and reduces the complications of diabetes. The published data show a positive association of HbA1c over the time period of the studies with increased risk of retinopathy, myocardial infarction and other complications of diabetes.

Therefore, HbA1c is a long-term measure of glucose metabolism and is recommended by WHO and diabetes associations as an essential indicator for the monitoring of blood glucose control.

WHO expressly recommends (2011) the HbA1c test for the diagnosis of diabetes:⁸

HbA1c can be used as a diagnostic test for diabetes, providing stringent quality assurance tests are in place, assays are standardized to criteria aligned to the international reference values, and there are no conditions present that preclude its accurate measurement. An HbA1c level of 6.5% (48 mmol/mol) is regarded as the cutoff point for diagnosing diabetes. A HbA1c value of < 6.5% (48 mmol/mol) does not exclude diabetes diagnosed using glucose tests.

Also, the International Expert Committee⁹ recommended in 2009 that diabetes be diagnosed in a subject whose blood HbA1c level has been tested to be $\geq 6.5\%$. Diagnosis should be confirmed with a repeat HbA1c test. A confirmation is not required in symptomatic subjects with plasma glucose levels > 200 mg/dL (> 11.1 mmol/L).

HbA1c testing is indicated in children in whom diabetes is suspected but the classic symptoms and a casual plasma glucose > 200 mg/dL (> 11.1 mmol/L) are not found. As for the diagnosis of diabetes, the HbA1c assay has several advantages over laboratory measures of glucose in identifying individuals at high risk for developing diabetes. The HbA1c test can be performed at any time of the day and does not require that the person fast and have preceding dietary preparations. These properties have made HbA1c the preferred test for assessing glycemic control in people with diabetes.

HbA1c testing should be performed routinely in all people with diabetes – first, to document the degree of glycemic control at initial assessment and then, as part of continuing care to determine whether a patient's metabolic control has reached and been maintained within the target range. The HbA1c test should be performed at least two times a year in patients who are meeting treatment goals (and who have stable glycemic control) and quarterly in patients whose therapy has changed or who are not meeting glycemic goals.

It has to be taken into account that a variety of genetic, hematologic and disease-related factors may lead to interferences in HbA1c assays. The most common important factors that affect HbA1c levels are disorders such as hemolytic anemia, sickle cell disease, thalassemia or polycythemia, disorders associated with accelerated red cell turnover (e.g., malaria), and hemoglobin variants (HbS, HbE, HbC and HbD, or HbF, depending on the assay employed). In cases when HbA1c levels are not reliable, long-term glucose control can be assessed by measuring fructosamine (glycated albumin).

Degree of glucose control	HbA1c	
	(%)	(mmol/mol)
Normal	< 5.6	< 39
Prediabetes	5.7–6.4	39–47
More stringent control (for select patients such as short duration of diabetes, type 2 diabetes treated with lifestyle or metformin only, long life expectancy, or no significant cardiovascular disease)	< 6.5	< 48 mmol/mol
Good therapeutic control	< 7	53 mmol/mol
Less stringent control (may be appropriate for patients with a history of severe hypoglycemia, limited life expectancy, advanced microvascular or macrovascular complications, extensive comorbid conditions, or long-standing diabetes in whom the goal is difficult to achieve)	< 8	64 mmol/mol

Table 6-1: Glucose control by glycohemoglobin.

The goal of diabetes therapy should be HbA1c concentration < 7%, and the treatment regimens should be reevaluated and significantly changed if HbA1c values are consistently > 8% (**Table 6-1**). These values apply only to assay methods that are certified as traceable to the accepted reference method(s) and certified by international/national harmonization programs. Glycated hemoglobin serves as a key predictor of the risk of developing diabetic complications. In addition, knowledge of HbA1c concentrations appears to alter the behavior of healthcare providers and/or patients, in turn improving glycemia and lowering HbA1c values.

The clinical relevance of HbA1c has been defined for four distinct clinical indications:

1. HbA1c is used as an independent marker of glycemic control and offers both the patient and healthcare providers a mean plasma glucose value over the previous 8–12 weeks. This value can indicate a need for alteration of diabetes therapy, which in turn results in lowered HbA1c values and improved glycemic control.
2. HbA1c is used as an indicator of the development of late diabetes complications. There exists an exponential relationship between percentage of HbA1c and risk of complications. This requires a more intensive therapy in diabetic patients aiming to reach an HbA1c value near normal. Every decrease in HbA1c values will benefit patients irrespective of age.
3. HbA1c can be used to set a treatment target in the management of a diabetic disorder.
4. HbA1c can be used to diagnose diabetes.

HbA1c INTERNATIONAL STANDARDIZATION

The IFCC Working Group on Standardization of HbA1c has established an international reference measurement system for HbA1c based on reference methods and prepared highly purified HbA1c reference material that has led to substantial improvements in inter-method and inter-laboratory variability. HbA1c values are reported as IFCC units in mmol/mol.

Reporting of HbA1c has been agreed at the international level by professional and diagnostic manufacturers, using the units of mmol/mol (IFCC) and percent (National Glycohemoglobin Standardization Program, NGSP), although the practice varies in different countries and regions of the world. The IFCC and NGSP reference systems coordinate their efforts to ensure continued stability of the reference methods and reference standards. This ongoing cooperation allows patient HbA1c results to be interconverted between NGSP and IFCC reporting units.

The relationship between the IFCC units and the values in percent by the NGSP can be described by a linear regression equation known as the Master Equation:

From IFCC (mmol/mol) to NGSP (%)	From NGSP (%) to IFCC (mmol/mol)
$NGSP = (0.09148 \times \text{mmol/mol}) + 2.152$	$IFCC = (10.93 \times \%) - 23.50$

Reference ranges: Nondiabetics: 4%–6% (20–42 mmol/mol); Diabetes: \geq 6.5% (48 mmol/mol)

URINE ALBUMIN (MICROALBUMINURIA)

The early signs of diabetic nephropathy cannot be detected by the routine screening tests for proteinuria, so more sensitive methods for detecting abnormal albumin excretion must be used. The early stage of albuminuria (microalbuminuria) is clinically defined as an albumin excretion rate of 30–300 mg/24 hours (20–200 $\mu\text{g}/\text{min}$), although true normal renal albumin excretion is lower than this. An elevated albumin excretion rate of $> 30\text{--}300$ mg/24 hours ($> 20\text{--}200$ $\mu\text{g}/\text{min}$, macroalbuminuria) is an indicator of cardiovascular risk in all subjects, especially for people with metabolic syndrome and type 2 diabetes. Microalbuminuria should be regarded as a predictor of both increased macro- and microvascular risk.

A classification of albuminuria is outlined in **Table 6-2**.

Microalbuminuria identifies people at risk for the development of diabetic nephropathy, hypertension, and both chronic renal disease and CVD. The risk of progression to renal disease in patients with microalbuminuria is twentyfold higher than in patients with normal excretion. Elevated blood pressure in type 2 patients is the major predictive factor for the development of microalbuminuria. The finding of microalbuminuria in 35%–40% of type 1 diabetes, 25%–60% of type 2 and 10%–15% of those without diabetes is associated with an increased relative risk for future cardiovascular events, including myocardial infarction (MI), stroke, cardiovascular death and CHF hospitalization. Thromboembolic or hemorrhagic stroke can be detected in 20%–25% of diabetic subjects with clinical proteinuria, 10% in those with borderline proteinuria and 7% without proteinuria. The increased risk of retinopathy and diabetic complications in patients with microalbuminuria can be reduced with better glycemic control.

Albumin excretion rate	µg/min	mg/24 h	mg/L	mg/mmol creatinine	mg/g creatinine
Normal	< 11	< 15	< 15	< 1.5	< 12
Clinically abnormal	20–200	30–300	30–300	> 3.5	> 24
Clinical nephropathy	> 200	> 300	> 300		

Table 6-2: A classification of abnormal urine albumin excretion rate.

SECTION 7

IMMUNODEFICIENCIES AND MONOCLONAL GAMMOPATHIES

DISORDERS OF THE IMMUNE SYSTEM: IMMUNODEFICIENCY AND MONOCLONAL GAMMOPATHY	71
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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Classify the immunodeficiency disorders.
- 2 Describe the different monoclonal gammopathies.
- 3 Identify the cryoglobulins and Bence-Jones protein.
- 4 Specify how serum free light chain (FLC) immunoassay can detect and quantify free light chains.
- 5 List examples of complement-mediated diseases.

DISORDERS OF THE IMMUNE SYSTEM: IMMUNODEFICIENCY AND MONOCLONAL GAMMOPATHY

IMMUNODEFICIENCY

Immunodeficiency is classified as primary, in which the cause is/was previously unknown or genetic, or secondary, in which there is a recognized cause for the immune defect, such as cancer, infection (especially HIV), drug treatment, radiotherapy or malnutrition.

Immunodeficiency disorders can be classified as:

- Antibody (B-cell) immunodeficiency disorders.
- Cellular (T-cell) immunodeficiency disorders.
- Combined antibody-mediated (B-cell) and cell-mediated (T-cell) immunodeficiency disorders.
- Phagocytic dysfunctions.

PRIMARY IMMUNODEFICIENCY

Primary immunodeficiencies include a variety of genetic disorders in which part of the body's immune system is missing or malfunctioning, thus making the patient more susceptible to infections. If left untreated, these infections may be fatal. The disorders constitute a spectrum of more than 80 defects of the body's immune system.

Primary immunodeficiencies are relatively uncommon genetic disorders and include disorders of humoral immunity (affecting B-cell differentiation or antibody production), T-cell defects, combined B- and T-cell defects, phagocytic disorders, and complement deficiencies. Major features of these disorders include recurrent or persistent infections, infections with unusual or opportunistic organisms, developmental delay, and a positive family history. Early recognition and diagnosis can alter the course of primary immunodeficiencies significantly and have an important effect on patient outcome.

Essential laboratory tests include complete and differential blood count, testing of all the major immunoglobulins, free light chain (FLC) assay, and antibody responses to immunization. Serum IgG levels are usually less than 20 mg/dL (2 g/L), with serum IgA, IgM and IgE being undetectable. Measurement of IgG subclasses is usually not helpful. The hereditary disease severe combined immunodeficiency (SCID) usually results in death from infections by 1 year. Screening for SCID has been added to standard newborn testing to identify cases, so they can receive lifesaving hematopoietic stem cell transplantation as infants.

DISORDERS OF THE WHITE BLOOD CELLS

Disorders of the white blood cells include a deficiency of leukocytes (leukopenia) and proliferative disorders. These disorders may be reactive (e.g., due to infection) or neoplastic and of hematopoietic and lymphoid origin (e.g., the lymphomas: Hodgkin's disease and non-Hodgkin's lymphoma), the leukemias, and the plasma cell dyscrasias (multiple myeloma).

MONOCLONAL GAMMOPATHIES

Monoclonal gammopathies (myeloproliferative disorders, plasma cell dyscrasias) constitute a group of diseases characterized by the proliferation of a single clone of plasma cells or B-lymphocytes that produces a homogeneous monoclonal protein (M-protein: M-component, monoclonal Ig-band or paraprotein). The M-protein, which may be a polymer, monomer or fragment of an immunoglobulin or only free light chains, is recognized as a discrete band of restricted migration — a narrow discrete spike — on electrophoresis. When the band represents a monoclonal free light chain, it is usually called a Bence-Jones protein (BJP). In some cases, more than one cell clone may produce monoclonal gammopathies (biclonal or very rarely triclinal).

Monoclonal gammopathies may be associated not only with malignant proliferations of lymphocytes or plasma cells (i.e., the B-cell malignancies, including multiple myeloma [MM] and Waldenström's macroglobulinemia [WM]) but also with benign disease such as the monoclonal gammopathy of undetermined significance (MGUS). Primary (AL) amyloidosis may be associated with multiple myeloma and rarely with lymphoid malignancies, but most cases can be considered as a particular form of monoclonal gammopathy, where the monoclonal free light chain causes damage by virtue of its amyloidogenic properties.

MALIGNANT LYMPHOMAS

The lymphomas, Hodgkin's disease and non-Hodgkin's lymphoma, represent solid tumors derived from neoplastic lymphoid tissue cells (i.e., lymphocytes or histiocytes and their precursors or derivatives). These malignant cells can produce monoclonal immunoglobulin, often of IgM type. Sometimes, patients with such disease also have decreased immunoglobulin levels, even in the absence of M-component.

PLASMA CELL DYSCRASIAS¹⁰

Plasma cell dyscrasias are characterized by expansion of a single clone of immunoglobulin-producing plasma cells, and as a result, an increase in the plasma level of a single monoclonal immunoglobulin or its fragments or only in a free light chain may be observed.

The plasma cell dyscrasias include multiple myeloma, localized plasmacytoma (solitary myeloma), lymphoplasmacytic lymphoma, AL amyloidosis and MGUS. These patients often have a typical monoclonal band when their serum is analyzed by electrophoretic methods.

MULTIPLE MYELOMA

MM is the most frequent of the malignant plasma cell dyscrasias, accounting for 1% of all cancers, 10% of all hematologic malignancies in Caucasians and 20% in Blacks.

Myeloma is characterized by proliferation of a malignant plasma cell clone in the bone marrow and osteolytic bone lesions throughout the skeletal system. The malignant plasma cells also can form plasmacytomas (plasma cell tumors) in bone and gastrointestinal tract. One of its hallmarks is the unregulated production of monoclonal M-component, which is IgG in about 60% of cases (IgG myeloma), IgA in 20% (IgA myeloma), and IgD and IgE in < 3%. In 17% of multiple myelomas, the abnormal cells produce only free light chains, the Bence-Jones proteins (light-chain disease), and lack the M-component in their plasma, but most often, a high level of the free light chain is found in urine. However, up to 80% of myeloma cells produce complete intact immunoglobulins, as well as excess free light chains, such that both M-component and BJP are

present. Less than 1% of myelomas are IgM, with most IgM cases being MGUS, lymphoma or Waldenström's macroglobulinemia. In nonsecretory myeloma, no M-component is detected in plasma/serum or urine on electrophoresis, but it can be shown in plasma cells by immunohistochemical methods.

The laboratory findings that suggest multiple myeloma are:

- Clonal bone marrow plasma cells > 10% on biopsy.
- M-component of IgG concentration > 2,000 mg/dL (> 20 g/L) or IgA concentration > 1,000 mg/dL (> 10 g/L) (elevated IgM in less than 1% of myeloma cases).
- Presence of IgD or IgE M-component in any concentration.
- Decreased uninvolved immunoglobulins (IgM < 50 mg/dL [< 0.5 g/L]; IgA < 100 mg/dL [< 1 g/L], IgG < 600 mg/dL [< 6 g/L]).
- Immunoglobulin fragments in the urine (BJP) or serum (such as monomeric IgM).
- Urinary BJP excretion of at least 1 g/24 hours.
- Progressive, often rapid, increase in the concentration of the M-component.
- Increase of serum beta 2-microglobulin > 6 mg/L.

PRIMARY (AL) AMYLOIDOSIS

Amyloidosis is a protein conformational disorder in which insoluble fibrous amyloid proteins are deposited diffusely in tissues throughout the body, causing disease largely through architectural distortion. Multiple varieties of amyloidosis exist, but here only the subtype associated with paraproteinemia (free light chains), namely primary light-chain amyloidosis (AL amyloid), will be discussed. In primary (AL) amyloidosis, a monoclonal population of bone marrow plasma cells is present and consistently produces abnormally processed lambda (λ) or kappa (κ) light-chain fragments. In association with the acute-phase reactant, serum amyloid A protein, these immunoglobulin light chains are aggregated and deposited in extracellular spaces and cause chronic inflammation and arthritis. While there may be gross proteinuria, no monoclonal component is necessarily detected in serum, and if there is one, it consists only of the monoclonal free light chain, which is also present in urine but in higher concentration.

Serum free light chain (FLC) estimation

The serum FLC immunoassay can detect and quantify free light chains in serum with remarkable specificity and sensitivity. The assay gives a positive result (increased level of either kappa or lambda, together with an altered ratio of free kappa to free lambda light chain) in 98% of patients with systemic AL amyloidosis, including those in whom a monoclonal immunoglobulin cannot be demonstrated by conventional electrophoretic means. This assay is not specific for AL amyloidosis, and monoclonal FLCs are present in about half of patients with uncomplicated MGUS and in virtually all patients with multiple myeloma.

WALDENSTRÖM'S MACROGLOBULINEMIA

Waldenström's macroglobulinemia (WM, also known as lymphoplasmacytic lymphoma) is a malignant proliferation of plasmacytoid lymphocytes corresponding to activated B-lymphocytes and caused by a clonal disorder of B-cells in which an excessive amount of an IgM M-component is produced.

Monoclonal IgM levels are typically > 1,000 mg/dL (10 g/L), and FLC is often demonstrated. Other laboratory findings include a very high erythrocyte sedimentation rate, often exceeding 100 mm/hr; hyperuricemia, hyperviscosity, usually when the IgM level is > 3,000 mg/dL (> 30 g/L); often the presence of cryoglobulins, rheumatoid factor or cold agglutinins; and in about 4%, hyponatremia and hypercalcemia. Several coagulation abnormalities and evidence of platelet function defect may be present. Plasmapheresis is used to quickly remove IgM from the plasma, lowering viscosity and improving blood flow.

HEAVY CHAIN DISEASE

Heavy chain disease (HCD) is a group of very rare plasma cell disorders producing excessive amounts of a monoclonal heavy (H-) chain but no light chain. Monoclonal production of each of the major immunoglobulin H-chain classes results in a different clinical presentation. There are three major forms:

- α -H-chain disease (Seligmann's disease)
- γ -H-chain disease (Franklin's disease)
- μ -H-chain disease

MANIFESTATIONS OF PLASMA CELL DYSCRASIA

CRYOGLOBULINS

Cryoglobulinemia results from abnormal proteins that precipitate at reduced temperatures. It is associated with plasma cell disorders and chronic inflammation. Cryoglobulins can be monoclonal immunoglobulins (type I) as in myeloma or Waldenström's, monoclonal proteins (usually IgM κ) complexed to IgG (type II), or polyclonal immune complexes with no monoclonal component (type III). Types II and III are associated with connective tissue and autoimmune disorders. Through the formation of protein gels at reduced body temperatures, precipitated cryoglobulins can occlude small vessels, giving rise to Raynaud's phenomenon, vascular purpura (cold urticaria) and arthralgia. Cold agglutinins, which are RBC antibodies, are different from cryoglobulins, although they may present as small IgM M-components.

BENCE-JONES PROTEIN

BJP has been established as being the same as monoclonal FLC, synthesized by a single clone of B-cells. Normal plasma cells appear to produce a slight excess of free light chains, whereas B-cell neoplasms can produce a much greater excess of these light chains. The Bence-Jones proteins are readily excreted into the urine because of their low molecular weight. The most frequent clinical associations are with multiple myeloma, Waldenström's macroglobulinemia, AL amyloidosis and light-chain deposition disease.

Once the tubular reabsorptive capacity of the kidney becomes saturated, BJP is excreted in urine (overflow proteinuria). The molecular mass of BJP is quite variable and can appear in urine as a monomer (22 kDa), a dimer (44 kDa) or low-molecular mass fragments or can show a high degree of polymerization. Testing for BJP is regarded as central to the initial assessment of patients presenting with a plasma M-component, although the presence of BJP is not necessarily diagnostic of multiple myeloma.

Bence-Jones protein is capable of causing, in addition to structural damage, functional disturbances of the tubular cells in patients without renal failure or any abnormalities on renal biopsy.

Immunofixation electrophoresis (IFE) is required to verify that the two fundamental characteristics of the BJP are present, namely that it is a free light chain (not bound to intact immunoglobulin) and that it is monoclonal (versus polyclonal or oligoclonal kappa and lambda light chains).

IFE combines an electrophoretic step to separate the serum or urine proteins with immunochemical typing.

BETA 2-MICROGLOBULIN

Beta 2-microglobulin (β 2-microglobulin, β 2M) is a structural component of the Class I HLA molecules, which are present in variable amounts on the surface of nucleated cells. β 2M is released during immune activation from the surface of lymphocytes and monocytes. Therefore, β 2M serum concentrations are often elevated in malignant lymphoproliferative diseases, due to increased cell synthesis. Its levels depend on tumor mass and renal function and can be used for staging of multiple myeloma. Increased serum concentrations of β 2M are good predictors of complete response and treatment failure in low-grade lymphoma. Elevated β 2M does not differentiate multiple myeloma and MGUS and is not diagnostic since the same pattern is seen in other lymphoproliferative disorders, but it is very useful to assess prognosis and response to therapy in multiple myeloma.

Reference ranges: < 60 years: 0.8–2.4 mg/L; > 60 years: 1.1–3.0 mg/L

COMPLEMENT-MEDIATED DISEASES

The development of new targeted therapies for complement-mediated diseases necessitates a proper understanding of the complement system. Indeed, activation of the complement system significantly contributes to the pathogenesis of various acute and chronic inflammatory diseases. Current strategies to inhibit complement include the replacement or substitution of endogenous soluble complement inhibitors and the administration of antibodies to block key proteins of the complement cascade or to neutralize the action of the complement-derived anaphylatoxins or the blockade of complement receptors.

Three examples of complement-mediated diseases are: (1) atypical hemolytic uremic syndrome, (2) paroxysmal nocturnal hemoglobinuria and (3) hereditary angioedema, in which mutations that affect some proteins of the complement system trigger the pathophysiology of these diseases.

When mutations or polymorphisms in the complement regulators are present, this results in reduced levels of complement proteins or in their ineffective action, thus boosting susceptibility to certain diseases and, especially, making the kidney more susceptible to complement attack. Additionally, complement deficiencies can predispose to disease (e.g., SLE) because of reduced clearance of apoptotic cells and successive generation of complement-activating autoantibodies, or boosted formation of convertases, resulting in intensified complement activation.

Activation of the complement system takes place largely via the classical and/or the alternative pathways. As complement C3c is common to both pathways, the C3c concentration can be used as a measure of the activation of the complement system. Activation is indicated by decreased concentrations of C3c. A total congenital C4 deficiency is rare, but a partial C4 deficiency is rather common. The main use of C4 measurement is to assess the course of complement-deficient conditions. Indeed, isolated low values of C4 can occur in hereditary and acquired angioneurotic edema.

C1 INHIBITOR

C1 inhibitor (C1-esterase inhibitor, C1-INH; C1EI; molecular mass: ~ 105 kDa) is a protease inhibitor whose main function is the inhibition of the complement system to prevent spontaneous activation. C1-inhibitor is an acute-phase protein whose levels rise approximately twofold during inflammation. C1-inhibitor irreversibly binds to and inactivates C1r and C1s proteases in the C1 complex of classical pathway of complement. C1-INH is an important indicator of hereditary angioedema. In fact, about 85% of the patients show low levels of C1-INH (< 4 mg/dL), while others with nonfunctional inhibitor express C1-INH concentrations of 20–60 mg/dL.

Reference range: 15–18 mg/dL (0.15–0.18 g/L)

SECTION 8

IRON METABOLISM DISORDERS AND ANEMIA

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the turnover, absorption, transport and storage of iron in the body.
- 2 Identify the different forms of anemia.
- 3 List the most important laboratory tests for disorders of iron metabolism.
- 4 Specify how transferrin saturation is calculated.

DISORDERS OF IRON METABOLISM

IRON TURNOVER

In the balanced state, 1–2 mg of iron is turned over each day. Adult males normally have approximately 50 mg of iron per kilogram of body weight, and adult females have approximately 35 mg/kg (**Figure 8-1**).

The approximate distribution of active iron for an average man is 70% in hemoglobin (2,100 mg), 10% in myoglobin (200 mg) and tissue (heme and nonheme), enzymes and cytochromes (150 mg), and 0.1%–0.2% (3 mg) in the transport-iron compartment, with 20% Fe stored in tissue cells as ferritin (700 mg) and as hemosiderin (300 mg).

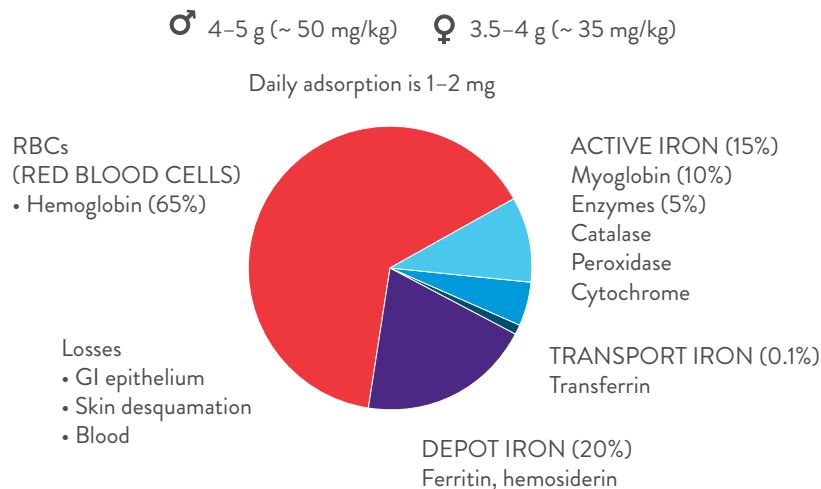


Figure 8-1: Total content of iron in the body.

IRON ABSORPTION

Because iron absorption is limited, iron is highly conserved by the body to handle its daily requirements. Aging red blood cells (RBCs) undergo phagocytosis by mononuclear phagocytes, resulting in release of the iron, which binds to transferrin for reutilization. This system of iron reutilization is very efficient with about 97% of the daily iron needs met from this storage pool and another 1 mg derived from intestinal absorption.

IRON TRANSPORT

Ionic iron or dietary heme iron is absorbed principally in the duodenum and to a lesser extent in the jejunum. Iron from the intestinal mucosal cell is transferred to the iron-transporting protein in the blood, transferrin,

as Fe^{3+} (ferric iron with a trivalent positive charge, in contrast to ferrous iron with a bivalent positive charge). Each transferrin molecule has two high-affinity binding sites for iron. This system transfers iron from cells (intestinal, macrophages) to specific receptors on different cell-types (e.g., erythroblasts, mucosal epithelial cells, placental cells, liver cells). Iron from the transport pool is utilized mainly for red cell production in the bone marrow. More than two-thirds of the iron in the body is incorporated into hemoglobin in the form of erythroid precursors and mature red cells. Each erythrocyte contains a billion atoms of iron. The transferrin-iron complex enters the RBC precursor via cellular transferrin receptors by endocytosis, with iron transferred to the mitochondria and inserted into protoporphyrin to produce heme, while the transferrin molecule is recycled. During iron deficiency, the liver synthesis of transferrin is increased to enhance the transport capacity of iron. The same is observed during pregnancy and during treatment with estrogens. During diseases with increased inflammatory activity (e.g., infectious diseases, RA, IBD, malignant diseases) and during starvation, the liver synthesis of transferrin is decreased. At the same time, iron is trapped within the iron storage cells, resulting in a low serum level of iron and a low iron saturation of transferrin (**Figure 8-2**).

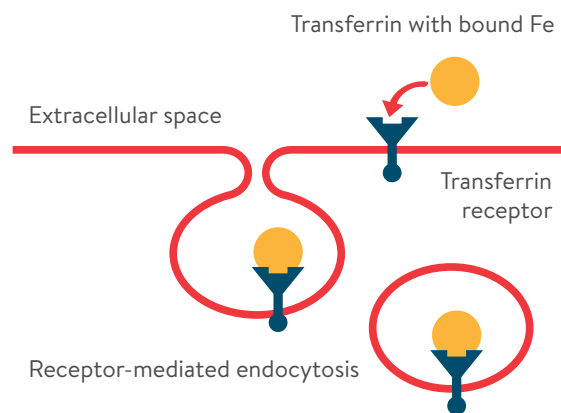


Figure 8-2: Iron transport by transferrin.

IRON STORAGE

The absorbed iron that is not used for erythropoiesis passes through the mucosal cell, binds to transferrin and is transferred to the iron storage pool. Iron is stored in parenchymal cells of the liver and in reticuloendothelial macrophages. The major proteins involved in cellular iron uptake and storage in the body are the transferrin receptor; ferritin; and the iron regulatory proteins, IRP1 and IRP2. Transferrin receptors are found on the surface membrane of all nucleated cells in numbers that are a function of cellular iron requirements. Accordingly, their number is greatest on erythroblasts, liver and placental cells. The levels of serum transferrin receptor reflect the activity of erythropoiesis in the marrow.

Ferritin functions as a compact storage site for iron, which can act as a readily accessible reserve when needed. It belongs to a heterogeneous family of proteins formed around an iron core and is a soluble and active storage fraction found in hepatocytes and macrophages of the bone marrow and spleen. The tissue-ferritin pool is very labile and readily available for any iron requirement in the body. Circulating (plasma) ferritin appears to originate in the mononuclear phagocyte (reticuloendothelial) system, and its circulating concentration parallels the size of the body stores (1 $\mu\text{g/L}$ equals 8 mg of iron in the storage pool). Hemosiderin represents the second iron storage pool in which iron is relatively insoluble and stored primarily in the liver (in Kupffer cells) and in the bone marrow macrophages. The iron regulatory proteins, IRP1 and IRP2, control expression of genes involved in the synthesis of both transferrin receptor and ferritin. They are sensitive to intracellular iron availability and coordinate iron metabolism by regulating the synthesis of transferrin receptor and/or apoferritin.

ANEMIA

Anemia is defined as a pathologic deficiency in the amount of oxygen-carrying hemoglobin in the red blood cells. It is a common problem for patients with various diseases or in different conditions, especially people with chronic kidney disease, cancer, heart disease, gastrointestinal disorders, rheumatic disease or diabetes. This condition is associated with fatigue, a feeling of weakness or reduced physical and mental capacity unrelieved by rest. Additional symptoms include diminished ability to perform daily functions and possibly impaired cognitive function, headache, dizziness, chest pain and shortness of breath, nausea, depression, and occasionally pain. These symptoms are often complicated by coexisting disease(s). There are many compromises that are necessary when one has symptomatic anemia. This can affect the tolerability of therapy. Anemia is also associated with a poorer prognosis and increased mortality.

IRON-DEFICIENCY ANEMIA

ANEMIA OF CHRONIC BLOOD LOSS, HYPOCHROMIC ANEMIA OF PREGNANCY, INFANCY AND CHILDHOOD

Anemia secondary to diseases with an inflammatory component, followed by pure iron-deficiency anemia, is the most common cause of anemia worldwide and is, therefore, of great socioeconomic importance. In pure iron-deficiency anemia, the earliest stage is depletion of body iron stores, which is asymptomatic and does not produce anemia. Women, especially in the childbearing years, have lower iron stores than men and are more at risk of developing iron deficiency. As iron deficiency progresses, iron-deficient red cell production (erythropoiesis) develops, followed by iron-deficiency anemia.

DISORDERS OF IRON DISTRIBUTION

ANEMIA OF CHRONIC DISEASE/ANEMIA OF CHRONIC INFLAMMATION

Worldwide, the anemia of chronic disease (also known as anemia of chronic inflammation) is the most common form of anemia. The pathophysiologic features appear transiently during virtually any infection or inflammation, and the clinical findings are usually those of the underlying disease.

DISORDERS OF IRON UTILIZATION

ANEMIA OF RENAL DISEASE

The extent of renal dysfunction correlates with the severity of anemia. The renal production of erythropoietin (EPO) generally parallels renal excretory function, and anemia occurs when the glomerular filtration rate (GFR) is $< 45 \text{ mL/min/1.73 m}^2$. Decreased erythropoiesis resulting from reduced EPO is expressed as a reduction of reticulocytes and a subnormal marrow response. Renal lesions, primarily in the glomerular region, generally result in the most severe anemia. Other mechanisms, such as mild hemolysis that occurs in uremia, may increase the severity of the anemia.

DISORDERS OF IRON OVERLOAD

Iron overload usually presents in one of two ways. In cases in which erythropoiesis is normal but the serum iron content exceeds the iron-binding capacity of transferrin (e.g., in cases of hereditary hemochromatosis), iron is deposited in the bone marrow and in parenchymal cells of the liver, the heart and a subgroup of endocrine tissues. In contrast, when iron overload results from the increased catabolism of erythrocytes (e.g., in cases of transfusional iron overload), iron accumulates first in reticuloendothelial macrophages and then later in parenchymal cells. Chronic iron overload is characterized by widespread accumulation of (histochemically stainable) iron within the tissues that does not lead to tissue damage and is generally termed **hemosiderosis**. When excess iron deposition is associated with cellular damage and functional insufficiency (i.e., hepatic fibrosis or cirrhosis), the term **hemochromatosis** is used.

Hereditary hemochromatosis is the classical disorder of iron overload. In its primary form, it is an HLA-linked genetic defect of iron absorption.

Focal hemosiderosis occurs in iron tissue stores, mainly in the lungs and kidneys. Renal hemosiderosis can result from extensive intravascular hemolysis caused by trauma to RBCs (e.g., chronic disseminated intravascular coagulation, defective or torn heart valve leaflets, prosthetic mechanical heart valves, or in paroxysmal nocturnal hemoglobinuria).

Transfusional iron overload occurs in conjunction with chronic anemia in patients who receive regular transfusions over many years.

LABORATORY TESTS FOR DISORDERS OF IRON METABOLISM

TRANSFERRIN

Transferrin (TRF, Tf, siderophilin) concentrations, together with serum iron, are used to assess iron status. The measurement of transferrin as transferrin saturation (TS) (i.e., the ratio of iron and TRF concentration expressed as a percentage) is the most accurate indicator of iron supply to the bone marrow. TS is very low in iron deficiency and very high in iron overload. In iron deficiency, the degree of transferrin saturation is an extremely sensitive indicator of functional iron depletion. TS is used in screening for hemochromatosis, for exclusion of iron overload, in iron distribution disorders (e.g., in liver diseases), and in monitoring the EPO treatment of patients with renal failure.

The total iron-binding capacity (TIBC) is calculated from transferrin as follows:

$$\text{TIBC } (\mu\text{mol/L}) = \text{transferrin } (\text{g/L}) \times 25.12 \text{ } (\mu\text{mol/g})$$

Nowadays, the transferrin saturation (TS) has replaced the total iron-binding capacity and is at present calculated approximately from transferrin, as follows:

$$\text{TS } (\%) = \text{iron } (\mu\text{mol/L}) \times 100 / \text{TIBC } (\mu\text{mol/L}) \text{ or } \text{TS } (\%) = 3.98 \times (\text{iron } [\mu\text{mol/L}] / \text{transferrin } [\text{g/L}])$$

TIBC is being replaced by the unsaturated iron-binding capacity (UIBC) test. $\text{TIBC} = \text{Fe} + \text{UIBC}$, so TIBC can be calculated using the results of the iron and UIBC assays. TS (%) can be estimated using TIBC derived from UIBC.

In screening for hereditary hemochromatosis, transferrin saturation provides a better indication of the homozygous genotype than does ferritin. The treatment of anemia with EPO in people with renal failure is only effective when sufficient depot iron is present and when inflammation is under control. The best monitoring procedure is to determine transferrin saturation during therapy. Transferrin saturation, in

conjunction with ferritin, gives a conclusive prediction of the exclusion of iron overload in patients with chronic liver disease. The limitations of using transferrin saturation reflect those of serum iron (i.e., wide diurnal variation and low specificity).

Transferrin is also a negative acute-phase protein, and its serum concentration is low during inflammatory and malignant diseases. Reduced transferrin levels are observed also in protein-losing enteropathy, severe liver diseases, malnutrition and nephrotic syndrome, and disorders of hemoglobin synthesis (e.g., in porphyria, thalassemia).

Reference ranges: Transferrin: 200–360 mg/dL (2.0–3.6 g/L); Transferrin saturation: 16%–45%

FERRITIN

Ferritin is the major storage protein of reserve iron for hemoglobin synthesis. Plasma contains mainly L-type subunit-rich ferritin present in the liver and spleen. Ferritin is synthesized in almost all body cells, where it sequesters iron in a soluble form, providing accessible reserves for synthesis of iron-containing compounds, such as hemoglobin. It is present in large amounts in macrophages and hepatocytes for storage purposes and in erythroblasts for metabolic purposes. The liver and bone marrow each hold about one-third of body iron stores in the form of ferritin (**Figure 8-3**).

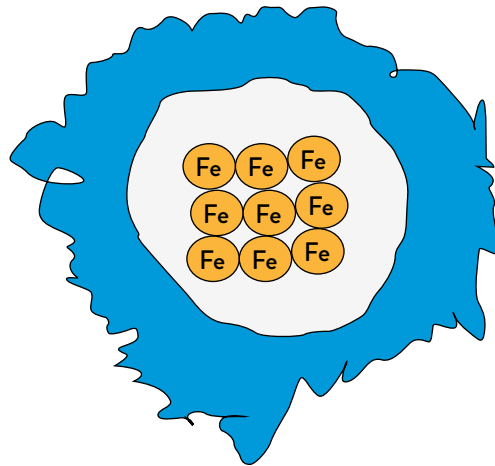


Figure 8-3: Iron storage by ferritin.

Ferritin is an important antioxidant, protecting cells against oxidative damage to DNA, proteins and lipids from free radicals generated in reactions catalyzed by iron released from ferritin.

Although not a transport protein like transferrin, ferritin is present in small concentrations in the serum, which is directly proportional to the body's total iron stores. This relationship makes the serum assay for ferritin an ideal noninvasive test of iron status. The measurement of ferritin in serum is useful in determining changes in body iron storage and clinically significant in the monitoring of the iron status of pregnant women, blood donors and renal dialysis patients. Measurement of the serum ferritin levels is the most sensitive measure of tissue iron depletion and indicates the earliest phase of iron deficiency. Whereas

serum iron demonstrates substantial circadian fluctuations and day-to-day variations of up to 30%, ferritin levels are constant over a long period of time in healthy individuals and do not show diurnal fluctuations. Therefore, a low serum ferritin level is virtually diagnostic of iron deficiency.

Elevated serum ferritin levels are typically derived from metabolically released ferritin rather than from acute tissue destruction. Consequently, increases in serum ferritin correlate with increased tissue iron stores. High ferritin levels may indicate iron overload without apparent liver damage, as occurs in the early stages of idiopathic hemochromatosis. Therapeutic phlebotomy, to remove excess iron from the body, results in the serum ferritin level returning to normal before the transferrin saturation. The donation of one unit of blood drops ferritin levels 40%–50% in males and to about 20% in females who have not previously donated blood.

An individual assay value must be interpreted in the context of the clinical setting, taking into consideration the patient history and other laboratory tests. Serum ferritin can also be increased due to inflammation, in malignant diseases, in effective erythropoiesis and as a result of oral iron therapy. It acts as an acute-phase protein, with its levels in serum being used to evaluate clinical conditions not related to iron storage, such as acute and chronic inflammation, chronic liver disease, and malignancy. Therefore, the significance of elevated ferritin levels must be evaluated carefully.

Reference ranges: Children: 15–120 $\mu\text{g/L}$; Adults: Men: 30–300 $\mu\text{g/L}$; Women: < 50 years: 10–160 $\mu\text{g/L}$; Women: > 50 years: 30–300 $\mu\text{g/L}$

SOLUBLE TRANSFERRIN RECEPTOR

Iron is transported in plasma by transferrin, which provides iron to cells through its interaction with a specific membrane receptor (TfR). A truncated form of the membrane-associated TfR, soluble transferrin receptor (sTfR), is a glycoprotein of approximately 85 kDa, which is detected in plasma as a result of externalization of TfR.

Circulating concentrations of sTfR are proportional to cellular expression of the membrane-associated TfR. sTfR is a sensitive indicator of iron deficiency and, in contrast to ferritin levels, not influenced by the acute-phase response. As such, it has been proposed as a laboratory test to identify iron deficiency in hospitalized and chronically ill patients and, thus, reduce the need for a bone marrow biopsy or trial of iron therapy. Ferritin levels reflect iron stores and sTfR levels, the degree of iron-deficient erythropoiesis. Unlike ferritin, sTfR remains normal in patients with acute or chronic inflammation or liver disease and appears to be effective in distinguishing iron-deficiency anemia from anemia of chronic disease.

sTfR may be a useful parameter also for the monitoring of erythropoiesis in various clinical settings (e.g., in the prediction of the hematological response to EPO treatment).

Reference ranges: Children < 16 years: 1.50–3.00 mg/L; Adults: 0.85–2.30 mg/L

SECTION 9

GASTROINTESTINAL, PANCREATIC AND LIVER DISEASE

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PANCREATIC DISEASE	85
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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Give examples of how useful acute-phase proteins can be in differentiating between active and inactive chronic inflammatory gastrointestinal disease.
- 2 Specify the reason why CRP testing has an independent prognostic value in monitoring acute pancreatitis.
- 3 List the inherited and acquired liver diseases.
- 4 Explain the clinical relevance of proteins in hepatic diseases.

GASTROINTESTINAL, PANCREATIC AND LIVER DISEASE

INFLAMMATORY BOWEL DISEASE

The term **protein-losing gastroenteropathies** refers to a variety of intestinal diseases (e.g., inflammatory bowel disease [IBD], such as Crohn's disease and ulcerative colitis) characterized by a severe loss of plasma proteins into the gastrointestinal tract during high-active phases of the diseases, causing hypoalbuminemia, often complicated by edema, ascites, and pleural and pericardial effusions. The diagnosis of protein-losing gastroenteropathy should be considered in all people with hypoproteinemia in whom other known causes, such as malnutrition, impaired protein synthesis or protein loss through other organs like the kidney or skin, have been excluded.

Albumin, prealbumin, ceruloplasmin, transferrin and immunoglobulins (IgG, IgA, IgM) levels are significantly decreased in protein-losing gastroenteropathies, while haptoglobin is decreased only when there is no acute-phase reaction, which increase may neutralize the negative response.

CRP and AAG are useful in differentiating between active and inactive chronic inflammatory gastrointestinal disease and monitoring the activity, especially of Crohn's disease (**Figure 9-1**). Because malabsorption is present in these diseases, albumin and prealbumin show decreased levels, due to the inflammatory process of the diseases.

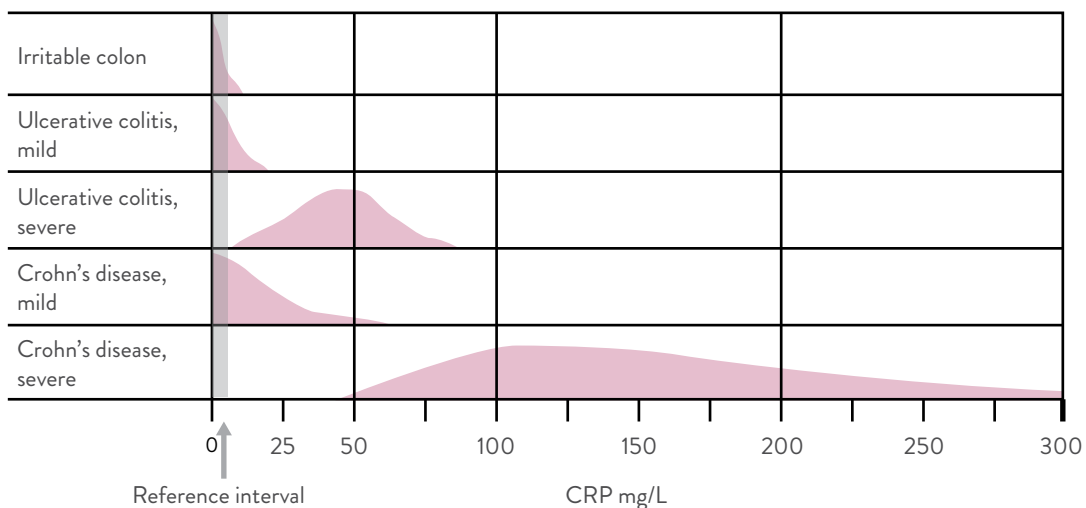


Figure 9-1: CRP in various gastrointestinal diseases.

COLORECTAL CANCER

People with ulcerative colitis or Crohn's disease have an increased risk of colorectal dysplasia and carcinoma in comparison to risk in the general population.

CRP and AAG are often elevated in gastrointestinal tumors, which are associated with acute-phase reaction and thus can be valuable in preoperative tumor staging. It is also well documented that increased inflammatory activity (CRP > 5 mg/L) of chronic stage disease increases the future risk for development of colorectal cancer. CRP, AAG and alpha-1 antitrypsin (AAT) can be used to monitor the inflammatory process during disease progression. Albumin, prealbumin and TF are decreased in people with colorectal cancer, especially when metastases occur.

PANCREATIC DISEASE

Inflammatory disease of the pancreas is a common problem in industrialized countries, with gallstones and alcohol being the major causes. Pancreatitis tends to present with abdominal pain (acute pancreatitis), which may improve with no sequelae or may run a more severe course that can lead to death. When the pancreas is continuously injured, such as with alcohol, a chronic condition (chronic pancreatitis) results in obstruction and fibrosis of the gland, leading to pancreatic insufficiency and chronic pain. Even one attack of pancreatitis from alcohol use can lead to some residual pancreatic damage.

Gallstones are the leading cause of acute pancreatitis in developed countries and, together with microlithiasis, accounts for more than 90% of cases of pancreatitis worldwide. Hyperlipoproteinemias are connected with the majority of lipid-associated cases of pancreatitis that occur in 15%–40% of patients. Hypercalcemia and hyperparathyroidism may also induce pancreatitis.

ACUTE PANCREATITIS

Acute pancreatitis results from an autodigestive process. Pancreatic digestive enzymes, vasoactive materials and other toxic materials flow out of the pancreas into the surrounding tissues, leading to widespread chemical irritation and resulting in simple edema to severe hemorrhage and necrosis. Trypsin and chymotrypsin are the initiating enzymes, with their release resulting in the release and activation of other proenzymes, including proelastase, procollagenase and phospholipases. Trypsin damages endothelial cells and mast cells, resulting in the release of histamine. This major inflammatory mediator enhances vascular permeability, leading to edema, hemorrhage and the activation of the kallikrein system, which in turn results in the production of vasoactive peptides or kinins. The typical markers of acute pancreatitis are the enzymes amylase and lipase, both of which are elevated in this condition.

In acute pancreatitis, CRP concentration has independent prognostic value. A peak of more than 200 mg/L on days 2–4, or more than 120 mg/L at the end of the first week, could be as predictive as the multiple factor scoring systems (**Figure 9-2**).

CRP measurements allow differentiating between mild and severe attacks, especially after the first week. CRP rises to higher concentrations in patients with severe disease, but it is the rate of decrease from peak concentrations that provide greater differentiation between grades of severity. High concentrations of CRP seem to give a warning of severe local inflammation in people whose initial illness is relatively mild and whose clinical course is apparently benign.

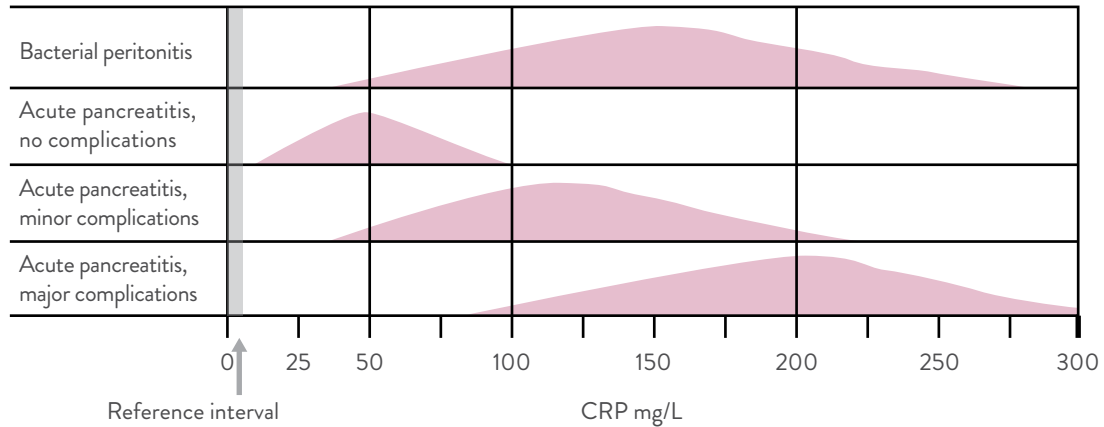


Figure 9-2: Typical serum CRP levels in people with acute pancreatitis in comparison with people with bacterial peritonitis.

CHRONIC PANCREATITIS

Chronic pancreatitis is characterized by progressive functional damage to the pancreas, with or without evidence of severe inflammation. There is permanent destruction of pancreatic tissue, and exocrine and endocrine pancreatic insufficiency usually follows. Excessive alcohol intake over long periods of time is the major etiologic factor, accounting for about 75% of cases. Other causes of chronic pancreatitis include diabetes, protein-calorie malnutrition, hereditary pancreatitis, cystic fibrosis, autoimmune disease, hypertriglyceridemia, hyperparathyroidism and obstruction of the pancreatic duct by tumor, genetic abnormalities and idiopathic causes.

CRP in serum is mostly normal or only marginally increased in patients with chronic pancreatitis. The exception is in a case with an acute exacerbation of a chronic pancreatitis.

LIVER DISEASE

The liver is the primary site for synthesis of many of the plasma proteins, including albumin, prealbumin, alpha-1 antitrypsin (AAT), alpha-1-acid glycoprotein, ceruloplasmin, haptoglobin, alpha-2-macroglobulin, transferrin and coagulation (and complement factors), but not immunoglobulins (which are produced by plasma cells and B-lymphocytes). The measurement of serum proteins gives important information about liver diseases.

There are different types of liver diseases, which can be inherited or acquired:

- Among the inherited liver diseases, the most common ones are hemochromatosis, Gilbert's syndrome, AAT deficiency, Budd-Chiari syndrome, glycogen storage disease type II and Wilson's disease.
- Among the acquired liver diseases, the most common ones are acute and chronic viral hepatitis, hepatic and primary biliary cirrhosis, and hepatic malignancies.

A low concentration of serum prealbumin is a more sensitive indicator of impaired liver function than serum albumin.

INHERITED LIVER DISEASES

Hemochromatosis

Hemochromatosis is one of the most common genetic disorders among Caucasians, although other ethnic groups are also affected. It is an iron-storage disorder in which there is inappropriate increase in the absorption of iron from the gut. This leads to iron deposition in various organs with eventual impairment, especially of the liver, pancreas, heart and pituitary gland.

People suspected of hemochromatosis or unexplained liver disease should be screened for the disease by testing serum ferritin and transferrin saturation for possible elevations. CRP measurement can help the proper diagnosis by identifying a possible acute-phase reaction that may falsely elevate ferritin and decrease transferrin levels. The diagnosis is obtained by genotyping of the hemochromatosis gene HFE for the two mutations C282Y and H63D.

In the treatment of hemochromatosis, with removal of excess body iron by regular phlebotomy, serum ferritin is monitored every 3 months to assess progress. When the serum ferritin is within the low normal range, the frequency of venesection is decreased to three or four per year. Siblings of people with hemochromatosis should be screened with serum ferritin and transferrin saturation, as the siblings have a one in four chance of being affected.

Alpha-1 antitrypsin deficiency

AAT deficiency is inherited, resulting in pulmonary emphysema and hepatic disease. Various presentations are possible, including neonatal hepatitis, chronic active hepatitis, cirrhosis and hepatocellular carcinoma. The risk of cirrhosis and primary liver cell carcinoma is increased for subjects who are homozygous for AAT deficiency. Heterozygosity may predispose to chronic obstructive lung disease, with development of emphysema qualitatively similar to that in homozygotes, but at a later age. Smoking will decrease the life expectancy in subjects with PiZZ phenotype by about 20 years.

Diagnosis of antitrypsin deficiency is suggested by a weak or absent α 1-band on protein electrophoresis or by AAT quantitation if the serum concentration is less than 60 mg/dL (0.60 g/L). A preliminary diagnosis is confirmed either by phenotyping using isoelectric focusing or by genotyping. A low concentration of serum prealbumin is a sensitive indicator of impaired liver function.

Wilson's disease

Wilson's disease is an inherited disorder characterized by the pathological accumulation of copper in the liver, central nervous system and other organs. It may present as pediatric liver disease, which may include fulminant hepatic failure (often with hemolysis), chronic active hepatitis and cirrhosis. Biochemical abnormalities include a low serum concentration of ceruloplasmin and high urinary copper concentration.

ACQUIRED LIVER DISEASE

Acute viral hepatitis

This represents an important group of diseases presenting with hepatitis (i.e., an inflammatory process of the liver). They share clinical, biochemical and morphologic features but are caused by different specific hepatotropic viruses. The most common causes of viral hepatitis are infections by hepatitis virus A (HAV), hepatitis virus B (HBV), hepatitis virus C (HCV), hepatitis virus D (HDV) and hepatitis virus E (HEV). However, viral hepatitis can also occur — although less commonly — with infections such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), adenovirus, herpes simplex (HSV) and Coxsackievirus.

In regard to serum proteins, IgM, but often also IgG, is elevated in patients in the acute viral phase. The immunoglobulin levels usually normalize within 8–12 weeks in uncomplicated cases. Because of the acute

inflammatory reaction in viral hepatitis, CRP and ferritin might be increased and prealbumin and albumin decreased. Often in these cases, AAT levels are increased, while those of haptoglobin are decreased, and those of AAG are relatively unchanged. β 2M concentration is often elevated in HCV due to cell necrosis.

HEPATIC AND PRIMARY BILIARY CIRRHOSIS

Hepatic cirrhosis is a chronic diffuse hepatic disease that is characterized by the widespread presence of nodules in the liver combined with fibrosis. It results from damage to liver cells from toxins, inflammation, metabolic derangements and other causes. In the Western world, alcohol abuse is the leading cause of liver cirrhosis, but cirrhosis can also result from chronic viral hepatitis, metabolic and biliary diseases. Cirrhosis can lead to end-stage liver disease. Anemia is fairly common in these patients. Low levels of serum prealbumin, albumin, C3 and C4 directly reflect disease severity and impaired hepatic function. Decrease of Apo B, Apo A-1 and Lp(a) is often detected in cirrhosis. When serum albumin concentration falls below approximately 2.5 g/dL (25 g/L), ascites and edema develop. Elevated AAT is a key finding in liver cirrhosis. Increase of IgA is a typical finding in people with alcohol-induced cirrhosis. Sometimes there is also an increase in serum IgG concentration.

Primary biliary cirrhosis (PBC) is an autoimmune disease that destroys bile ductules, leading to intrahepatic cholestasis with ultimate progression to fibrotic liver disease.

Early findings are cholestasis with markedly elevated alkaline phosphatase and gamma-glutamyltransferase (GGT). Serum bilirubin, enzymes ALT and AST, and albumin are often normal early in the course of the disease. In people with PBC, very high IgM levels are quite common with less IgG elevations, while IgA is generally unchanged. In the early disease, C3 and haptoglobin levels are elevated, but with hepatocellular damage, they decrease. Serum cholesterol and lipoprotein levels are also increased initially (**Table 9-1**).

Protein	Elevated	Decreased
Albumin		in cirrhosis
Prealbumin (transthyretin)		in cirrhosis
Alpha-1 antitrypsin (AAT)		in deficiency (PiZZ)
Alpha-1-acid glycoprotein (AAG)		in severe liver disease
Apolipoprotein A-I (Apo A-1)		in liver disease
Ceruloplasmin (CER)	in cholestasis	in Wilson's disease
Haptoglobin (HP)		in liver disease
Transferrin (TRF)	in hemochromatosis, transferrin is decreased or normal; transferrin saturation is increased; ferritin is increased	in cirrhosis; desialylated in ethanol abuse
Apolipoprotein B (Apo B)	in cholestasis	in cirrhosis
IgA	in alcoholic liver disease	
IgG	in cirrhosis and CAH	
IgM	in viral hepatitis and PBC	

CAH = chronic active hepatitis; PBC = primary biliary cirrhosis

Table 9-1: Serum proteins and their clinical relevance in hepatic diseases.

SPECIFIC ANTIBODIES IN GASTROINTESTINAL AND HEPATIC DISEASES

In the clinical evaluation of inflammatory bowel disease (IBD), anti-*Saccharomyces cerevisiae* antibodies (ASCA) tend to occur in Crohn's disease, while neutrophil-specific antibodies (e.g., p-ANCA for perinuclear anti-neutrophil cytoplasmic antibody) are associated with ulcerative colitis. Diagnosis of these disorders is confirmed by histopathologic examination of intestinal biopsy. A powerful use of ASCA and p-ANCA is to distinguish IBD from irritable bowel syndrome (IBS), in which the autoantibodies are absent.

The detection of some specific autoantibodies can be very useful for diagnosing autoimmune hepatitis (AIH) and distinguishing it from viral hepatitis and other liver diseases. Patients with AIH exhibit antinuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), and anti-liver kidney microsomal (anti-LKM) antibodies. Anti-soluble liver antigen (anti-SLA) autoantibody is also found in AIH. Because AIH is a chronic progressive condition for which treatment can be successful, diagnosis should be made promptly to initiate proper therapy.

Primary biliary cirrhosis (PBC) is marked by the presence of anti-mitochondrial antibodies (AMA). The related disorder primary sclerosing cholangitis (PSC) does not show AMA but can have several different autoantibodies. Some patients with AIH also have an overlap syndrome with PBC or PSC and mixed serologic findings. Diagnosis of these hepatic diseases is confirmed with liver biopsy.

SECTION 10

PROTEIN LOSS,
KIDNEY DISEASES AND
PROTEINURIA

KIDNEY DISEASES 91



LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 Describe the factors that can cause an increase of protein excretion in the urine.
- 2 Identify the types of proteinuria and the marker proteins in urine for this condition.
- 3 List the markers of tubular nephropathies in serum and urine.
- 4 Give examples of diseases and conditions in which the detection of microalbuminuria has a diagnostic relevance.
- 5 Explain why the measurement of cystatin C in serum is superior to creatinine for the estimation of the glomerular filtration rate.

KIDNEY DISEASES

Uroscopy (i.e., the practice of diagnosing disease through the visual examination of the urine) was one of the earliest laboratory procedures used by physicians. It was indeed the starting point for detection of a specific disease.

Kidney diseases are classified mainly into glomerular and tubular diseases, according to the two essential structures of the nephron (**Figure 10-1**) (i.e., the glomerulus and the tubule), and are called glomerulopathies and tubulopathies, respectively.

Kidney disease can also be divided according to either failure of kidney function or, without such failure, with further division into chronic and acute forms. Additionally, diseases that involve the kidneys include kidney stones and renal carcinomas.

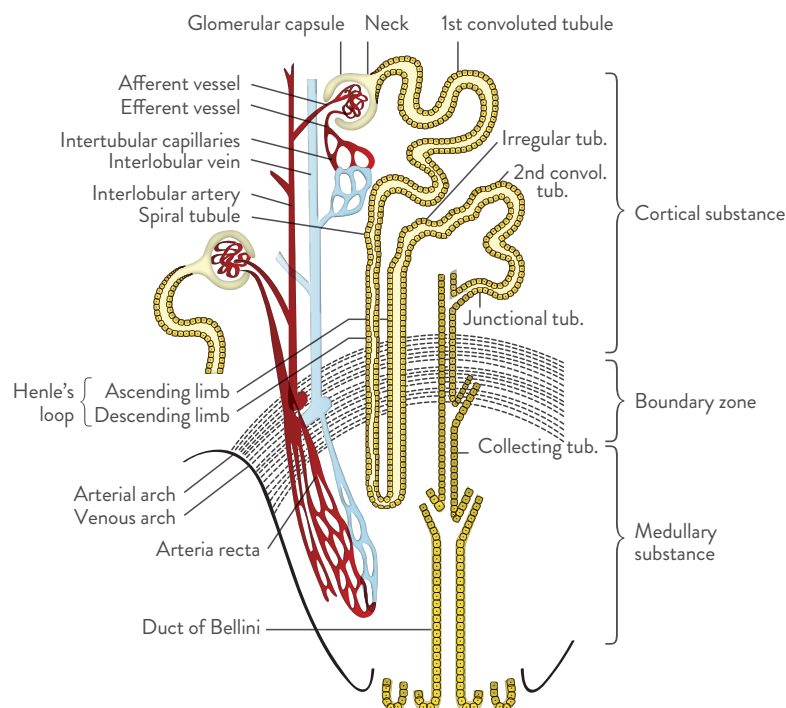


Figure 10-1: Anatomy of the kidney.

Kidney diseases in which glomerular or tubular function is impaired are associated with protein loss and thus an increased excretion of proteins of different sizes into the urine (i.e., proteinuria). However, proteinuria is not proof of a kidney disease, nor does its absence exclude such a disease. The detection of protein in urine necessitates further evaluation to determine the pathological cause. Today, it is obsolete to use old unspecific total protein methods to quantify protein in urine. It is recommended to use only specific immunochemical methods to measure specific proteins in urine.

Urine normally contains proteins, and two-thirds of the normal urine protein content is made up of filtered plasma proteins, such as albumin, transferrin, low molecular weight proteins (LMW; e.g., alpha-1-microglobulin [α 1M], RBP, beta 2-microglobulin [β 2M], and some immunoglobulin components, such as polyclonal free kappa and lambda light chains, IgG and IgA). The remainder, including Tamm-Horsfall glycoprotein, is derived from the urinary tract itself.

An increase of protein excretion in the urine may be caused by several factors including:

- Orthostatic proteinuria.
- Changes in glomerular capillary permeability.
- Alteration in size and charge selectivity of the glomerular basement membrane.
- Decreases in tubular reabsorption and/or catabolism of protein, especially with excretion of low molecular weight proteins in the range of 10–50 kDa.
- Increases in serum levels of certain low molecular weight proteins that are normally retained by the glomerulus.
- Postrenal leakage of protein.

From a clinical point of view, the proteinuria can be classified in different types: prerenal, renal (glomerular and tubulointerstitial) and postrenal proteinuria. Proteinuria should also prompt microscopic examination of the urine to detect any erythrocytes, leukocytes or casts of the tubules that indicate renal disease.

PRERENAL PROTEINURIA AND OVERFLOW PROTEINURIA

Prerenal proteinuria is associated with elevated concentrations of certain proteins in urine and sometimes in plasma. These forms are in connection with nonrenal disorders, when excess LMW proteins associated with myeloma and myelocytic/monocytic leukemia or after trauma are filtered by the glomerulus and exceed the reabsorptive capacity of the tubule.

Common types of overflow proteinuria are:

- Bence-Jones proteinuria (excretion of monoclonal immunoglobulin light chains).
- Hemoglobinuria (in acute hemolytic syndrome).
- Myoglobinuria (in rhabdomyolysis, after multiple injuries or seizures).
- Lysozymuria (in Hodgkin's disease, myelomonocytic leukemias, myelofibrosis, sarcoidosis).
- Beta-2 microglobulinuria (resulting from a large increase in lymphocyte turnover).

GLOMERULAR PROTEINURIA

Glomerular proteinuria occurs when proteins with a molecular weight greater than 40–65 kilodaltons (kDa) are excreted. Severe glomerular proteinuria is often associated with nephrotic syndrome. A special form of proteinuria with increased tubular cell secretion is Tamm-Horsfall proteinuria, which is associated with febrile states.

Proteins such as albumin, IgG and transferrin are quantitated in the urine to differentiate nonselective from selective glomerular proteinuria and its mixed forms. Albumin and IgG are measures of the molecular sieve function of the kidney, whereas albumin is specifically used to check the anion filter function.

TUBULAR PROTEINURIA

Tubulopathies or tubulointerstitial diseases occur in most renal disorders. Tubular proteinuria results when glomerular function is normal but the proximal tubules have a reduced capacity to reabsorb and catabolize proteins, resulting in an increased urinary excretion of the low molecular weight proteins that normally pass through the glomerulus. There are many causes of tubular proteinuria, with damage to the cells of the proximal tubule largely due to infections and nephrotoxic substances.

A tubular pattern is either tubular dysfunction (genetic, toxic or infectious), overflow (increased concentration of LMW proteins in blood [e.g., myoglobin, lysozyme, Bence-Jones, free globin chains]) or secondary to end-stage renal disease, in which the normal filtration of LMW proteins is inhibited, causing an increase of the LMW proteins in plasma and, secondary to this, an overflow proteinuria in the few nephrons that still function.

The following proteins are most commonly used as markers of tubular proteinuria: α 1M, RBP, cystatin C and free light chains kappa and lambda. β 2M is not recommended, as it is easily degraded in urine during transportation and storage. It is important to evaluate these proteins together with albumin in urine. In cases with increased glomerular proteinuria and more than 0.5 g/L of albumin in urine, albumin will block the total tubular capacity to reabsorb proteins.

MIXED GLOMERULAR AND TUBULAR PROTEINURIA

When both glomerular and tubular functions are compromised—as seen in chronic renal failure, chronic pyelonephritis and other conditions affecting the entire nephron—a mixed urine protein pattern will result. The urine then contains increased amounts of high molecular weight (HMW) proteins (e.g., albumin, IgG, TRF), together with LMW proteins, such as α 1M, cystatin C and β 2M.

POSTRENAL PROTEINURIA

A postrenal pattern is seen in postglomerular hematuria, in an infection/inflammation or in tumors of the urinary tract. In postrenal proteinuria, an increased excretion of all serum proteins, including HMW proteins of molecular mass greater than 250 kDa, is observed and can simulate a renal disease, such as glomerular proteinuria.

LABORATORY INVESTIGATIONS OF PROTEINURIA

A proteinuria may be transient or persistent and necessitates further investigation when it persists. The important marker proteins in urine are listed in **Table 10-1**.

Modern laboratory diagnostic procedures for detection of proteinuria should allow not only detection of any pathologic protein excretion but also correlate the clinical findings with the amount, pattern and composition of urine proteins.

Type of disorder	Mechanism	Marker protein
Glomerular	Size selectivity; increased permeability/size selectivity	IgG; transferrin; albumin
Tubular	Decreased reabsorption/tubular dysfunction	α 1M; β 2M; cystatin C; RBP; polyclonal Ig light chains
Overflow/prerenal	Increased filtration due to excess plasma levels	Ig light chains; myoglobin; hemoglobin; lysozyme
Postrenal	Increased excretion	α -2-macroglobulin

Table 10-1: Type of proteinuria and marker proteins in urine.

URINE ALBUMIN (MICROALBUMINURIA)

The excretion of small amounts of albumin in the early stages of renal disease is important clinically, and with the advent of sensitive immunoassays for urinary albumin, normal or small increases in its excretion can now be monitored. The increase in urinary albumin excretion, below 200 mg/L, has been defined as **microalbuminuria** and has been shown to precede and predict clinical proteinuria and end-stage renal failure, both in diabetics and people with hypertension.

It has also been shown that low-grade albuminuria is a very strong predictor of future cardiovascular events, including myocardial infarction.

The increased albumin excretion is of glomerular origin and can be reversed in its early stages, when the treatment of the underlying disease is optimized, with subsequent improvement of kidney function using several medical measures and normalization of the albuminuria, at least for a certain time.

Microalbuminuria is defined as the condition in which albumin is excreted (24-hour urine or timed day collection) at a rate between 20 and 200 μ g/min, or 30–300 mg/24 hours. A normal albumin excretion rate (AER) should amount to 2–20 μ g/min, or 3–30 mg/24 hours, while a clinical albuminuria or macroalbuminuria is present at an excretion rate above 200 μ g/min (approximately 300 mg/L), or > 300 mg/24 hours. Urinary albumin excretion has a high intraindividual variability, even when individuals refrain from physical exercise on the day before the collection of urine and when overnight collections are used. Therefore, multiple measurements are usually required to make a diagnosis, and repeated collections over time are required to establish the progression of albumin excretion. A result is considered to be diagnostic when two out of three urine specimens are positive within a 3-month period.

In current practice, it is more practical to test early morning urine samples and measure the albumin/creatinine ratio (ACR). Microalbuminuria is considered to be present when the ratio of albumin/creatinine in urine reaches a level greater than 30 mg/g (> 3.0 mg/mmol creatinine). This method may theoretically avoid falsely negative results due to polyuria and spurious reductions in urinary albumin concentration.

The screening of diabetic patients for renal disease is recommended at least once a year by measuring the albumin/creatinine ratio in an early morning urine sample (or a random urine sample). A positive result should be confirmed by repeat testing of at least one of two more samples in a 6-month period.

The detection of microalbuminuria is clinically relevant not only for diabetic nephropathy but also as a marker for:

- Increased risk for cardiovascular disease.
- Increased risk of early mortality in patients with acute myocardial infarction.
- Control of gestational diabetes (for the follow-up of diabetic women and their neonates).

- Control of pregnancy complications (e.g., preeclampsia).
- Early detection of a glomerular proteinuria of other origins (e.g., hereditary) or associated with vascular diseases or infectious diseases.
- Early detection of kidney dysfunction in hypertensive patients.

Reference ranges: Albumin excretion rate in urine: < 20–200 $\mu\text{g}/\text{min}$ or < 30–300 mg/24 h; Albumin concentration (early morning urine): < 30–300 mg/L; Albumin/creatinine ratio in urine: < 30–300 mg/g; < 3.0 g/mol creatinine

MARKERS OF TUBULAR NEPHROPATHIES

Alpha-1-microglobulin

The measurement of alpha-1-microglobulin (α1M , protein HC) (molecular mass, 33 kDa) in urine is a useful laboratory test for the early diagnosis of renal tubular disorders and for monitoring the reabsorptive function of the tubules. The reasons for this are its relatively high concentration in urine (5–12 mg/L) and its stability, even at low urine pH. Furthermore, the concentration of α1M in the urine shows a low biological variance because its serum levels are less subject to external changes compared with other low molecular weight proteins such as β2M or RBP. Slight variations in α1M due to circadian rhythm can be eliminated by relating its urinary excretion to urine creatinine. Alpha-1-microglobulin in serum forms complexes with antitrypsin and IgA.

Reference ranges: Urine: second morning urine: < 14 mg/g creatinine or < 1.58 g/mol creatinine; 24-hour urine: < 12 mg/L or < 20 mg/24 h; Serum: < 10 mg/L

Beta 2-microglobulin (in serum and urine)

Beta 2-microglobulin (β2M ; molecular mass, 11.8 kDa) is a polypeptide, which is a component of the HLA proteins and, as such, is present on the surface of all nucleated cells. In body fluids, it occurs in both the free form and bound as HLA antigen, although the HLA proportion is very low. β2M is rapidly eliminated from the circulation exclusively via the kidneys (half-life, 40 minutes), and 99.8% is reabsorbed in the proximal tubule and catabolized to amino acids.

β2M can be used to assess glomerular filtration rate, but nowadays cystatin C is preferred. β2M is also used to monitor the renal tubular function, especially the tubulotoxic effects of various substances, such as the heavy metals cadmium and lead, and as a screening test for the early identification of Balkan nephritis in regions in which it is endemic. However, β2M in urine has the disadvantage of being unstable below pH 6. Therefore, it is recommended to administer bicarbonate to the subjects before urine collection in order to properly alkalinize the urine. Unfortunately, these recommendations are rarely followed. Furthermore, β2M is a less reliable urine marker than other LMW proteins, like α1M . This is due to the fact that various diseases, such as multiple myeloma, Waldenström's macroglobulinemia, B-cell lymphomas, primary systemic amyloidosis and HIV infection raise the β2M serum level, which in turn increases renal elimination. Nevertheless, β2M measurement in serum is a useful test to monitor kidney transplant patients, as increased β2M levels may give an early indication of transplant rejection. Additionally, β2M is also a very useful marker to monitor people with lymphoproliferative malignancies. In fact, an increase in β2M in people with myeloma indicates an adverse prognosis and a more fulminant disease.

Reference ranges: Serum: Age-dependent: < 60 years: 0.8–2.4 mg/L; > 60 years: 1.1–3.0 mg/L; Urine: 0.004–0.3 mg/L (median 0.08 mg/L); 30–360 mg/24 h; < 0.20 mg/g creatinine

RETINOL-BINDING PROTEIN (IN URINE)

Retinol-binding protein (RBP) is a small polypeptide (21 kDa), which is largely bound to prealbumin in serum and serves as a transport protein for vitamin A. Its serum level depends on various conditions, including protein intake, liver disease and acute-phase reaction. In patients with tubular nephropathies, the RBP concentration in urine increases more so than other urinary proteins, but the clinical sensitivity for tubular disease is similar to that of other tubular markers.

Reference range: Urine: < 0.5 mg/L

CYSTATIN C (IN SERUM)

Cystatin C (previously called post-gamma globulin or gamma-trace protein) has a low molecular mass of 13.3 kDa. Its characteristics, which include a constant rate of endogenous production in all nucleated cells, free filtration by the glomerulus, no secretion but reabsorption by the renal tubules, and no extrarenal excretion, make it a very useful marker of GFR and renal function. In patients with impaired GFR, the serum level of cystatin C increases. Its concentration in serum is totally independent of inflammation, and cystatin C exhibits no circadian variation. Elevation of cystatin C is, however, reported in patients with malignant disease without renal impairment, probably secondary to the increased number of cells. It has also been shown that the cystatin C level in serum is increased in hyperthyroidism and decreased in hypothyroidism. Oral or intravenous treatment with high doses of corticosteroids also increases the cystatin C concentration in serum.

A positive correlation between serum cystatin C and creatinine levels has been demonstrated in patients with renal disease. Cystatin C has advantages over the standard serum creatinine test and other protein markers because it is not influenced by muscle mass, diet or acute inflammatory processes. For these reasons, cystatin C is a much earlier indicator of decreased GFR compared to serum creatinine, which is not increased until after the GFR has fallen by 50%, or below 60 mL/min/1.73 m². Quite the reverse, cystatin C-based formulae for estimation of GFR (eGFR) can be used to monitor accurately the renal function from within the reference range and down to less than 4 mL/min/1.73 m². As already reported, the Modification of Diet in Renal Disease (MDRD) study equation can be only used to estimate GFR when the GFR is below 60 mL/min/1.73 m².

Cystatin C provides a good means of evaluating renal function in pediatric patients because levels reach adult values slightly before 1 year of age. Creatinine, in contrast, does not reach stable adult levels until after puberty because of increasing muscle mass, and this contributes to a decrease in its sensitivity as a GFR marker. In addition, creatinine overestimates GFR in patients with muscle wasting, in paraplegic patients, in patients with ascites (which increases body weight) or edema, and in those who have difficulty in converting creatine to creatinine. Furthermore, creatinine and MDRD underestimate GFR in bodybuilders with severely increased muscle mass. It has been suggested that estimating GFR using both creatinine and cystatin C may provide additional information to define a patient's clinical condition. Equations for eGFR using both analytes are readily available, and the calculations can be automatically performed.

Assays for cystatin C are not influenced by analytical interferences like bilirubin and hemoglobin, as is creatinine, which is an advantage in icteric samples from jaundiced neonates and *in vitro* hemolysis occurring during pediatric sample collection. A new cystatin C Certified Reference Material, ERM-DA471/IFCC, is now internationally available for the standardization of cystatin C assays. Other work is in progress with the contribution of the major diagnostic companies for harmonization of commercial cystatin C assays, aiming to develop one eGFR equation that can be applied overall when using cystatin C as a marker for GFR.

Reference ranges: Male: < 50 years: 0.3–0.8 mg/L; Male: > 50 years: 0.4–1.0 mg/L; Female: 0.4–1.0 mg/L

SECTION 11

PULMONARY DISEASES AND ALLERGIC DISEASES

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 List the most important pulmonary diseases and their causes.
- 2 Explain the importance of diagnosing an Alpha-1 Antitrypsin deficiency to decrease the development of pulmonary emphysema.
- 3 Describe the relationship between elevated IgE levels and atopic diseases.

PULMONARY DISEASES

Chronic obstructive pulmonary disease (COPD) is broadly defined and includes several clinical and pathologic entities, primarily chronic bronchitis and emphysema. It is characterized by airflow obstruction that is chronic, progressive and not fully reversible. It is associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking.

When COPD is associated with infection and there is an acute-phase reaction, CRP and C4 values are typically elevated. In COPD due to chronic bronchitis, decreased C4 values correlate with the degree of emphysema. Furthermore, in COPD, an inverse correlation between total IgE and lung function is observed.

Chronic bronchitis is a chronic inflammation of the bronchi in the lungs, defined clinically as chronic persistent cough with production of bronchial secretions, enough to cause expectoration, duration of which is 3 months in each of 2 successive years after exclusion of other causes.

Emphysema is a long-term progressive and obstructive disease of the lungs that is defined pathologically as a permanent destructive enlargement of the air spaces distal to the terminal bronchioles without obvious fibrosis. It causes primarily shortness of breath due to overinflation of the alveoli.

AAT deficiency can raise the risk of developing a pulmonary emphysema in young people and adults in their 40s and 50s, especially if they smoke.

The emphysema is caused by activation of macrophages and granulocytes in the pulmonary tree and thus by release of proteolytic enzymes, especially elastase, which, in the case of AAT deficiency, is not inhibited by AAT. The elastase then leads to a degradation of the elastic tissue in the lungs. If a subject presenting AAT deficiency is a smoker, then certain substances deriving from the smoke activate the macrophages in the lungs and simultaneously destroy the protease-inhibiting activity of AAT. To decrease the development of emphysema, it is important to diagnose AAT deficiency at an early age. Therefore, AAT testing should be performed in young patients (4th or 5th grade) with a strong family history who can develop COPD. A family screening is also recommended. Such subjects should avoid working in dusty environments, and they need pulmonary protection by early and adequate antibiotics treatment of infections in the respiratory tract and in the lungs.

In homozygous AAT deficiency, adult smokers develop dyspnea or emphysema on an average 20 years earlier than nonsmokers.

Asthma is a chronic inflammatory disorder of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, repeated episodes of wheezing and breathlessness, chest tightness, and coughing. It differs from COPD in its pathogenic and therapeutic response. In asthma, IgG, IgM, total IgE as

well as haptoglobin and C3, are elevated, and in asthma caused by allergy, allergen-specific IgE is increased.

In asthma, inflammation in all airways is involved but usually without involvement of the lung parenchyma, whereas COPD shows a predominant involvement of peripheral airways (bronchioles) and lung parenchyma.

LUNG CANCER

In industrialized countries, lung cancer is the most common cancer in men and is rapidly approaching the same incidence in women. Lung cancer represents the prototype of a tumor induced by chemical carcinogens, especially those inhaled by cigarette smoking.

CRP is often increased and albumin decreased with lung cancer, and ferritin may be elevated due to inflammation caused by the malignancy.

ALLERGIC DISEASES

Allergy denotes specific diseases caused by a reaction of the immune system to environmental antigens, which induces a hypersensitivity reaction characterized by tissue inflammation and organ dysfunction. The clinical features of each allergic disease reflect the immunologically induced inflammatory response in the organ or tissue involved and are usually independent of the antigen properties. The diversity of allergic responses arises from the involvement of different immunologic effector pathways, each of which generates a unique pattern of inflammation.

Allergy can be either antibody- or cell-mediated. The antibody accountable for an allergic reaction belongs usually to the IgE isotype, and the affected subjects may be referred to as having an IgE-mediated allergy, whereas in non-IgE-mediated allergy, the responsible antibody can belong to the IgG isotype.

DIAGNOSIS OF ALLERGIES AND LABORATORY TESTING

The diagnosis of allergy is often thought to be difficult, but in the majority of cases, a thorough clinical history, combined with relatively simple investigations, will prove adequate. The history is an important component, and one typically looks for allergic symptoms occurring consistently after exposure to the same agent and improvement of symptoms during periods of avoidance. Where doubt remains, further investigation by skin-prick testing and/or the detection of allergen-specific IgE is necessary.

IMMUNOGLOBULIN E (IgE)

Immunoglobulin E (IgE) (reaginic, skin-sensitizing or anaphylactic antibody) is found primarily in respiratory and gastrointestinal mucous secretions.

IgE levels are elevated in atopic diseases (e.g., allergic or extrinsic asthma, hay fever, atopic dermatitis, parasitic diseases, advanced Hodgkin's disease, IgE-monoclonal myeloma).

The IgE serum concentration in a patient is dependent on both the extent of the allergic reaction and the number of different allergens to which he is sensitized. Nonallergic healthy individuals have IgE concentrations that vary widely and increase steadily during childhood, reaching their highest levels at ages 15–20 years and thereafter remaining constant until about age 60, when they slowly decline.

Reference range: Healthy adults: < 100 IU/mL (kU/L) (< 0.3 mg/L)

SECTION 12

PLASMA/SERUM PROTEIN PROFILES AND DISEASE MANAGEMENT USING SERUM PROTEIN TESTING

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 List some useful plasma/serum protein profiles.
- 2 Give examples of diagnostic strategies for disease management using selected serum proteins.
- 3 Specify which proteins can indicate the presence of an inflammatory (acute-phase) reaction.
- 4 Describe which serum protein changes/deficiencies point out an increased susceptibility for infectious diseases.

SOME USEFUL PLASMA/SERUM PROTEIN PROFILES

Profile	PAL	RBP	ALB	TF	AT	AAG	HP	FIB	CRP	C3, C4	GAM	CP
Complete protein profile	(X)		X		X	X	X	X	X	(X)	X	
Single inflammation marker								(X)	X			
Inflammation (restricted profile)						(X)		X	X			
Inflammation (extended profile)	(X)		X			X	X	X	X			
Infection	(X)		X			X		(X)	X	(X)	X	
Nutritional	X	(X)	X	X		(X)		(X)	X			
Complement consumption						X		(X)	X	X		
Hemolysis						X	X			X		
Anemia				X		X	X	X		X	X	
Liver	X		X	X	X	X	X			(X)	X	X
Vascular								X	X	(X)		
Preeclampsia/eclampsia								X	(X)	(X)		
Immunological										(X)	X	
Infection susceptibility					X	X			X	X	X	
Myeloma/lymphoma			X			X	X		X		X	
Autoimmune diseases			X			X		X	X	X	X	

Table 12-1: Protein associated with various disorders.

EXAMPLES OF DIAGNOSTIC STRATEGIES FOR DISEASE MANAGEMENT USING SELECTED PROTEINS

INTERPRETATION OF A PLASMA/SERUM PROTEIN PROFILE

Increase in protein turnover vs. decrease in protein synthesis

An increased protein turnover can be seen in people with long-standing, ongoing inflammatory diseases, malnutrition or proteinuria and in people with severe leakage of proteins to the intestine, to ascitic fluid or through the skin. Patients with inflammatory diseases or malnutrition especially have decreased synthesis of some proteins, such as albumin, prealbumin and transferrin. Of these proteins, prealbumin decreases and increases faster than the other proteins with a half-life of 24 hours and a rapid (within days) response to therapy. In patients with increased protein loss, the various proteins are lost in relation to their respective molecular mass, shape and electrical net charge. Low serum concentration of albumin and prealbumin in the absence of an acute or chronic inflammatory process and in the absence of increased protein loss indicates decreased production capacity of proteins in the liver, as it is seen in liver disease, eventually with progressive liver cirrhosis.

Presence of an inflammatory (acute-phase) reaction

In order to detect and evaluate an inflammatory process, the determination of proteins such as CRP, SAA, FIB and AAG is optimal. Measurement of CRP can also be performed, even through point-of-care testing, within minutes and with sufficient precision and accuracy. Measurement of a combination of certain proteins (e.g., ALB, PAL, TRF, CRP, FIB, HP) often gives detailed information about the disease process.

If it is necessary to detect or monitor an acute inflammatory disease, such as a bacterial infection (e.g., in appendicitis; **Figure 12-1**), CRP testing is sufficient, and the addition of ESR and white blood cell count does not add any valuable information in most situations.

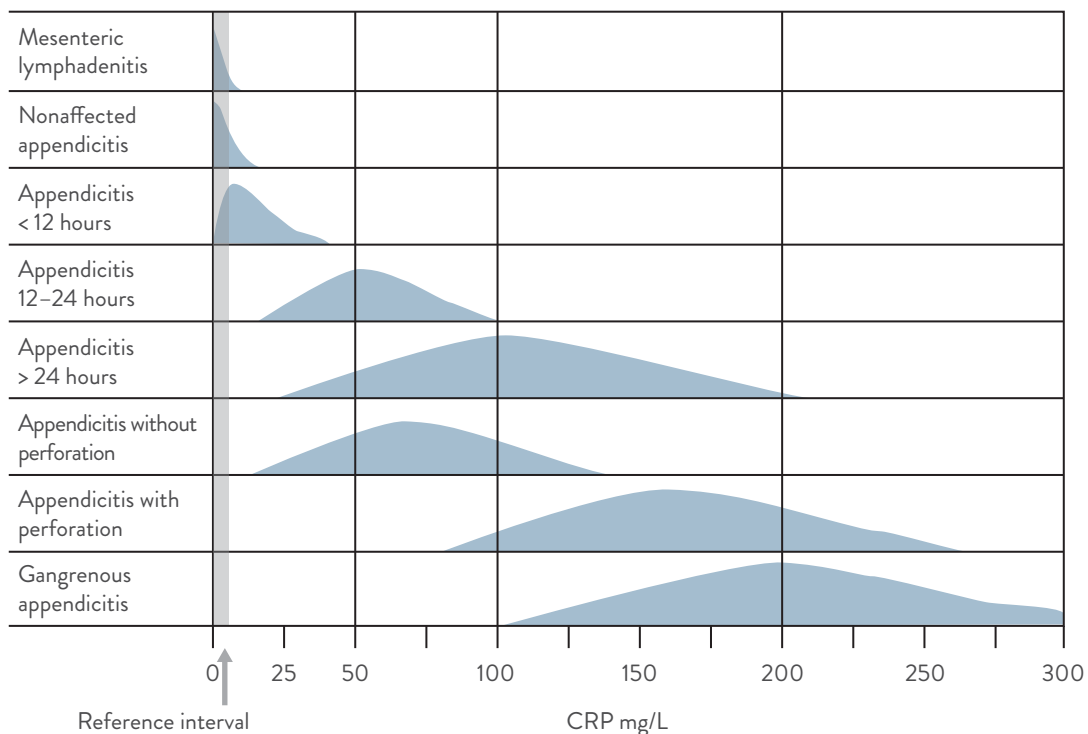


Figure 12-1: CRP in various types of appendicitis.

Increased erythrocyte turnover (hemolysis)

A low serum concentration of HP is regarded to be a marker of increased erythrocyte turnover (hemolysis). This is based on the HP function, which is to bind and eliminate hemoglobin (Hb) released from the erythrocytes. The half-life of the HP-Hb complex is much shorter than the half-life for Hp, 0.5 hours compared to 3–5 days. HP can bind one-half hemoglobin (α - β) molecule per HP- β -chain. The formed HP-Hb complex is eliminated by the Kupffer cells in the liver. This results in a decrease of the HP serum concentration in relation to the degree of hemolysis.

About 5%–10% of the newly produced erythrocytes are defective and are destroyed in the bone marrow, so-called ineffective erythropoiesis. This hemolysis is responsible for approximately half of the normal daily production of HP. If ineffective erythropoiesis is doubled in people without an inflammatory process, the HP concentration in serum will decrease toward 0 g/L. To be able to draw conclusions about the erythrocyte turnover from HP measurements, the result should be evaluated after having tested at least one of the other APPs in serum.

Two problems concerning the interpretation of haptoglobin results are that HP serum levels are type-dependent, with the lowest level for the phenotype HP 2-2 and the highest level for the HP 1-1 phenotype, and that the serum HP concentration is age-dependent, with very low levels at the ages of 5–18 years.

Increased immunoglobulin synthesis of mono-, oligo- or polyclonal nature

The concentration of IgG, IgA or IgM does not increase more than up to 150%–200% of the respective mean level during the majority of infectious diseases. However, some autoimmune diseases and some viral infections can cause the immunoglobulins to increase up to 200%–400%. Selective IgA increase is seen in people with respiratory tract infections, pneumonia, other lung diseases, joint diseases (RA), some inflammatory bowel disease and alcohol-induced liver diseases, such as fatty liver or cirrhosis. Selective increase of IgM is typical, especially for viral infections (e.g., hepatitis, EBV, CMV), malaria and trypanosomiasis infections and in people with biliary liver diseases, including primary biliary cirrhosis, where extremely high serum levels of IgM can be seen. Selective increase in IgG concentration is seen in SLE and chronic active hepatitis.

Changes indicating increased susceptibility for infectious diseases

Increased susceptibility for infections can either be inherited or acquired. This susceptibility can be due to functional defects or deficiency of cellular or plasma factors. The most important plasma protein deficiencies related to increased susceptibility for infections are hypo- or agamma-globulinemia of one or several of the immunoglobulins, complement factor deficiency, and AAT deficiency (secondary to emphysema).

The most common immunoglobulin deficiency is selective IgA deficiency. Besides hereditary immunoglobulin deficiencies, a deficiency of or suppressed immunoglobulin level can be secondary to a lymphoproliferative malignancy. It is therefore important to search for changes in immunoglobulin level, M-components or light chain disease (i.e., Bence-Jones myeloma [Bence-Jones proteinuria]). Treatment with corticosteroids often results in reduction of immunoglobulin levels.

Complement deficiencies are rare but important causes of increased susceptibility for severe infections. Complement deficiencies seem to be related to increased prevalence of certain types of bacterial infections and also an increased prevalence of autoimmune diseases, such as SLE.

Liver pattern

It is known that liver diseases often, but not always, present with a typical serum protein pattern, which can give valuable clinical information. This typical liver pattern includes a relative increase of AAT and FIB while the HP level is decreased in relation to other APPs. ALB, TRF and PAL are often very low in patients with liver diseases, especially in severe cases. These changes can be seen in people with different types of both hepatitis and cirrhosis. In people with liver diseases, these protein changes are often combined with changes in the serum levels of IgG, IgA and IgM (**Figure 12-2**). An increased IgA serum concentration, together with a typical liver pattern APPs and decreased concentrations of ALB, TRF and PAL, can often be seen in patients with fatty liver or liver cirrhosis induced by long-standing alcohol abuse (or overweight). An increased concentration of IgM in serum is common in people with biliary liver diseases and viral hepatitis. Solitary increased serum concentration of IgG, together with decreased concentration of ALB, TRF and PAL and typical APPs liver pattern, are common in patients with chronic active hepatitis or lupoid hepatitis.

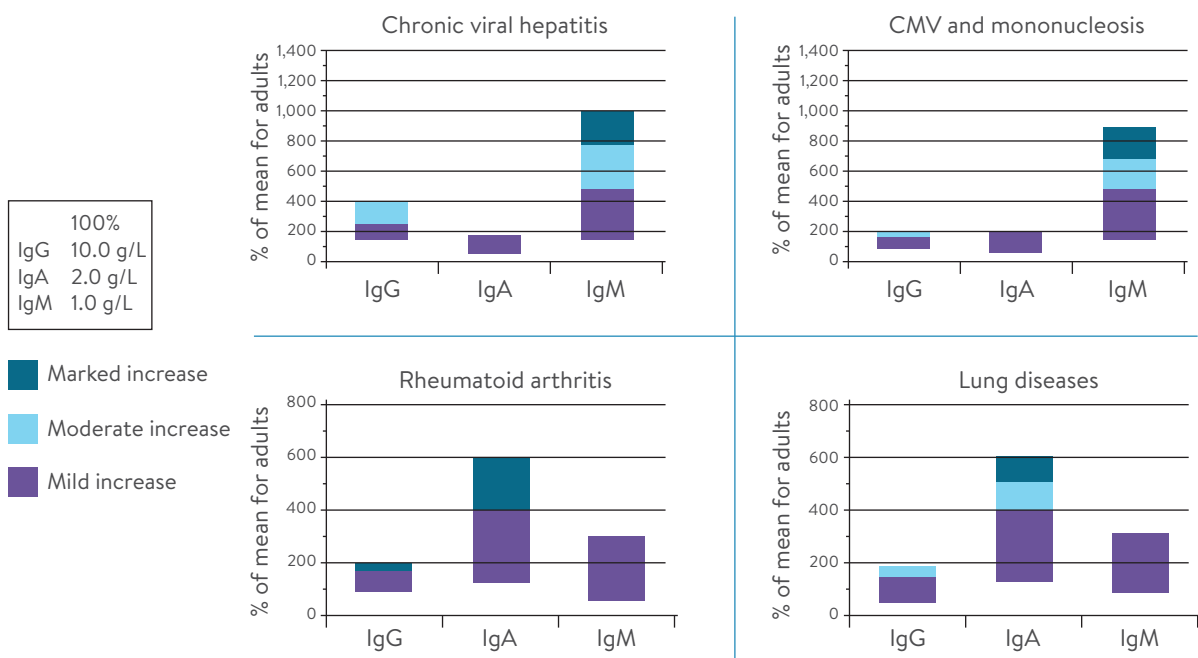


Figure 12-2: Immunoglobulin patterns in various diseases.

Estrogens have an influence on the APPs pattern in a similar way as liver diseases, except that the HP level is not influenced by estrogens. It may be that the APPs liver pattern is partly caused by an altered estrogen metabolism in the liver in the course of liver diseases and partly by a decreased HP synthesis or an increased HP elimination secondary to increased erythrocyte turnover.

Increased estrogen effect on some of the plasma proteins is seen during pregnancy, estrogen or antiestrogen treatment.

Vascular and connective tissue pattern

Inflammatory diseases in connective tissue or involving the vascular system often have very high serum levels of FIB and HP, especially in relation to AAT. This pattern is often seen in people with active RA, temporal arteritis, vasculitis, polymyalgia rheumatica and certain malignant diseases (e.g., hypernephroma, malignant melanoma).

REVIEW QUESTIONS: SECTIONS 5–12

Answers are provided at the end of this Learning Guide.

1. Which of the following is associated with increased cardiovascular risk?
 - A Elevated serum Apo A-1 and Apo B
 - B Decreased serum Apo A-1 and Apo B
 - C Decreased serum Apo A-1 and increased Apo B

2. Which of the following occurs when the immune system mistakenly attacks the body's own tissues?
 - A Autoimmunity
 - B Allergy
 - C AIDS
 - D Immunity

3. Which of the following is an autoimmune disease that occurs when the immune system attacks the body's own tissues?
 - A Stroke
 - B Diabetes
 - C Cancer
 - D Glandular fever

4. A characteristic of IgE is:
 - A Responsible for the symptoms of allergy
 - B Exists as monomer and dimer in plasma
 - C Four subclasses
 - D Pentameric structure

5. Which is the best method to monitor diabetic glucose control over an 8- to 12-week period?
 - A Glucose
 - B Glycated hemoglobin
 - C Haptoglobin
 - D Prealbumin

6. When measuring CRP as an indirect risk factor for CVD, what does an elevated CRP serum level represent? (Choose all that apply.)
- A The severity of the inflammatory response in atherosclerotic vessels
 - B The extent of inflammation related to myocardial ischemia
 - C The amount of lipids in blood
 - D The activity of the coagulation system
7. Which is an immunologic feature of rheumatoid arthritis?
- A Presence of IgE rheumatoid factors in serum
 - B Increase of the serum IgD concentration
 - C Presence of anti-CCP antibodies in serum
 - D Decreased cystatin C in synovial fluid
8. Which laboratory findings suggest the presence of multiple myeloma?
- A M-component of IgG concentration > 2,000 mg/dL or IgA concentration > 1,000 mg/dL in serum
 - B Immunoglobulin fragments in urine (Bence-Jones protein) or serum
 - C Increase of serum beta 2-microglobulin over 6 mg/L
 - D All of the above
9. Which protein is useful when measured in the urine to indicate the presence of a proteinuria?
- A C-reactive protein
 - B IgM
 - C Albumin
 - D Lipoprotein(a)
 - E None of the above
10. The deficiency of which protein can cause severe pulmonary emphysema?
- A IgM
 - B α 1-antitrypsin
 - C Complement C3
 - D Transferrin
 - E None of the above

SECTION 13

METHODS OF PROTEIN
MEASUREMENTS,
STANDARDIZATION AND
FACTORS AFFECTING TEST
RESULTS

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LEARNING OBJECTIVES

After completing this section, you will be able to:

- 1 List and describe different technologies used to identify and quantify serum proteins.
- 2 Describe the differences between immunonephelometry and immunoturbidimetry.
- 3 Explain antigen excess and how it can affect patient results.
- 4 Identify important steps for establishing common reference systems.
- 5 Explain how the metrological traceability chain can ensure traceability of results.
- 6 Describe preanalytical factors that affect serum protein concentrations.
- 7 Indicate how to store the collected blood samples prior to assay.
- 8 List different analytical considerations specific to protein analysis.

PRINCIPLE AND METHODS OF PROTEIN MEASUREMENTS

Protein measuring techniques

With the application of modern separation techniques, an increasing number of different proteins has been recognized. The potential number of proteins in body fluids and cellular elements is at least 1,000 and possibly more than 10,000. Protein methodology has evolved over time and resulted in new diagnostic applications (**Table 13-1**). Clinically useful techniques for the determination of the total serum protein concentration were introduced by modification of Kjeldahl's assay for nitrogen. Howe's precipitation technique, established in 1921, and its modifications allowed the salting out of several protein fractions and facilitated the systematic study of serum proteins. By employing various concentrations of sodium sulfate, three fractions were identified: albumin, euglobulin (the globulin that precipitates out in distilled water) and pseudoglobulin. The two globulin fractions identified by this technique have been used in the clinical laboratory for the so-called euglobulin lysis test. The albumin/globulin (A/G) ratio was also based on this precipitation technique.

By reactivity	Biuret reaction, Lowry method
By chemical properties	Absorption at $\lambda = 260$ nm (Phe) or 280 nm (Tyr, Trp)
By physical properties	Charge: electrophoresis
By activity	Enzymatic reactions
By immunogenicity	Immunochemical methods + different signal generators
By a combination of two of the above-reported methods	For example, immunoelectrophoresis and electroimmunoassay

Table 13-1: Different methods for measuring/identifying proteins.

IMMUNOCHEMICAL METHODS – IMMUNOASSAYS

Immunoassay is now the fastest-growing analytical technology in use for the detection and quantification of biomolecules used in the diagnosis and management of disease. Nowadays, as part of the daily routine, the clinical laboratory performs a wide range of immunoassays for measurement of specific proteins. Certain labor-intensive quantitative immunochemical gel precipitation techniques, such as the single radial immunodiffusion (RID), are still employed in some small laboratories or for rare proteins. Such techniques have been completely replaced by faster, highly automated immunochemical assays that take place in liquid phase and are based on optical detection of immunoprecipitates (**Figure 13-1**).

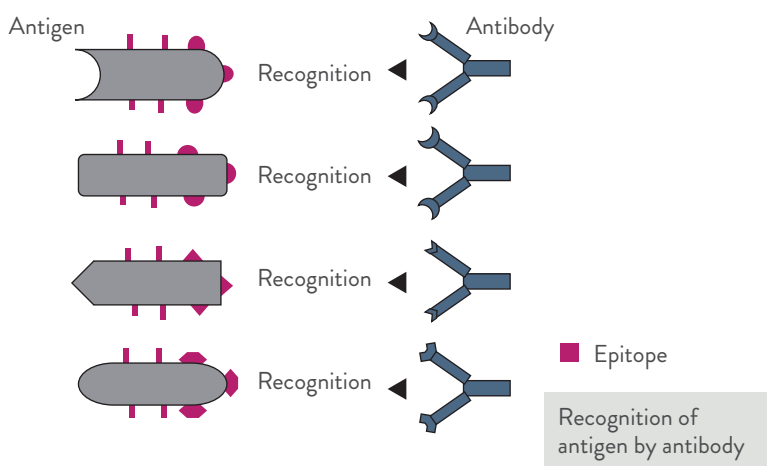


Figure 13-1: Specificity of antibody binding to target epitopes enables immunoprecipitate formation with antigens.

Immunochemical reactions form the basis of a diverse range of sensitive and specific clinical assays. In a typical immunoassay, the molecule of interest, the **antigen** (Ag), is detected by means of a complementary molecule, the **antibody** (Ab), which is used as the reagent. The high specificity and affinity of antibodies for specific antigens, coupled with the unique ability of antibodies to cross-link antigens, allows the identification and quantitation of specific substances by a variety of immunochemical methods. The antigen may be a small molecule (a hapten) or a large protein. However, only one portion of the antigen, the **epitope**, binds to the **paratope** of the antibody. A small antigen may contain several epitopes but only be capable of binding one antibody molecule, the remaining surface of the molecule being unavailable for binding because of steric hindrance from the first antibody molecule. By comparison, a large biomolecule may contain many independent epitopes and often copies of the same epitope, thus enabling it to bind to several antibody molecules simultaneously.

PRINCIPLE OF IMMUNOCHEMICAL MEASUREMENT OF PROTEINS

Reaction between antigen and antibody

In an immunochemical analysis, either the antibody is used as a reagent to detect the antigen of interest or the reagent is an antigen used for identification and quantitation of a specific antibody. A protein antigen can be considered as polyvalent, with possible multiple copies of the same epitope, as well as different and distinct epitopes.

The binding of such a protein antigen to its corresponding antibody in an immunochemical assay is an equilibrium reaction, which occurs in a series of different steps, as follows:

1. In the initial reaction, Ag and Ab bind rapidly.
2. An immune complex Ag-Ab forms and slowly grows.
3. The number of antigen and antibody molecules increases in the immune complex, which finally precipitates once a critical size is reached.

HEIDELBERGER AND KENDALL IMMUNOPRECIPITIN REACTION

Michael Heidelberger and Forrest Kendall first described the immunoprecipitin reaction in quantitative terms in 1935. The general form of the relationship between antigen concentration and antibody precipitated is shown in **Figure 13-2**. This is often referred to as the Heidelberger-Kendall curve and is divided into three distinct zones, as follows:

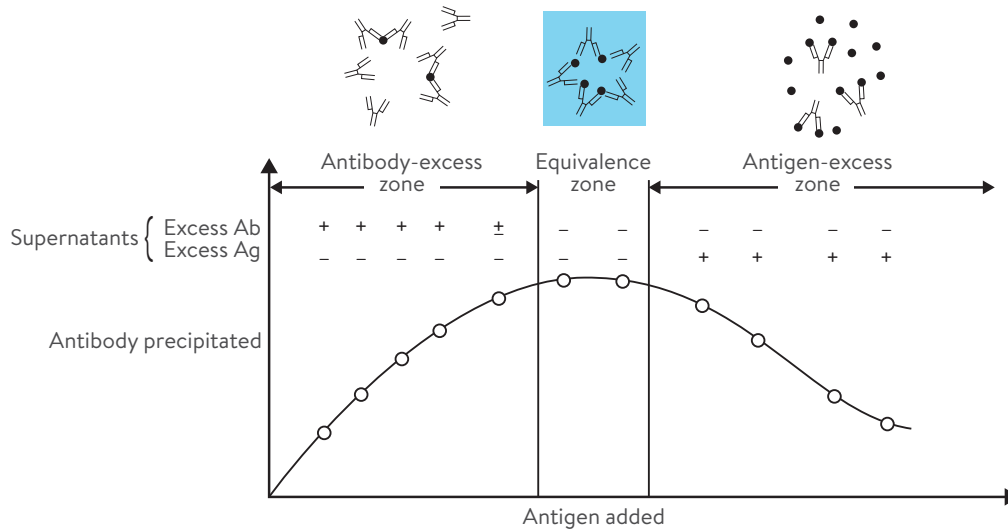


Figure 13-2: Heidelberger and Kendall immunoprecipitin curve.

Antibody excess (prozone or prezone) is the first zone (A), in which a low Ag/Ab ratio exists. As the antigen concentration increases, small and soluble immune complexes are formed. Their concentration is measured as an absorption signal (immunoturbidimetry) or by a scattered light signal (immunonephelometry) and is proportional to the antigen concentration.

Equivalence zone is the second zone (B), in which there is an optimal ratio of antibody bridging to antigen concentration. As more antigen molecules bind to the antibody, the point of maximum precipitate formation is reached. Little or no free antigen or free antibodies are present in solution.

Antigen excess (postzone) is the third zone (C), in which the Ag/Ab ratio increases and there is an increased amount of free antigen and a reduced amount of antibody. Because of the high antigen concentration, the formation of small, soluble immune complexes is favored over the larger precipitate. The small complexes are not large enough to scatter light, and the apparent light-scattering signal is less than at the point of equivalence.

An important consequence of these observations is that there are two possible antigen concentrations, which will generate the same apparent signal/concentration reading: one in the antibody excess zone and the other in the antigen excess zone. Sample dilution ranges in these immunoassays are selected to obtain a signal that is proportional to the antigen concentration in the antibody excess zone (i.e., slope A of the Heidelberger-Kendall curve). In all immunoprecipitation methods in which an antigen in excess migrates into an antibody-containing gel, the systems are adjusted in such a way that the ratio between antigen and antibody is equal, thus allowing the tests to stay within the equivalence zone. The antigen-antibody complexes migrate until they reach a size where they can no longer stay in solution in the gel, thus forming an immunoprecipitate.

Polyclonal and monoclonal antibodies

Historically, most immunoassays have been developed by using heterogeneous mixtures of antibodies obtained from the serum of animals (usually rabbits, goats, sheep, horses or, nowadays, hens) immunized with the antigen of interest.

A polyclonal antibody is produced by many cell clones in a foreign (usually animal) host in response to the administration of a defined antigen/immunogen (a protein or a substance coupled to a carrier), which elicits antibody formation. The polyclonal antiserum is a heterogeneous mixture of antibodies obtained from the serum of the animal. These polyclonal antibodies can differ from each other in terms of their specificity, avidity, affinity and binding kinetics to different epitopes on the antigen. The avidity of polyclonal antibodies is difficult to determine, due to the diversity of the antibody population.

A monoclonal antibody (Mab/mab) is in contrast to the product of a single clone or plasma cell line in culture, and Mabs were first prepared in 1975 by Georges Köhler and César Milstein. Mabs are produced by removing cells from the spleens of animals (usually mice or rats) immunized with antigen, fusing these cells with a myeloma cell line and growing the resulting hybridoma cells in culture. The hybridoma cells are usually screened repeatedly to select those cells producing the desired antibody; then the selected cells are injected into the peritoneal cavities of other mice to produce larger quantities of the specific antibody. Because only one cell line is used to produce the desired antibody, in general, monoclonal antibodies have inherently more uniform properties of affinity and antigen recognition than polyclonal antibodies, which are produced from a combination of cell lines. In addition, Mabs-producing cells can be frozen for long-term storage, reducing lot-to-lot variations. The unique ability of a monoclonal antibody to react with a single epitope on polyvalent antigen means that the majority of monoclonal antibodies will not cross-link and precipitate macromolecular antigens. The use of a monoclonal antibody in an immunoaggregation reaction is more dependent on the frequency of the complementary epitope on the antigen. By the appropriate choice of a mixture of monoclonal antibodies, it is possible to adjust the apparent affinity/polyvalency, in order to obtain conditions such that the mixed monoclonal antibodies can have an equivalent behavior to that of a polyclonal antiserum.

IMMUNOASSAY METHODOLOGY

Competitive and noncompetitive immunoassays

The amount of antigen is inversely or directly proportional to the amount of signal. Noncompetitive assay format is referred to as a “sandwich” assay because the analyte is bound (sandwiched) between two highly specific antibody reagents.

Homogeneous and heterogeneous immunoassays

The homogeneous assays do not require the separation of unbound complexes from bound complexes. The heterogeneous assays require the separation of unbound complexes, often utilizing a solid-phase reagent, such as a plastic tube, plastic bead or magnetic particle.

Homogeneous immunoassays

The first practical homogeneous immunoassay was based on latex agglutination. Micrometer-size polystyrene latex particles coated with adsorbed immunoglobulin were used and shown to quickly agglutinate with rheumatoid factor. Latex agglutination assays have since been developed with both antibody- and antigen-coated particles and are used to measure a wide variety of clinically important substances.

Turbidimetry and nephelometry are techniques based on optical detection systems that measure immune complex formation when an antigen solution is mixed with a solution of the corresponding antibody.

Turbidimetry is based on the principle of measuring the intensity of transmitted light, whereas **nephelometry** is based on the principle of measuring the intensity of scattered light. The immune complex formation is dependent on a number of factors, including reagent pH, ionic strength, temperature and macromolecular precipitation enhancers (e.g., polyethylene glycol 6000 [PEG]). PEG is used to accelerate the reaction and precipitate soluble immune complexes. Polymers are added to the buffers in these assays in the form of polyethylene glycol (e.g., PEG 6000). Nonionic detergents (e.g., Tween 20) are often used in the buffer to decrease unspecific precipitations. For these types of assays, it is important to remember that when the light

is transmitted or scattered, Beer's Law (i.e., the formula that describes the relationship between concentration and absorbed light) does not apply and a standard curve is required.

Immunonephelometric assays (INA)

In INA methods, the formation of immune complexes obtained after addition of buffer, specific antiserum and specimen containing the protein of interest is measured in a nephelometric system. A beam from a light source (e.g., a laser) is directed through the cuvette in which the immunoreaction takes place and is scattered by the antigen-antibody complex in solution (i.e., the formed immune complex). The scattered light intensity, which is measured at an angle to the incident light beam, is converted into protein concentration using a standard curve. In kinetic or rate-immunonephelometric methods, changes in the scattered light are measured at short time intervals, while in an end point method, the reaction is measured over a defined period of time.

Immunoturbidimetric assays (ITA)

In ITA methods, turbidity develops when soluble immune complexes are formed by the addition of the sample containing the protein of interest to a specific antiserum and a buffer containing an accelerator, PEG. The resulting turbidity is measured in a photometric system as the change in the absorption of a light beam passing through the suspension (e.g., the increase in absorption at 334 or 340 nm within a defined period of time). ITA measurements can be made using any modern analyzer. The high quality of such photometric systems enables precise measurement of very small changes in turbidity/transmitted light against a higher background scatter. The amount of light scattered shows an inverse relationship to the wavelength of the incident light, and the optimum wavelength for turbidimetric monitoring increases with the size of the immune complex. Thus, a wavelength of 340 nm (or less) is preferred for the monitoring of protein antigen-antibody complex formation, as it more quickly detects the early stages of complex formation.

ITA measurements can be performed either by end point or kinetic procedures if an instrument is capable of gathering light-intensity data at precise time intervals after the initiation of the reaction. The major benefit of kinetic monitoring is the ability to take both reagent and sample blank readings immediately after initiation of the reaction (less than 5 seconds). A kinetic mode for reaction monitoring may involve only two data points, with the choice of read points very important, as it can influence accuracy, especially when differences in reaction kinetics between sample and calibrator exist. In general, the use of the second data collection point near the apparent end point minimizes the influence of sample-to-sample variations in reaction kinetics. An alternative approach is to continuously monitor the reaction to show that the peak rate of change of light scatter is related to antigen concentration.

Particle-enhanced immunoassays/particle-enhanced turbidimetric immunoassays (PETIA)

The benefit of a particle-enhanced agglutination test compared to a nonenhanced method is the increased sensitivity achieved by increasing the relative light-scattering signal. The reagent antigen or antibody is coupled mostly to latex microparticles (0.01–0.8 μm in diameter) in suspension that are chemically derivatized with a desired binding reactant. When an analyte is added to the coupled-particle suspension, the particles form immune complexes with the sample antibody or antigen and then agglutinate with the formation of aggregates detected by either a change in electrical resistance or in light scattering. In the case of light scattering using either turbidimetry or nephelometry, the degree of light scattering depends on the size of the particle (**Figure 13-3**).

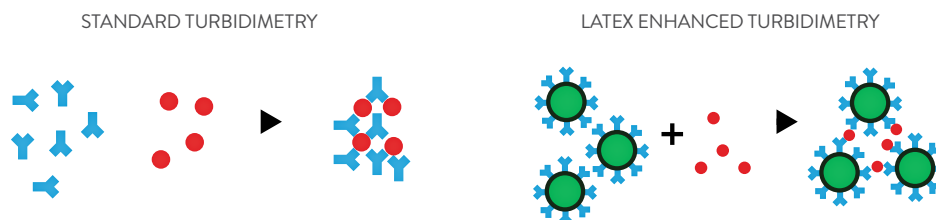


Figure 13-3: Two turbidimetry methodologies.

NEPHELOMETRY VERSUS TURBIDIMETRY

It is possible to measure the loss of light passing straight through the solution (called turbidimetry) or the increase of light reflected in a different direction (called nephelometry).

In turbidimetry, the detector is placed in a direct line with the incident light, and the light sensed by the detector decreases as the number of analyte particles increases. The amount of unaltered light is measured, as well as the amount of light directly in forward scattering. The loss of light due to scattering at angles different from 180° appears as a virtual absorbance. Turbidimetric signals are therefore measured in the same units as true absorbance measurements, although they cannot be expressed in molecular units.

In nephelometry, the detector is placed at a constant angle to the light path to avoid detection of light passing through the sample. The nephelometric detector senses light scattered by the particles. The amount of light reaching the detector increases as the number of analyte particles increases. In nephelometry, two types of instrumentation for measuring scattered light at different angles are in use today. One is a rate nephelometer, measuring at an angle of about 60° , and the other is a fixed-time nephelometer measuring at an angle of about 10° . Nephelometric signals cannot be expressed as molecular or molar constants; they are expressed as instrument readouts in millivolts or bits.

The different detector positions of turbidimetric or nephelometric design of measurement are illustrated in **Figure 13-4**.

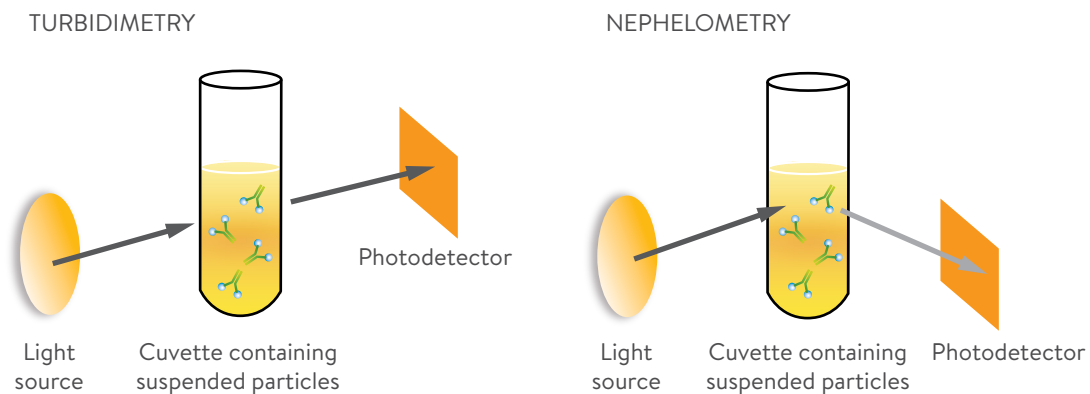


Figure 13-4: Turbidimetry and nephelometry methodologies.

Turbidimetry and nephelometry are looking at the same phenomenon with different arrangements of detectors, and there have been many discussions as to which method might be more useful or more sensitive to measure immunoprecipitation.

From theoretical considerations, nephelometric measurements have the advantage, in that they can be measured against a dark background and with very sensitive detectors like photomultipliers. While this is true for many reasons, this expensive equipment is not used in routine instrumentation.

The performance of photometers in clinical chemistry analyzers has improved considerably over the past two decades, as have software and pipetting systems. Also, due to workflow considerations, turbidimetry has become much more popular because it can be applied to routine clinical chemistry analyzers. Immunoturbidimetry also has the advantage of better precision because immunonephelometry uses higher sample dilution, which might have a negative impact on precision.

MEASUREMENT OF LOW-CONCENTRATION PROTEINS

When proteins are too low in concentration for detection by immunoprecipitation methods, other immunoassay procedures can be effective. Such approaches are frequently based on solid-phase reagent antibody bound to plastic plates or particles. After the protein antigen binds to this first antibody, a second antibody to the antigen is added with some type of label attached. The label can be a radioactive isotope, an enzyme (such as alkaline phosphatase), a fluorescent molecule (such as fluorescein) or a chemiluminescent molecule (such as dioxetane). Configurations of these assays have been widely adapted to automated instrumentation for measurement of many different proteins at relatively low concentrations in serum.

Still more innovative is the use of mass spectrometry for quantification of plasma proteins, which requires very sophisticated instrumentation and software for data analysis. This application of proteomics has the huge advantage of not requiring analyte-specific reagents, such as antibodies, and thus can be adapted to essentially any target protein.¹¹

STANDARDIZATION/HARMONIZATION OF PROTEIN ASSAYS

INTERNATIONAL STANDARDIZATION/HARMONIZATION ACTIVITIES

In order to establish internationally standardized operating systems for quality assessment in laboratory medicine, several institutions, professional societies and diagnostic companies in this field are working together to effectively promote the standardization of laboratory tests, as well as reference and control materials.

The following steps are important for establishing common reference systems and include:

- Agreement on steps for registration and approval of reference materials and reference methods.
- Exchange of information on the development of new reference materials and reference methods.
- Support of programs for the preparation of reference materials.
- Interlinking of existing national programs for establishing guidelines.
- An international database of reference materials, reference methods and laboratories that maintain the reference method procedures that is available to the clinical laboratory field.

Thus, a worldwide reference system needs to be established in collaboration with organizations producing or certifying reference materials, metrology institutes, regulatory bodies, the diagnostic industry, accredited reference laboratories and professional organizations.

International programs for standardization of various protein immunoassays are presently conducted by various organizations and professional societies.

Such activities are directed to:

- The development of optimized and standardized methods.
- The production and evaluation of appropriate reference materials.
- The establishment of appropriate reference ranges and cutoff values for specific analytes.

Traceability in laboratory medicine

Correct analytical performance of quantitative laboratory assays is fundamental for the accurate reporting of results for patient samples. An important component in the ensuring of accuracy and comparability of laboratory measurements is the metrological traceability of the measurement results to internationally recognized standards.

Traceability is defined by ISO as “the property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international, through an unbroken chain of comparisons all having stated uncertainties.”

The implementation of the European legislation for the CE marking of *in vitro* diagnostics (IVDs) — the EU IVD Directive (98/79/EC) — includes the requirement that manufacturers of IVD assays must ensure the traceability of results measured with them (i.e., that the values assigned to calibrators and/or control materials, and thus traceability of results by routine methods, must be ensured through appropriate reference measurement procedures and/or reference materials/standards of higher metrological order).

For measurement results expressed in SI units, traceability is the mechanism used to track the path of a measurement from the SI unit to the actual result, each link having a known uncertainty of measurement. The application of the traceability concept to all IVDs implies the availability of a well-designed reference system to which routine laboratory assays can be traced, as well as networks of accredited reference measurement laboratories.

It must be recognized that conventional units are commonly used in some countries to report test results. SI units are preferred, but results can be readily converted to conventional units.

Due to the lack of reference materials or methods, some analytes cannot be reported in SI units. Establishing traceability chains for these analytes is nevertheless necessary.

This implies the availability of a well-designed reference system to which routine laboratory assays can be traced. Such a reference system should consist of the following essential elements:

- Clear biochemical definition of the analyte with regard to the intended clinical use
- Reference measurement procedures that specifically measure the analyte (or quantity) in human samples
- Suitable primary and secondary matrix-based reference materials
- Networks of accredited reference measurement laboratories
- Information regarding medical traceability of the reference system
- A statement concerning the uncertainty of the measurement

The main use of reference materials is for the transfer of their accurately assigned values to master calibrators of diagnostic companies for calibration of commercially available test systems.

International standardization activities and strategies allow for a standardized or at least harmonized approach to disease management, using more reliable laboratory testing based on metrological traceability.

Traceability chain

To ensure traceability of results by routine methods, a hierarchical unbroken chain of traceable comparisons (referred to as the metrological traceability chain) has to be applied. It refers to the International System of Units (abbreviated SI from the French *Le Système International d’Unités*), which is the modern form of the metric system. The structure of such a traceability chain is presented in **Figure 13-5**.

The SI traceability chain starts at the top with the definition of the appropriate SI unit for the analyte, followed by the transfer of the SI unit via a primary reference measurement procedure and a primary calibrator. The accuracy or trueness of a value, in ISO terminology, is based on the primary reference measurement procedure and the primary reference material.

Primary reference measurement procedures represent the ultimate in accuracy and are based on measurement principles proven to be analytically specific, have a low uncertainty of measurement, and provide direct traceability to an SI unit of measurement without reference to a calibrator.

Since primary reference materials are expensive, and primary reference measurement procedures are not practical for routine use, intermediate calibrators and methods are needed to continue the transfer of accuracy to the product calibrators and, ultimately, to the patient specimens. Each transfer step increases the error or uncertainty of the final measurement. Therefore, the number of transfer steps in the traceability chain should be as few as practical.

The uncertainty associated with each step is cumulative and incremental and is shown graphically at the left side of **Figure 13-5**.

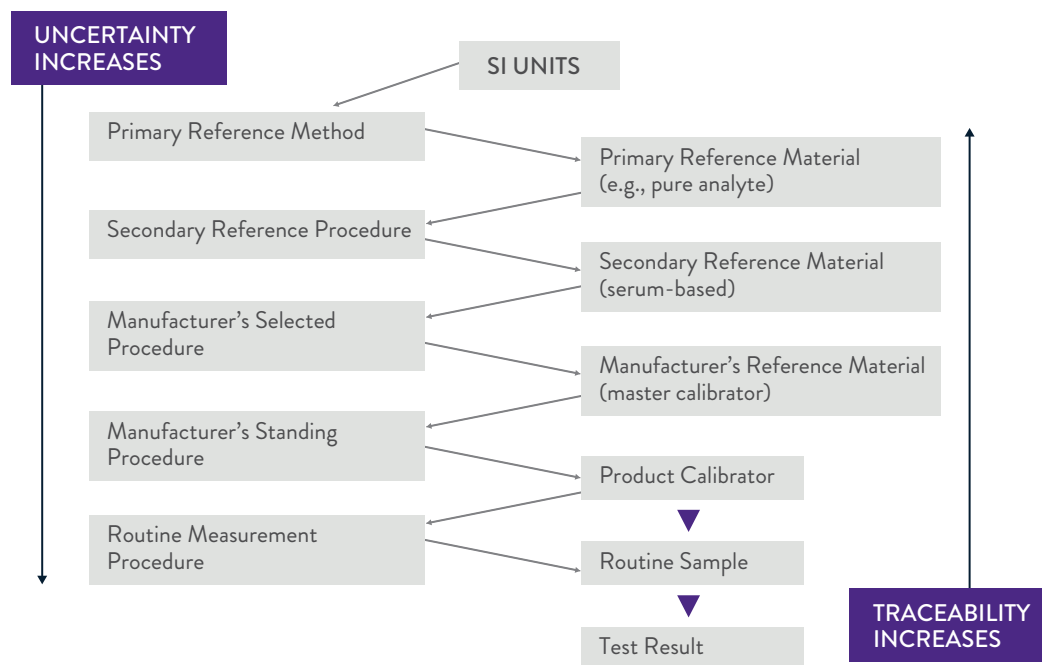


Figure 13-5: The metrological traceability chain.

In some cases, it may be practical to assign values directly to the manufacturer's in-house working calibrator, eliminating the need for a secondary reference material and thereby reducing the final uncertainty. However, attempts to shorten the chain are hampered by the lack of reference materials that are commutable with human serum. Consequently, additional intermediate steps that involve the use of panels of human samples are sometimes required. The traceability chain ends with the routine measurement procedure used to produce a result from a sample.

Metrological commutability refers to the comparability of test results if a reference material, such as an assay calibrator, is tested by a reference method and one or more field methods (i.e., routine clinical laboratory assays).

FACTORS CONNECTED WITH VARIATIONS OF PROTEINS IN BODY FLUIDS AND EFFECTS ON TEST RESULTS

The quality of patient care depends on the quality of all the information that a physician uses in making treatment decisions. As clinicians come to rely more and more on biochemical markers for early detection, diagnosis and prognosis (about 80% of medical decisions are based on laboratory findings/measurements), the accuracy of the clinical laboratory result is an increasingly important component of quality patient care. Therefore, it is very important that clinicians understand how a reliable result can be obtained and how important the contribution of the medical staff is for a proper preparation of the patient and successful blood collection, with fast and safe specimen delivery to the laboratory.

All analytes that are measured in body fluids by the clinical laboratory for medical purposes are subject to several sources of variation, including:

- Physiological and genetic variation.
- Pathophysiological variation.
- Preanalytical variation (specimen collection and processing).
- Analytical variation (methodological bias and imprecision).
- Postanalytical variation (reporting of test results).

The most frequent preanalytical errors are the inappropriate choice of laboratory tests or panel of tests, and the most frequent postanalytical errors derive from inappropriate interpretation and utilization of laboratory results.

An identification of all errors is extremely difficult for laboratories to do because many errors will neither produce detectable abnormal results nor raise questions from the user.

PHYSIOLOGICAL VARIATION

Age of subjects

Protein levels vary with age, especially in neonates, children and the elderly. Aging may generate biomarker profiles similar to that in malignancy.

Gender

Female and male hormones affect the levels of many transport proteins (e.g., apolipoproteins, haptoglobin, transferrin and, to a lesser extent, the immunoglobulin IgM).

Medications

Oral contraceptives, hormone replacement therapy (HRT), estrogens, androgens, corticosteroids, antihypertensives and nonsteroidal anti-inflammatory drugs affect the levels of many proteins. When estrogens are used, decreased concentrations of alpha-1-acid glycoprotein and haptoglobin and increased concentrations of fibrinogen, alpha-1 antitrypsin, ceruloplasmin, thyroxine-binding globulin and transferrin are observed.

Exercise

Strenuous exercise can change the levels of many proteins (e.g., myoglobin). The magnitude of change is related to the amount of exercise done.

Genetics

Genetic polymorphisms and single-gene mutations can affect protein levels (e.g., low or high antibody responders, phenotypes associated with certain races and deficiencies).

Diet/nutrition

Nutritional status can affect protein level (e.g., prealbumin, RBP, albumin, transferrin).

Pregnancy

Levels of various proteins can be affected by pregnancy. There are increases of fibrinogen, transferrin, thyroxine-binding globulin, sex hormone-binding globulin (SHBG), alpha-1 antitrypsin, apolipoproteins, ceruloplasmin, complement proteins and CRP and decreases of alpha-1-acid glycoprotein, albumin, ferritin, haptoglobin, IgA and IgG.

Bed rest

Prolonged bed rest can change the levels of proteins.

Environment

Increased levels of immunoglobulins occur in tropical regions compared to cooler climates.

Biological variation

Biological variation, the natural fluctuation of body fluid constituents around the homeostatic setting point, has two components: within- and between-subject variation. There are clearly large differences among the set points of individuals, and this is termed between-subject biological variation.

PATHOPHYSIOLOGICAL VARIATION**Increased loss of proteins**

Pathological loss of plasma proteins occurs via a malfunctioning organ (e.g., in nephrotic syndrome, glomerular and tubular proteinurias), burns or gastroenteropathies.

Altered synthesis

Alteration in synthetic plasma protein rate occurs during inflammatory diseases (e.g., infections, autoimmune diseases) but also in liver and renal diseases, or it is due to phenotypic deficiencies.

Volume

Alterations in blood/fluid volume occur due to loss or dehydration increase in protein concentration.

Catabolism

Increased catabolic rate occurs in inflammation and in renal diseases due to the increased consumption of some plasma proteins.

Compensatory mechanisms

Increased high molecular weight proteins (e.g., alpha-2-macroglobulin, Apo B), compensate for the loss of other lower molecular weight plasma proteins (e.g., albumin in nephrotic syndrome).

Preanalytical variation

To obtain valid results in diagnostic laboratory tests, it is very important that the substance in the body fluid under investigation be preserved as unchanged as possible throughout the analytical procedure. During the process of coagulation of blood, when obtaining serum for assaying, certain substances/biomarkers, platelets and coagulation factors are activated. These processes continue during the transport of the sample to the laboratory for testing, especially when the sample containers used have no anticoagulants.

Some preanalytical factors that may influence the analytical results of the sample:

- Age, gender, race and pregnancy – consult age- and gender-specific reference ranges, especially for immunoglobulins; hormonal influence in pregnancy stimulates synthesis of some proteins
- Diet, starvation and physical activity – malnutrition reduces protein synthesis; lipids and apolipoproteins decreased; fluid balance disruption changes concentrations of proteins
- Caffeine, tobacco and alcohol – acute-phase proteins can increase; liver function decreased; dehydration effects
- Timing of sampling – diurnal variation in fluid balance affects protein concentration; also changes in concentration after meals
- Diagnostic and therapeutic measures – proper timing necessary to compare baseline and responses to treatments
- Posture and tourniquet – hemoconcentration increases protein concentration due to prolonged tourniquet pressure; fractional increase of proteins in sitting position compared to recumbent
- Site of sampling – fingerstick collection can have tissue fluid mixed with blood sample
- Anticoagulants – coagulation testing requires clean atraumatic collection of blood
- Transportation of samples – quick transport to laboratory by courier or pneumatic tube to avoid deterioration of sample
- Storage, processing, centrifugation and distribution – timely separation of blood into serum and clot or plasma and cells to maintain integrity of analytes; immediate analysis or proper storage temperature to avoid degradation of proteins
- Effects of lipemia, hemolysis and hyperbilirubinemia – optical interferences with colorimetric or turbidimetric assays; recollect if hemolyzed

Preanalytical aspects on lipoprotein measurements:

- Sample should be collected after 12 hours' fasting and reduced activity (not valid for Apo A-1 and Apo B).
- Drugs that modify lipid metabolism should not be taken the day before blood sampling.
- Avoid prolonged tourniquet application.
- Use plasma preferably (serum values: ~ 4% higher).
- Lipids and lipoproteins are usually stable several hours at -24°C to +20°C.
- Lipids and lipoproteins are usually stable several days at +4°C to +8°C.
- Perform a control after 2–4 weeks if therapy has been changed.

ANALYTICAL VARIATION

There are various causes for analytical variability (**Table 13-2**) that contribute to a certain extent to overall variability.

In particular disease states, certain substances present in the patient's blood (e.g., circulating endogenous antibodies) can interfere in some immunoassays within the analytical process.

A careful analysis is necessary in such cases to elaborate the reasons for certain discrepant test results and to improve the applied assay procedure.

Heterogeneity of analyte	Reagents used in the assay	The analytical instrument
Sample collection and processing	– Ab specificity – Ab species and nature – Stability of epitopes	– Curve-fitting algorithm – Technique of measurement – Laboratory environment
Matrix effects of sample		
Hyperlipidemia		
Hemolysis	Reagent lot variation	Maintenance of the instrument
Rheumatoid factors	Aging of reagents and calibrators	Aging of light source
M-components	Separation technique	Mixing of the sample
Heterophilic antibodies	Standards and controls	
	Calibration procedure	

Table 13-2: Causes of analytical variability in immunoassays.

Postanalytical variation and errors

It should be stressed that clinicians should receive a proper education in biochemical pathophysiology, as well as effective clinical training on interpretation of laboratory test results. This could contribute substantially to reduce postanalytical errors in the clinical routine.

Moreover, an increasing body of evidence demonstrates the importance of the postanalytical phase and the importance of decreasing the turnaround time (TAT), so that effective therapy can be applied as early as possible. It is also essential to improve the appropriateness of reference intervals (age and gender specific) and to allow more objective validation and interpretation of data by use of expert systems.

Interpretation of laboratory results and plausibility checks are very important. For example, the measurement of immunoglobulin concentrations in a sample result far below the reference range should induce a check for the presence of a monoclonal protein, which could have produced an excess antigen situation.

REVIEW QUESTIONS: SECTION 13

Answers are provided at the end of this Learning Guide.

1. Which immunoassay method for serum protein analysis measures the intensity of directly transmitted light?
 - A Nephelometry
 - B Fluorescence
 - C Turbidimetry
2. Which of the following patient behaviors is (are) known to affect serum protein concentration?
 - A Exercise
 - B Amount of sleep
 - C Position
 - D Posture
 - E Both A and C
 - F All of the above
3. Fasting before blood sampling is recommended because eating may:
 - A Increase triglycerides and interfere with assays
 - B Increase levels of certain proteins
4. Protein concentrations in a given individual are consistent year-round.
 - A True
 - B False
5. Serum samples may be safely stored at room temperature for up to 1 week before assaying proteins.
 - A True
 - B False

6. Checking for antigen excess is important for:
- A** Samples containing analyte concentrations that approach the high end of the assay range
 - B** Albumin since concentrations are so high in most patients
 - C** Polyclonal IgG, even if concentrations are normal
7. Monoclonal antibodies and polyclonal antibodies:
- A** Can provide specificity and sensitivity for measuring analytes
 - B** Are immunoglobulins
 - C** Bind antigen through the Fab binding region
 - D** All of the above
8. In the reaction between antigen and antibody, what is the antigen excess zone?
- A** The zone in which a low antigen/antibody ratio exists
 - B** The zone in which there is an optimal ratio between antigen and antibody
 - C** The zone in which there is an increased amount of free antigen and a reduced amount of antibody
9. Identify the two sources of variation to which all analytes measured in body fluids are subject.
- A** Physiological and genetic variation
 - B** Pathophysiological variation
 - C** Preanalytical variation (specimen collection and processing)
 - D** Analytical variation (methodological bias and imprecision)
 - E** Postanalytical variation (reporting of test results)
10. The age and gender of a subject are sources of:
- A** Physiological variation
 - B** Pathophysiological variation
 - C** Preanalytical variation
 - D** Analytical variation
 - E** Postanalytical variation

APPENDIX

- APPENDIX A:
SUMMARY OF SPECIFIC PROTEINS AND THEIR CHARACTERISTICS
- APPENDIX B: REFERENCES
- APPENDIX C: SUGGESTED RESOURCES
- APPENDIX D: CORRECT RESPONSES

APPENDIX A

SUMMARY OF SPECIFIC PROTEINS AND THEIR CHARACTERISTICS

Specific protein	Approximate molecular mass function	States with increased concentration	States with decreased concentration
Albumin ALB RR: Serum: 35–52 g/L Urine: < 20 mg/L	66.3 kDa – Maintenance of colloid osmotic pressure – Transport of hormones, Ca, Mg, Zn, fatty acids, organic dyes, drugs, bilirubin, bile acids, vitamins, etc. – Antioxidant – Protein reserve	In serum: – Dehydration – Intravenous infusion – Anabolic steroids In urine: – Glomerular kidney disease – Diabetic nephropathy – Congestive heart failure – Toxemia of pregnancy – Rheumatic fever – Increased CVD risk In cerebrospinal fluid: – Damage of the blood-brain barrier	Acute blood loss, anabolic steroids Reduced synthesis: – Liver cirrhosis, malnutrition, analbuminemia Increased catabolism: – Inflammation, infection, trauma, Cushing’s syndrome, hyperthyroidism, hypercortisonism Abnormal loss: – Shock, bleeding, loss through gastrointestinal system, kidney and skin (burns) Pathological redistribution: – Postoperatively, burns, peritoneal carcinosis
Alpha-1-Acid Glycoprotein (Orosomucoid) AAG RR: 0.5–1.2 g/L	40 kDa – Binds basic drugs and hormones and consists of different polymorphs with varying carbohydrate content – Inhibits lymphocyte function by down regulation of the immune response	– Inflammation, acute infection, rheumatoid arthritis, SLE, IBD, malignant diseases, burns, fever, trauma, AMI – Corticosteroid administration – Slight reduction of GFR	– Malnutrition, hepatic damage, severe protein-losing gastroenteropathies – Helpful in differentiating elevated levels due to inflammatory conditions from those due to estrogen effects in which AAG levels are normal
Alpha-1-Antichymotrypsin ACT RR: 0.3–0.6 mg/L	55–68 kDa – Inhibits cathepsin G, mast cell chymase – Possible immunoregulatory functions	Acute-phase response in inflammatory conditions (e.g., burns, surgical trauma, bacterial infection, AMI)	– Hereditary deficiency – Infancy – Nephrotic syndrome – Asthma
Alpha-1 Antitrypsin AAT RR: 0.9–2.0 g/L	52 kDa Inhibits serine proteases, especially neutrophil elastase	– Acute infection and inflammation, advanced malignant diseases (especially liver metastases), pancreatitis, acute hepatitis and liver cirrhosis – Anabolic steroid therapy	– Hereditary deficiency – Chronic obstructive pulmonary disease (lung emphysema), nephrotic syndrome, protein-losing gastroenteropathies
Alpha-2-Macroglobulin α ₂ M RR: Serum: 1.3–3.0 g/L Urine: 10 mg/g creatinine	725 kDa Proteinase inhibitor (plasmin, pepsin, trypsin, chymotrypsin, cathepsin D, endopeptidases, PSA)	In serum: Liver diseases, diabetes mellitus, nephrotic syndrome, inflammation, neural tube defects In urine: Post-renal proteinuria/post-renal hematuria	Fibrinolysis, DIC, acute pancreatitis, biliary or renal stones, liver tumors, gastroduodenal ulcers, AMI, protein malnutrition, protein-losing gastroenteropathies
Apolipoprotein A-1 Apo A1 RR: M: 0.11–0.17 g/L F: 0.12–0.19 g/L	28.3 kDa – Main component of HDL – Ligand for HDL-receptor – Activator of lecithin-cholesterol-acyltransferase (LCAT) – Stabilizes prostacyclin	– Increase is associated with decreased risk for atherosclerosis – Familial hyperalphalipoproteinemia – Estrogens, pregnancy, exercise, familial increase, liver disease – Moderate alcohol intake	– Decrease associated with increased atherosclerosis risk – Familial hypoalphalipoproteinemia – Tangier disease (hereditary), “fish-eye” disease, cholestasis, sepsis

Specific protein	Approximate molecular mass function	States with increased concentration	States with decreased concentration
Apolipoprotein B Apo B RR: M: 0.80–1.55 g/L F: 0.75–1.50 g/L	Apo B-100: 549 kDa Apo B-48: 265 kDa – Primary protein component of low-density lipoprotein (LDL), VLDL and chylomicrons – Cholesterol transport – Ligand for LDL (Apo B/E) receptors	– Increase is associated with increased risk for atherosclerosis – Familial hypercholesterolemia – Familial hyperapobetalipoproteinemia – Nephrotic syndrome – Biliary obstruction – Type II hyperlipidemia	– Familial hypobetalipoproteinemia – Abetalipoproteinemia – Liver disease – Neuromuscular degeneration
Beta 2-microglobulin β2-Microglobulin β2M RR: (in mg/L) Serum: < 60 yrs: 0.8–2.4 > 60 yrs: 1.1–3.0 Urine: < 0.3	11.8 kDa Component of HLA proteins and present on the surface of nucleated cells	In serum: – Decrease of glomerular filtration rate and renal tubular function – Multiple myeloma – B-cell lymphomas – HIV infection, amyloidosis – Allograft rejection In urine: Tubular damage (unstable below pH 6)	Not known
Ceruloplasmin CP RR: < 5d: 0.05–0.40 g/L Adults: 0.20–0.60 g/L	151 kDa – Binds and transports copper – Essential in the regulation of redox potential and utilization of iron	– Cholestasis, primary biliary cirrhosis, SLE, leukemia, rheumatoid arthritis – Estrogen therapy, pregnancy – Chronic alcoholism – Acute-phase response	– Wilson’s disease and connective tissue diseases (e.g., Menke’s syndrome) – Protein loss in nephrotic syndrome, protein-losing enteropathy and malabsorption – Decreased synthesis in advanced liver disease
Complement C3 RR: 0.90–1.80 g/L (in fresh samples, C3 values are lower)	185 kDa – Activation of the complement system via classical and alternative pathways (C3c is common to both pathways) – Opsonization, chemotaxis – Monitoring clinical progression and activity in SLE, membranous proliferative glomerulonephritis, immune complex diseases	– Inflammatory conditions, but increase is only slight to moderate (e.g., bacteremia) – Systemic infections, biliary obstruction, amyloidosis, nephrotic syndrome – Corticosteroid therapy	Hereditary deficiency states or complement-activating diseases: – Decreased synthesis in severe protein malnutrition – Hypercatabolism in membranoproliferative glomerulonephritis, SLE, Sjögren’s syndrome, rheumatoid arthritis, autoimmune hemolytic anemia, gram-negative bacteremia, neonatal respiratory distress syndrome (NRDS) – Loss in protein-losing gastroenteropathies, burns
Complement C4 RR: 0.10–0.40 g/L	206 kDa – Activation of the classical pathway only – Anaphylatoxin – Monitoring clinical progression and activity in SLE, membranous proliferative glomerulonephritis, immune complex diseases	– Acute inflammatory process – Estrogens, pregnancy	– Autoimmune disease – Immune complex diseases – Hereditary deficiency states (e.g., hereditary angioneurotic edema, SLE with nephritis) – Cryoglobulinemia – Acute glomerulonephritis

Specific protein	Approximate molecular mass function	States with increased concentration	States with decreased concentration
C-Reactive Protein CRP RR: 0.06–3.0 mg/L, median: 0.59 mg/L	105 kDa – Activates complement (Ca ²⁺ dependent) and thus phagocytosis resulting in early, nonimmune clearance of gram-negative bacteria and tissue breakdown products – Activates monocytes – Enhances uptake of LDL into macrophages in the atherosclerotic plaques	Inflammation, acute and chronic tissue destruction, tumors In most circumstances, CRP is the only APP measurement required: – 10–40 mg/L in viral infection and mild bacterial infection – 40–200 mg/L in acute inflammation, moderate bacterial infection – 300–700 mg/L in extensive trauma, burns or severe sepsis	Not known
Cystatin C RR: (in mg/L) Serum: M: < 50 yrs: 0.3–0.8 > 50 yrs: 0.4–1.0 F: 0.4–1.0 Urine: 0.03–0.3	13 kDa A cysteine protease inhibitor involved in processing prohormones and the catabolism of collagen, which is produced by most nucleated cells	In serum: – Indicator of decreased glomerular filtration rate – Prognostic indicator of secondary cardiovascular events, metabolic syndrome and type 2 diabetes, loss of functionality at old age In urine: Tubular damage	Not known
Ferritin FER RR: (in µg/L) Children: 15–120 Adults: M: 20–280 F: < 50 yrs: 15–150 > 50 yrs: (20–280)	~ 450 kDa – Major storage protein of reserve iron for hemoglobin synthesis – Sequesters iron in a soluble form providing accessible reserves for synthesis of iron-containing compounds – Important antioxidant	– Increase tissue iron stores – Iron overload – Inflammatory diseases, chronic liver disease, malignancy – Ineffective erythropoiesis – Result of oral iron therapy	– Decreased tissue iron, iron deficiency – A ferritin decrease is diagnostic of iron deficiency
Fibrinogen FIB RR: 1.8–3.5 g/L	350 kDa Precursor of fibrin, the major constituent of blood clots	– Inflammatory diseases – Risk factor for cardiovascular diseases – Estrogens, pregnancy, oral contraceptives	DIC, liver disease, hereditary deficiency, infancy
Haptoglobin HP RR: 0.30–2.0 g/L	85–400 kDa Binds free hemoglobin in plasma, preventing loss of iron	Inflammation, autoimmune diseases, collagenosis, neoplastic processes, Hodgkin's disease, liver disease	– During childhood – Decreased synthesis in severe liver disease and estrogen therapy/contraceptives – Increased metabolism in intravascular hemolysis and in increased ineffective erythropoiesis
Hemopexin Hpx RR: 0.5–1.15 g/L	63 kDa – Binds heme and transports it to hepatocytes – Antioxidant action	– Acute-phase response – Cancers (especially melanomas) – Rheumatoid arthritis, hemochromatosis	Severe intravascular hemolysis during inflammation or injury, severe malnutrition, cirrhosis, hemorrhagic pancreatitis, dengue hemorrhagic fever, erythropoietic protoporphyria
Immunoglobulin A IgA RR: 0.7–4.0 g/L Age: Related during childhood	~ 160 kDa Predominant Ig class at mucosal sites (saliva, tears, respiratory, genito-urethral and gastrointestinal tract, colostrum), where it provides an early antibacterial and antiviral defense	– Chronic liver diseases – Chronic infections (especially in the lungs and gut) – Autoimmune disorders (e.g., rheumatoid arthritis, SLE), sarcoidosis and Wiskott-Aldrich syndrome – IgA myeloma	– Congenital and acquired immunodeficiency diseases (e.g., Bruton-type agammaglobulinemia, ataxia telangiectasia) – Non-IgA myeloma, Waldenström's macroglobulinemia – Protein-losing enteropathies, loss through skin from burns

Specific protein	Approximate molecular mass function	States with increased concentration	States with decreased concentration
Immunoglobulin E IgE RR: < 0.3 mg/L, 100 IU/mL	188 kDa - Antibody (reagin) - IgE attaches via its Fc region to receptors on mast cells - Bridging of two IgE molecules by allergen-induced release of mediators from mast cell granules causing clinical features of acute allergy	- Atopic diseases (e.g., allergic or extrinsic asthma, hay fever and atopic dermatitis) - Parasitic diseases - Advanced Hodgkin's disease - IgE myeloma - Some malignant diseases	- Hereditary deficiencies - Acquired immunodeficiency - Ataxia telangiectasia - Non-IgE myeloma
Immunoglobulin G IgG RR: 7–16 g/L	145 kDa - Secondary immune responses - Antibody (especially to viruses, bacteria, toxins) - Only Ig that crosses the placenta (active transport) Subclasses: IgG1, IgG2, IgG3, IgG4	- Chronic liver disease (including cirrhosis), chronic infection, parasitic disease, autoimmune disease (especially SLE and CAH), sarcoidosis - IgG myeloma	- Acquired immunodeficiency - Hereditary deficiencies (class or subclass) - Protein-losing syndromes - Pregnancy - Non-IgG myeloma, Waldenström's macroglobulinemia - Lymphomas
Immunoglobulin M IgM RR: 0.4–2.3 g/L Age: Related during childhood	~ 970 kDa - Predominant antibody in primary immune responses - Constitutes the initial line of defense	- Acute/chronic infection (especially viral infections), liver disease (especially PBC), autoimmune disease, sarcoidosis - Waldenström's macroglobulinemia - Estrogens, pregnancy - Hyper-IgM dysgammaglobulinemia - Congenital infection (in newborns)	- Acquired immunodeficiency - Hereditary deficiencies - Protein-losing syndromes - Non-IgM myeloma - Infancy, early childhood
Lipoprotein(a) Lp(a) RR: < 0.3 g/L	300–700 kDa - Lp(a) consists of Apo(a) bound to Apo B via disulfide linkage - Partial sequence homology with plasminogen; blocks plasminogen binding of fibrin clots	- Hereditary elevation - Increase is associated with increased risk for coronary artery diseases - Nephrotic syndrome - Acute-phase response	- Hereditary decrease - Primary biliary cirrhosis - Anabolic steroids - Hypertriglyceridemia
Prealbumin (Transthyretin) PAL Transthyretin RR: 0.2–0.4 g/L	55 kDa Transport protein for thyroxine (T4) triiodothyronine (T3) and transports vitamin A in the presence of retinol-binding protein	In serum: - Glucocorticosteroids, nonsteroidal anti-inflammatory therapy - Hodgkin's disease - Alcoholism (without hepatocellular disease) In urine: - Glomerular and tubular proteinuria	- Inflammatory diseases - Sensitive indicator of protein-caloric malnutrition and early response to nutritional treatment - Nephrotic syndrome - Liver disease
Procalcitonin PCT RR: < 0.5 µg/L	12.6 kDa - Prohormone of calcitonin - Macrophages and monocytic cells of liver are involved in synthesis and release of PCT under conditions of systemic inflammatory response	- Inflammatory diseases, infection, sepsis or multiple organ dysfunction syndrome (MODS, > 2 mg/L) - Severe sepsis and/or shock (> 10 mg/L) - Major surgical procedures, polytrauma or burns, prolonged circulatory failure (0.5–2.0 µg/L)	Sheehan's syndrome (pituitary apoplexy)

Specific protein	Approximate molecular mass function	States with increased concentration	States with decreased concentration
Retinol-binding Protein RBP RR: Serum: 30–60 mg/L Urine: < 0.5 mg/L	21 kDa Transport of retinol from liver to various target tissues to peripheral tissues, protecting retinol from oxidative damage	<ul style="list-style-type: none"> – Glucocorticosteroids – Oral contraceptives – Renal failure In urine: Tubular proteinuria	<ul style="list-style-type: none"> – Short-term changes in energy and protein deficiency – Reflects nutritional status – Rapid decreases in response to starvation – Inflammatory diseases – Vitamin A deficiency, liver disease, hyperthyroidism
Serum Amyloid A SAA RR: < 6.4 mg/L	12–14 kDa <ul style="list-style-type: none"> – Lipid transport and metabolism (present in HDL fraction) – Alteration of HDL protective action in APR – Precursor of amyloid A fibrils 	<ul style="list-style-type: none"> – Inflammatory diseases – Monitoring AA amyloidosis, as well as transplant rejection 	Not known
Soluble Transferrin Receptor sTfR RR: Assay 1 Children: < 16 yrs: 1.5–3.0 mg/L Adults: M: 2.2–5.0 mg/L F (premenopausal): 1.9–4.4 mg/L Assay 2 0.83–1.76 mg/L	190 kDa <ul style="list-style-type: none"> – Iron transported in plasma by TF is donated to cells through interaction with a specific membrane receptor (TfR); the truncated form of TfR, sTfR, is detected in plasma as a result of externalization – sTfR is proportional to the mass of cellular TfR and originates mostly from erythroblasts; its levels reflect the degree of iron-deficient erythropoiesis 	<ul style="list-style-type: none"> – Iron-deficient anemia – Prediction of the hematological response to EPO treatment – b-thalassemia and autoimmune hemolytic anemia 	<ul style="list-style-type: none"> – Anemia of chronic disease/ inflammation – Hypoplastic anemia – Chronic renal failure – Hereditary hemochromatosis
Transferrin TRF RR: 2.0–3.6 g/L Transferrin saturation: 16%–45%	79.6 kDa Binding and transport of iron (Fe^{3+}) between tissues (mainly liver) and bone marrow, representing the total iron-binding capacity (TIBC)	<ul style="list-style-type: none"> – Iron deficiency – Estrogens, pregnancy 	<ul style="list-style-type: none"> – Inflammatory diseases – Acute-phase response – Disorders of hemoglobin synthesis – Hemochromatosis – Liver diseases, malnutrition – Anemia of chronic infection – Tumors

Selected parameters	Function	States with increased concentration	States with decreased concentration
Anti-CCP RR: < 5.0 U/L	A family of autoantibodies directed against citrulline-containing proteins; these antibodies are known by different names: – APF (anti-perinuclear factor) – AFA (anti-flaggrin autoantibodies) – AKA (anti-keratin autoantibodies) – Anti-CCP (cyclic citrullinated peptide) – Cyclic citrullinated peptides (CCP) are very specific for RA Abs (96%)	Rheumatoid arthritis is confirmed by the presence of autoantibodies, such as rheumatoid factor (RF), and anti-citrullinated protein antibody (ACPA) (tested as anti-cyclic citrullinated peptide [anti-CCP])	Healthy individuals, the normal situation
Anti-streptolysin O ASO RR: Adults: < 200 IU/mL Children: < 150 IU/mL	The ASL/ASO represents specific antibodies to the extracellular products of <i>Streptococcus pyogenes</i> (Group A streptococcus: GAS)	Increased in rheumatic fever, poststreptococcal acute glomerulonephritis, rheumatoid arthritis, ankylosing arthritis	Healthy individuals, the normal situation
Rheumatoid Factor RF RR: < 15 IU/mL	RF is a group of autoantibodies belonging to all Ig classes and directed against Ag determinants on the Fc fragment of altered or complexed IgG molecules	Increased in rheumatic disease, chronic bacterial infections, viral infections, parasitic infections, sarcoidosis, lymphomas, chronic liver disease	Healthy individuals, the normal situation

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ORGANIZATIONS PROVIDING SERVICES AND EDUCATIONAL MATERIALS

- American Diabetes Association® (ADA): www.diabetes.org
- American National Standards Institute® (ANSI): www.ansi.org
- Clinical and Laboratory Standards Institute® (CLSI® – formerly NCCLS): www.clsi.org
- Institute for Reference Materials and Methods (IRMM): www.irmm.jrc.be
- International Bureau of Weights and Measures (BIPM®): www.bipm.org
- International Federation of Clinical Chemistry (IFCC): www.ifcc.org
- Lab Tests Online. U.S. site, www.labtestsonline.org, provides links for sites in other countries and in a number of languages
- National Cholesterol Education Program (NCEP): www.nhlbi.nih.gov/about/ncep
- National Institute for Biologic Standards and Control (NIBSC): www.nibsc.ac.uk
- National Institutes of Standardization and Technology (NIST®): www.nist.gov
- National Kidney Foundation® (NKF®): www.kidney.org/kidneydisease/ckd/knowgfr.cfm
- World Health Organization (WHO): www.who.int/en/

SUGGESTED RESOURCES

ORGANIZATIONS PROVIDING STANDARDIZATION PROGRAMS

Cholesterol Reference Method Laboratory Network: www.cdc.gov/labstandards/crmln.html

IFCC HbA1c Standardization Program: www.ifcchba1c.net

National Glycohemoglobin Standardization Program: www.ngsp.org

ONLINE RESOURCES FOR BIOLOGIC VARIATION AND SETTING TARGET ACCURACY

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APPENDIX D: CORRECT RESPONSES

SECTIONS 1-4

- 1. F
- 2. A
- 3. B
- 4. C
- 5. E
- 6. D
- 7. A
- 8. B
- 9. B
- 10. C
- 11. B
- 12. F

SECTIONS 5-12

- 1. C
- 2. A
- 3. B
- 4. A
- 5. B
- 6. A, B
- 7. C
- 8. D
- 9. C
- 10. B

SECTION 13

- 1. C
- 2. F
- 3. A
- 4. B
- 5. B
- 6. A
- 7. D
- 8. C
- 9. C, E
- 10. A

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