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Acute care testing
handbook

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Authors:
Corina Seeger, MSc Biochemistry, Scientific advisor, Radiometer Medical ApS
Chris Higgins, MSc Medical Biochemistry, Medical writer, UK

Illustrations by medical illustrator Dr. Lotte Clevin, Denmark
Layout and graphics by Hertz and Radiometer Medical ApS

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Preface

Rapid access to blood tests is a mainstay in the diagnosis and treatment of acute disease. Oxygenation status and acid-base balance are determined by arterial blood gas analysis and constitute a central part of modern evidence-based treatment algorithms in critical care. Furthermore, devices, intended for critical care testing, allow for assessment of e.g. renal function (creatinine) and electrolytes, inflammation (C-reactive protein) and cardiac biomarkers.

This handbook is a concise, well-organized guide covering the spectrum of parameters provided by state-of-the-art devices. Each parameter's physiological role and pathophysiology is explained, and reference intervals are provided, as well as the most likely causes of abnormalities. Quick access to important information is complemented by colorful illustrations, essential lists and tables. This handbook is intended to be a supplement to comprehensive text books, offering an easy guidance to some blood tests and simplifying the healthcare providers' decision-making process. It provides instant reminders of vitally important clinical facts for students, nurses, residents and other medical professionals who are required to interpret blood test within critical care.

Frank Christian Pott, MD, DMSc, assoc. prof., senior consultant

Bispebjerg Hospital, Department of Anesthesia and Intensive Care

University of Copenhagen

Disclaimer

This handbook is based on a review of scientific publications at the time of publication. The goal of this handbook is to provide educational information, within blood gas and acute care testing, to healthcare professionals. It is not intended to define a standard of care. Neither should this book be interpreted as prescribing an exclusive course of clinical and medical management.

Every healthcare professional making use of this handbook is responsible for evaluating the appropriateness of applying it in the setting of any particular clinical situation.

The reference intervals, causes and symptoms listed in the different parameter sections are all non-exhaustive.

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Content

Oxygen status

Life depends on the continuous delivery of oxygen, present in inspired air, to tissue cells. This is achieved by the synergistic action of the respiratory and cardiovascular systems in a process that is marked by three sequential events:

- Uptake of oxygen to blood from alveolar air in the lungs
- Transport/delivery of oxygen in blood from lungs to tissues
- Release of oxygen from blood to tissues

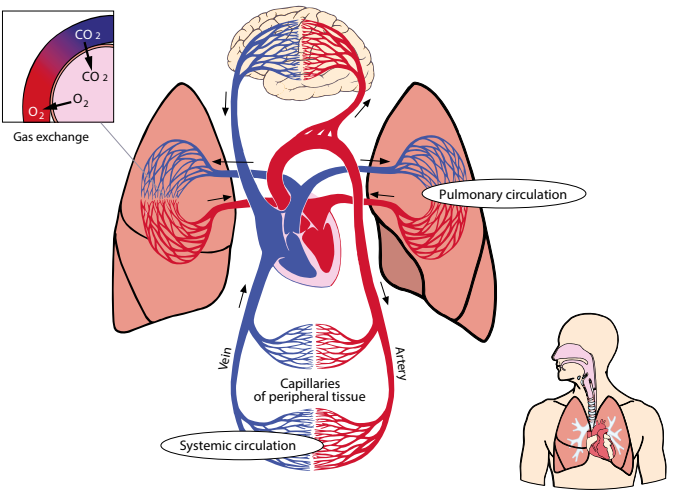


FIG. 1: The blood flow through the pulmonary and the systemic circulation.

The heart pumps deoxygenated blood into the pulmonary circulation and oxygenated blood into the systemic circulation (Fig. 1). In the pulmonary circuit, venous blood that is high in carbon dioxide (CO_2) and low in oxygen (O_2) flows from the right ventricle of the heart to the lungs. In the alveolar capillaries of the lungs, where pulmonary gas exchange occurs, carbon dioxide diffuses from blood to alveoli and oxygen diffuses from alveoli to blood. The now oxygenated

arterial blood flows from the lungs to the left atrium of the heart and onwards via the left ventricle and the systemic circulation to peripheral tissue. Here, oxygen diffuses from blood to tissue cells and carbon dioxide diffuses from tissue cells to blood. Deoxygenated venous blood then returns to the right atrium of the heart, completing the circuit.

The interactions in the pulmonary and the systemic circulation are rather complex, and under altered pathophysiologic conditions it may be difficult to predict the consequences of impaired oxygen uptake, impaired oxygen transport or impaired oxygen release. It is therefore imperative to evaluate all three to get the necessary information for adequate patient management. Oxygen partial pressure (pO_2) can be used to evaluate oxygen uptake. Oxygen content (ctO_2) can be used to evaluate oxygen transport. $p50$ can be used to evaluate the ability of oxygen release.

A description of the parameters mentioned below is found in the following sections of this handbook.

Oxygen uptake

In arterial blood, oxygen partial pressure (pO_2) is the result of oxygen uptake via diffusion through the alveolar-capillary membrane from the lungs to the blood. Thus, pO_2 (see pO_2) is the key parameter for the evaluation of oxygen uptake. pO_2 of arterial blood is influenced by:

- Alveolar oxygen pressure, which may be influenced by altitude, fraction of oxygen in inspired air ($FO_2(I)$), and alveolar pCO_2
- Degree of intra- and extrapulmonary shunting (FShunt)
- Diffusion capacity of the lung tissue

Oxygen transport/delivery

An adequate blood oxygen transport capacity is necessary to supply sufficient oxygen from the lungs to the peripheral tissue. Thus, the total concentration of oxygen in the arterial blood, ctO_2 (see ctO_2) is the key parameter for evaluating oxygen transport. ctO_2 is influenced by:

- Concentration of hemoglobin in the blood (ctHb)
- Concentration of dyshemoglobins (COHb, MetHb)
- Arterial oxygen pressure (pO_2)
- Arterial oxygen saturation (sO_2)

Oxygen delivery (DO_2) is the rate of supply of oxygen by arterial blood to organs and tissues. DO_2 is defined as the product of cardiac output (Q) and oxygen content of arterial blood ($ctO_2(a)$). This relationship reflects the synergistic importance of both Q and $ctO_2(a)$ for the adequacy of tissue oxygenation, and highlights that oxygen delivery depends on the combined function of blood, heart and lungs.

Oxygen release

To utilize the transported oxygen, it must be released to the peripheral tissues. Thus, the oxygen affinity to hemoglobin expressed by *p*50 (see *p*50) is the key parameter for evaluating the arterial blood's capability of releasing oxygen to the peripheral tissues. Oxygen release depends primarily on:

- Arterial and end-capillary oxygen pressure
- Oxygen content (ctO_2)
- Hemoglobin affinity for oxygen

Lactate and tissue oxygenation

Lactate is present in excess in blood mainly when there is insufficient oxygen in tissues to support normal aerobic cell metabolism (see lactate). Blood lactate thus serves as a marker of imbalance between tissue oxygen demand and oxygen supply. Elevated blood lactate may be caused by e.g. hypoperfusion, severely impaired arterial oxygen supply, or a combination of the two.

In general, an elevated or increasing lactate concentration is an early-warning indicator of impaired tissue oxygenation, occurring before other evidence of clinical shock or vital organ dysfunction. Decreasing or persistently low levels of blood lactate during critical illness is an improvement indicator of the patient status [1].

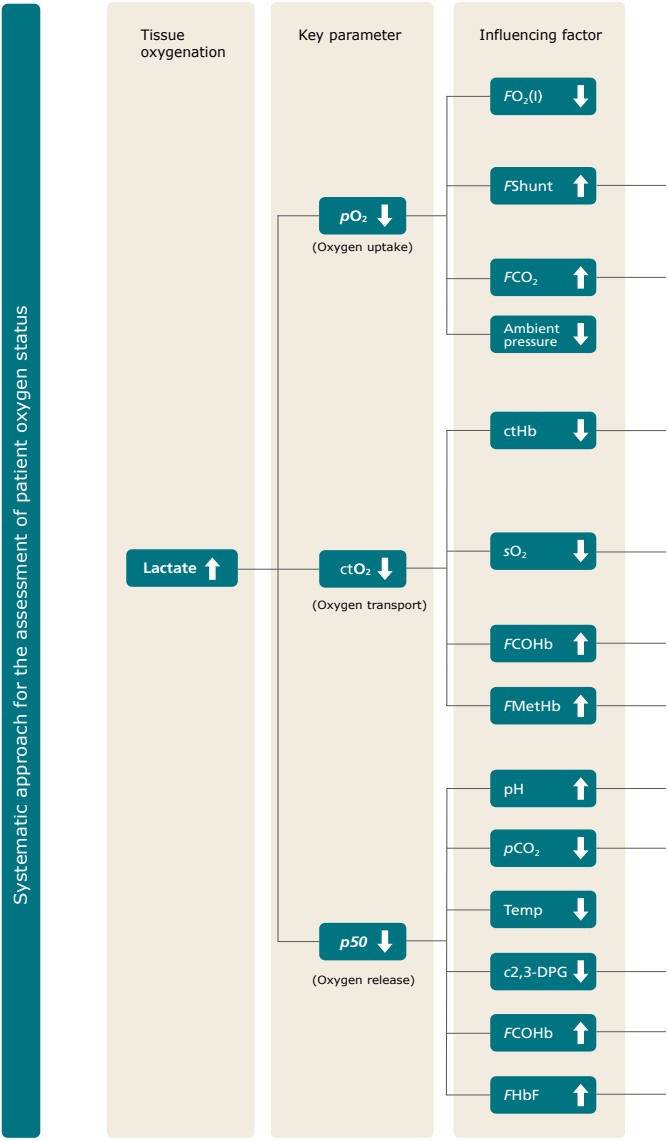

Systematic approach for the assessment of patient oxygen status
Tissue oxygenation
Key parameter
Influencing factor
Lactate ↑
pO2 ↓
(Oxygen uptake)
ctO2 ↓
(Oxygen transport)
p50 ↓
(Oxygen release)
FO2(l) ↓
FShunt ↑
FCO2 ↑
Ambient pressure ↓
ctHb ↓
sO2 ↓
FCOHb ↑
FMetHb ↑
pH ↑
pCO2 ↓
Temp ↓
c2,3-DPG ↓
FCOHb ↑
FHbF ↑

FIG. 2: Flow chart of the patient's oxygen status. The chart indicates the changes when arterial oxygen availability is impaired and shows how deviations in parameters interact. ↑: Increasing value. ↓: Decreasing value. ➡
Adapted from various textbooks and [2].

Description of the flow chart

The parameters in the flow chart (Fig. 2) are prioritized according to the order of evaluation. To make the flow chart practical in the clinical situation, only the most clinically relevant parameters and interactions are included.

The key parameters (pO_2, ctO_2, $p50$) have the highest priority, with the level of priority decreasing to the right. Evaluation should therefore begin with pO_2. If pO_2 is within the normal range, evaluate the next key parameter (ctO_2) and so forth.

If the key parameter being evaluated deviates from the expected value, look in the columns to the right of that parameter. Find the factors influencing the key parameter. One or more of these are possibly causing the deviation.

If lactate is the first parameter to be evaluated, and found to be too high, the next step will be to look at the key parameters to the right to find the possible cause of the high lactate concentration.

To get the most accurate picture of a patient's oxygen status, it is important that all parameters involved in oxygen uptake, transport, delivery and release are evaluated.

Oxygen partial pressure – pO_2

The amount of oxygen in blood is controlled by many variables, e.g. ventilation/perfusion. pO_2 is the partial pressure of oxygen in a gas phase in equilibrium with the blood. pO_2 only reflects a small fraction (1–2 %) of total oxygen in blood that is dissolved in blood plasma [3]. The remaining 98–99 % of oxygen present in blood is bound to the hemoglobin in the erythrocytes.

pO_2 primarily reflects the oxygen uptake in the lungs.

Reference interval pO_2 – examples

Adult/Child (wb, a)	kPa	mmHg
2 days–60 years	11.0–14.4	83–108
>60 years	>10.6	>80
>70 years	>9.3	>70
>80 years	>8.0	>60
>90 years	>6.65	>50
Newborn (wb, a)	**kPa**	**mmHg**
Birth	1.1–3.2	8–24
5–10 minutes	4.4–10.0	33–75
30 minutes	4.1–11.3	31–85
1 hour	7.3–10.7	55–80
1 day	7.2–12.7	54–95
Cord blood	**kPa**	**mmHg**
Arterial (a)	0.8–4.1	6–31
Venous (v)	2.3–5.5	17–41

[4] wb: whole blood; a: arterial

pO_2(a) decreases at the rate of ~0.29 kPa (2.2 mmHg) per decade after the age of 40 [5].

Physiological significance of pO_2

Life depends on the continuous supply of oxygen to tissue cells, which in turn depends on the continuous oxygenation of venous blood in the lungs. Oxygen diffuses down a pressure gradient from a relatively high level (21.2 kPa (159 mmHg) at sea level) in inspired air, to progressively lower levels in the respiratory tract, the alveolar gas, the arterial blood, capillaries and finally the cell/mitochondria, where the lowest pO_2 level (1–1.5 kPa (7.5–11.5 mmHg)) is observed. This decrease in pO_2 from inspired air to the mitochondria is called the oxygen cascade (Fig. 3). The pressure gradient of the oxygen cascade is physiologically essential for the delivery of inspired oxygen to tissues, and a pathological disturbance of the cascade, such as that which occurs in hypoventilation, can result in tissue hypoxia [6, 7].

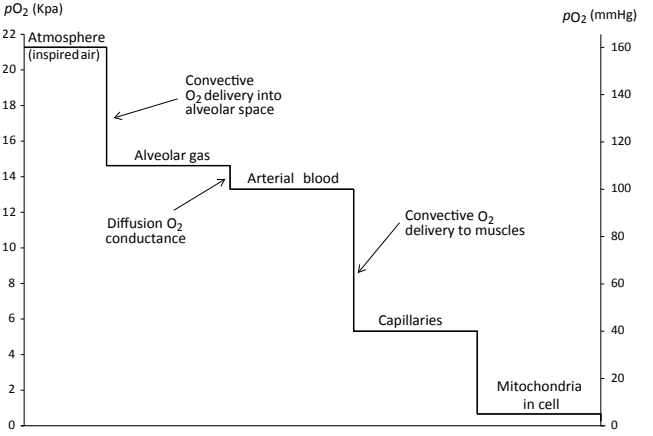


FIG. 3: The oxygen cascade.

Although pO_2 represents only a very small fraction of the total oxygen (ctO_2) (see ctO_2) being transported in arterial blood, it is highly significant as it is the major determinant of the amount of

oxygen bound to hemoglobin (see sO_2) and thereby the total amount of oxygen transported by arterial blood and made available to tissue cells. The relationship between pO_2 and sO_2 is described by the oxyhemoglobin dissociation curve (ODC) (Fig. 4). When pO_2(a) is higher than 10–11 kPa (75–83 mmHg), hemoglobin binds near-maximal amounts of oxygen (i.e. sO_2(a) >95 %). However, if pO_2(a) falls below ~10 kPa (75 mmHg) there is a marked decrease in sO_2 and therefore a sharp decline in the oxygen-carrying capacity of blood. The delivery of oxygen to tissues becomes increasingly compromised as pO_2(a) falls below ~10 kPa (75 mmHg), not primarily because pO_2(a) is decreased but because hemoglobin is carrying significantly less oxygen.

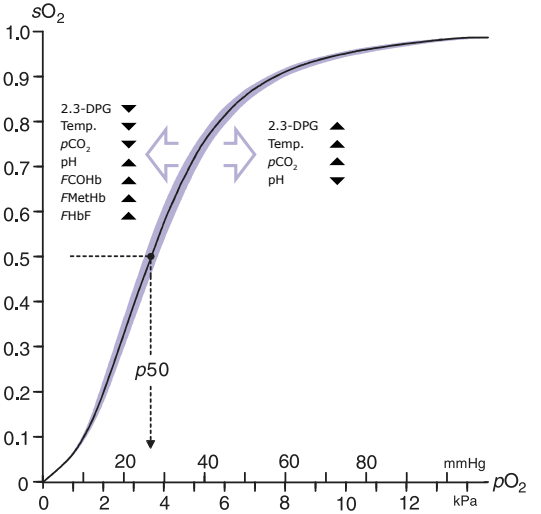


FIG. 4: Oxyhemoglobin dissociation curve, including extraneous factors determining left and right shift. For more information about the ODC see *p*50.
2,3-DPG: 2,3-diphosphoglycerate

Why measure pO_2?

The pO_2 is a reflection of the oxygen uptake in the lungs.

- It is the key parameter for assessing the adequacy of blood oxygenation, i.e. the transfer of environmental oxygen from lungs (alveoli) to blood (see oxygen status)
- It provides the means for diagnosing respiratory failure
- It provides the means for monitoring supplemental-oxygen therapy

When should pO_2 be measured?

Measurement of pO_2 is clinically useful in the diagnosis, assessment and monitoring of patients with severe acute or chronic respiratory disease or respiratory failure due to conditions other than respiratory disease (e.g. trauma to brain or chest, drug overdose).

Clinical interpretation

Terms used in interpretation

Hypoxemia is decreased oxygen content (see ctO_2) in blood (Table I). There are two main causes: impaired oxygenation of blood in the lungs and anemia. The first is evident as decreased pO_2 and the second is evident as decreased hemoglobin. It is important to be aware that although hypoxemia is usually associated with decreased pO_2, it can occur e.g. in patients with severe anemia, carbon monoxide poisoning and methemoglobinemia, despite normal pO_2 [8, 9].

Hypoxia [10] refers to the potentially life-threatening state in which the oxygen delivery to tissue cells is not sufficient to maintain normal aerobic metabolism. Affected tissue cells produce excess lactic acid, leading to increasing lactate levels in blood and resulting in metabolic acidosis (see lactate). Four types of hypoxia are recognized; they are:

Hypoxemic hypoxia: Defective mechanism of oxygenation in the lungs, resulting in insufficient oxygen content in the blood ($ctO_2(a)$ is low due to low $pO_2(a)$)

Ischemic hypoxia: Insufficient transport of oxygen to tissues, due to inadequate blood flow

Anemic hypoxia: Insufficient oxygen content in the blood due to decreased amount of hemoglobin able to carry oxygen

Histotoxic hypoxia: Impaired use of oxygen by tissues

Whatever the mechanism, it can, if sufficiently severe, lead to anoxia (cessation of oxygen supply) and tissue cell death. Myocardial infarction is an example of potentially fatal local tissue hypoxia caused not by hypoxemia, but by ischemia, due to thrombosis of a coronary artery [11].

Hyperoxemia is increased pO_2 in blood, i.e. $pO_2(a)$ >16.0 kPa (120 mmHg) (Table I). This can only occur in a clinical setting with administration of supplemental oxygen. It can lead to hyperoxia (increased oxygen content in tissues). Hyperoxia can be associated with oxygen toxicity; premature neonates are particularly vulnerable to the toxic effects of oxygen [12].

Respiratory failure is failure of the lungs to adequately perform pulmonary gas exchange. It is defined by $pO_2(a)$ <8 kPa (60 mmHg). Below the degree of hypoxemia that this level represents, there is increasing risk of hypoxia, even if cardiac output is not compromised. This degree of hypoxemia would usually trigger prescription of supplemental-oxygen therapy to ensure adequate tissue oxygenation. (see CO_2 definition of type I and type II respiratory failure).

Term	pO_2(a)		sO_2(a) approximate
	kPa	mmHg	%
Normoxemia	10.6	80	~96
	13.3	100	~98
Hypoxemia (mild)	9.3	70	~94
Hypoxemia (moderate)	8.0	60	~91
Hypoxemia (severe)	6.0	45	~80
Hyperoxemia	16.0	120	~98
Hyperoxemia (marked)	20.0	150	~99–100

TABLE I: Overview of pO_2(a) and corresponding sO_2(a) values that characterize normal blood oxygen (normoxemia), hypoxemia and hyperoxemia [11]. a: arterial

Causes of hypoxemia [13]

- Mechanical causes (e.g. airway obstruction, chest trauma)
- Neuromuscular diseases (e.g. Guillain-Barré syndrome, myasthenia gravis)
- Drugs that depress the respiratory center (e.g. opioids)
- Pneumonia
- Pulmonary embolism
- Pulmonary edema
- Acute asthma
- Acute respiratory distress syndrome (ARDS)
- Chronic obstructive pulmonary disease (COPD)
- Pulmonary disease (e.g. fibrosis)
- Pneumothorax

Symptoms associated with hypoxemia [14]

- Breathlessness on minimal exertion
- Shortness of breath/difficulty breathing/respiratory distress (dyspnea)
- Increased respiratory rate (tachypnea)

- Cyanosis
- Nasal flaring
- Wheeze/crackles on auscultation
- Increased sweating (diaphoresis)
- Confusion, disorientation, somnolence
- Coma
- Decreased SpO_2 (measured by pulse oximetry)
- Increased red cell count (polycythemia) with prolonged chronic hypoxemia

Causes of hyperoxemia

Oxygen therapy and pO_2

Too much oxygen may be toxic, causing endothelial damage in the lungs and other tissues. Increased pO_2 can only occur if the fraction of inspired oxygen (FO_2(I)) and therefore pO_2 of alveolar air is increased. The only clinical cause of increased pO_2(a) is supplemental-oxygen therapy [15].

At sea level FO_2(I) of ambient air is 21 %. Depending on the mode of delivery, oxygen therapy is associated with FO_2(I) up to 100 % (pure oxygen). Oxygen therapy poses an issue for interpreting pO_2, i.e. to decide if the pO_2 is appropriately high for the increased FO_2(I). A useful rule of thumb is that the difference between FO_2(I) (%) and pO_2 measured in kPa should not exceed 10 [16]. If this difference is significantly higher than 10, oxygenation is impaired.

Example

Consider two adult patients receiving oxygen therapy that provides FO_2(I) of 30 %. The first patient's pO_2(a) is 13 kPa and the second patient's pO_2(a) is 22 kPa. Using the rule of thumb it is clear that the first patient has impaired oxygenation despite the normal pO_2(a). The second patient does not; this patients pO_2(a) is appropriately high for the oxygen administration.

Hemoglobin – Hb

The concentration of total hemoglobin (*c*tHb) in blood includes oxyhemoglobin (cO_2Hb), deoxyhemoglobin (*c*HHb), as well as the dysfunctional hemoglobin species that are incapable of binding oxygen: carboxyhemoglobin (*c*COHb) (see COHb), methemoglobin (*c*MetHb) (see MetHb) and sulfhemoglobin (*c*SulfHb). Thus:

$$c\mathrm{tHb} = c\mathrm{O_2\,Hb} + c\mathrm{HHb} + c\mathrm{COHb} + c\mathrm{MetHb} + c\mathrm{SulfHb}$$

The rare sulfHb is not included in the reported ctHb in most oximeters.

Reference interval Hb – examples

	mmol/L	g/dL
Adult male	8.4 – 10.9	13.5 – 17.5
Adult female	7.1 – 9.6	11.4 – 15.5
Child	6.8 – 8.7	11.0 – 14.0
Neonate (at birth)	8.7 – 14.9	14.0 – 24.0

[17]

Hemoglobin: Structure and function

A small amount (<2 %) of the oxygen transported in blood is dissolved in blood plasma, but most of it (>98 %) is transported bound to the protein hemoglobin, contained within erythrocytes. The hemoglobin molecule (~280 million per erythrocyte) is composed of four polypeptide chains, the "globin" portion of the molecule. Each of these chains has an attached flat ringed molecule called heme. At the center of each heme group is an iron atom in the ferrous state (Fe^{2+}) [18] that forms a reversible bond with an oxygen molecule [19] resulting in a structural change of the hemoglobin molecule, thereby increase hemoglobins affinity for oxygen on the remaining binding sites. Each hemoglobin molecule has the capacity to bind up to four

oxygen molecules and can, theoretically, be 0, 25, 50, 75 or 100 % saturated with oxygen. The binding of hemoglobin with oxygen (see sO_2) occurs in the pulmonary capillaries, so that blood leaving the lungs and throughout the arterial system is normally close to 100 % saturated with oxygen. Each gram of hemoglobin can carry up to 1.34 mL of oxygen. Assuming a normal hemoglobin concentration of 150 g/L, and a normal sO_2 of 98 %, the oxygen-carrying capacity of hemoglobin in arterial blood is (1.34 × 0.98 × 150) ~200 mL O_2/L blood. Due to the low solubility of oxygen in water, a maximum of only 3 mL O_2/L blood can be carried dissolved in blood plasma. Thus, hemoglobin increases the oxygen-carrying capacity of blood from around 3 to ~200 mL O_2/L blood, and adequate oxygenation of tissue cells depends crucially on maintaining an adequate amount of hemoglobin in blood.

For otherwise healthy individuals the critical *c*tHb value below which tissue (anemic) hypoxia inevitably occurs is <3.1 mmol/L (5.0 g/dL) [20]; this critical *c*tHb is higher for those with cardiovascular or respiratory disease that limits their ability to compensate for decreased *c*tHb [20].

In the tissue microvasculature conditions (pH, pCO_2, temperature etc.) favor the release of oxygen from O_2Hb to cells, so that venous blood returning from the tissues to the lungs typically contains hemoglobin that is ~75 % saturated with oxygen (*c*tHb in venous blood comprises ~75 % O_2Hb and ~25 % HHb) [21].

In addition to its prime oxygen-carrying function, hemoglobin serves a minor role in the carriage of carbon dioxide (CO_2) in blood [22]. HHb can also bind hydrogen ions and this buffering action determines a role for hemoglobin in maintaining the pH of blood within normal limits.

Dyshemoglobin

Around 1 – 3 % of the measured *c*tHb is incapable of transporting oxygen reflecting the trace presence of COHb (see COHb) and MetHb

(see MetHb). This lack of function is reflected in their collective name: The dyshemoglobins [8, 9]. Given that MetHb and COHb normally constitute less than 3 % of *c*tHb in healthy individuals, their effect on the oxygen-carrying capacity of hemoglobin is minimal. However, a pathological increase in either MetHb or COHb decreases the oxygen-carrying capacity of blood. If this increase is sufficiently severe the resulting reduction in the oxygen carrying capacity of blood and concomitant tissue hypoxia is potentially fatal. A normal *c*tHb indicates that the oxygen-carrying capacity of blood is sufficient to meet the tissue demand for oxygen; that is not the case if dyshemoglobins are increased.

Why measure *c*tHb?

- It allows the diagnosis of anemia and assessment of its severity
- It is a necessary parameter for calculating the concentration of total oxygen content in blood (see ctO_2) and thereby assessing the risk of tissue hypoxia
- It is the principal index for assessing the clinical need for red blood cell transfusion

Causes of decreased *c*tHb

Anemia is the clinical syndrome that results from decreased *c*tHb. A diagnosis of anemia is seen in woman if *c*tHb <7.1 mmol/L (11.4 g/dL) and in men if *c*tHb <8.3 mmol/L (13.4 g/dL) [17].

The many causes of anemia include:

- Blood loss (hemorrhage)
- Iron deficiency
- Vitamin B12 and/or folate deficiency
- Increased red cell destruction (hemolytic anemia)
- Chronic inflammatory disease, cancer, chronic kidney disease
- Impaired erythrocyte production in the bone marrow (aplastic anemia, leukemia)
- Prematurity (a major risk factor for anemia during the neonatal period)
- Hemoglobinopathies (e.g. sickle cell anemia or thalassemia)

Symptoms of decreased ctHb

The principal pathological effect of anemia is decreased delivery of oxygen to the tissue cells and risk of tissue hypoxia (anemic hypoxia), but the extent to which this causes symptoms depends on several factors [17], including:

Severity of the anemia: Patients with mild anemia (ctHb >6.2 mmol/L (10.0 g/dL)) usually have no symptoms, but severe anemia (ctHb <3.7 mmol/L (6.0 g/dL)) is almost always symptomatic

Coexisting disease: The normal physiological (compensatory) response to anemia, which ensures, so far as is possible, continued delivery of oxygen to the tissues despite decreased ctHb, depends on functioning respiratory and cardiovascular systems

Those with coexisting respiratory and/or cardiovascular disease are particularly vulnerable to the effects of anemia, and more likely to manifest symptoms.

Symptoms include:

- Pallor
- Increased heart rate (tachycardia), palpitations
- Shortness of breath, particularly on exertion
- Tiredness and lethargy
- Dizziness, fainting
- Headaches

Causes of increased *c*tHb

Hemoglobin at higher-than-normal levels can be observed in people living at high altitudes [23].

Other infrequent causes are:
- Polycythemia vera
- Chronic lung disease (e.g. emphysema)
- Certain tumors
- Dehydration
- Drug abuse (e.g. erythropoietin (EPO) by athletes)
- Smoking

Symptoms of increased *c*tHb

- Weakness
- Fatigue
- Headache
- Itching
- Bruising
- Joint pain
- Dizziness
- Abdominal pain
- Shortness of breath
- Chronic cough
- Sleep disturbance (sleep apnea)
- Dizziness
- Poor exercise tolerance

Oxygen saturation – sO_2

Oxygen saturation (sO_2) is the ratio of oxyhemoglobin concentration to concentration of functional hemoglobin (i.e. oxyhemoglobin (O_2Hb) and deoxyhemoglobin (HHb) capable of carrying oxygen. Thus [24]:

$$sO_2\ \% = \frac{c\mathrm{O_2Hb}}{c\mathrm{O_2Hb} + c\mathrm{HHb}} \times 100$$

The sO_2 reflects utilization of the currently available oxygen transport capacity.

In arterial blood 98–99 % of oxygen is transported in erythrocytes bound to hemoglobin. The remaining 1–2 % of the oxygen transported in blood is dissolved in the blood plasma – this is the portion reported as partial pressure of oxygen (pO_2) [6]. (See pO_2)

Reference interval sO_2 – examples

Adult/child (wb, a):	94–98 %
Newborn (wb, a):	40–90 %

[4] wb: whole blood, a: arterial

A sO_2 of less than 80 % in adults are regarded as lift-threatening [25].

Physiological background – sO_2

Each hemoglobin molecule can bind a maximum of four oxygen molecules to form O_2Hb (see Hb, O_2Hb). The oxygen-delivery function of hemoglobin, i.e. its ability to "uptake" oxygen in the microvasculature of the lungs, transport it in arterial blood and "release" it in the microvasculature of the tissue cells, is made possible by a reversible change in the structure of hemoglobin which alters its affinity for oxygen (see Hb) [18]. One significant factor that determines

hemoglobin's affinity for oxygen is the pO_2 of the blood. The relationship between pO_2 and sO_2 is described by the oxyhemoglobin dissociation curve (ODC) (Fig. 5), which reveals in essence that the higher the pO_2, the higher is the hemoglobin affinity for oxygen, evident as increasing sO_2 [21].

The level of pO_2 is highest in arterial blood in the lungs due to the diffusion of inspired oxygen across the alveolar membrane from alveoli to blood (Fig. 3). Consequently, hemoglobin has the highest affinity for oxygen here, and it rapidly binds oxygen resulting in ~100 % saturated hemoglobin (sO_2(a)) due to blood from thebesian vein. By contrast in the tissues, where pO_2 is lower, hemoglobin has decreased affinity for oxygen, resulting in release of oxygen to tissue cells. The sO_2 of venous blood (sO_2(v)) returning from the tissues to the lungs is consequently decreased (~75 %) [21]. Illustrated in Fig. 5.

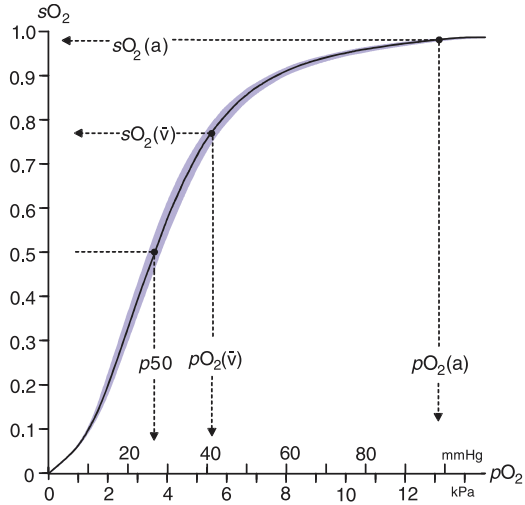


FIG .5: Oxyhemoglobin dissociation curve.
For more information about the ODC see *p*50
a: arterial; v̄: venous

Why measure sO_2?

sO_2 together with pO_2 provides the means for assessing blood oxygenation. Due to the sigmoidal shape of the ODC, sO_2 is less informative than pO_2, when blood is adequately oxygenated. In the upper flat portion of the curve large changes in pO_2 are reflected in much smaller changes in sO_2. Indeed, in cases of hyperoxemia (see pO_2) sO_2 may reach 100 %, and any additional oxygen will only be the oxygen dissolved in blood plasma. Here pO_2 will remain the only means of assessing blood oxygenation.

sO_2 is a major determinant of total oxygen content (see ctO_2).

sO_2 is a useful parameter for monitoring supplemental-oxygen therapy.

When should sO_2 be measured?

Measurement of sO_2 is clinically useful in the diagnosis, assessment and monitoring of patients with severe acute or chronic respiratory disease or respiratory failure due to conditions other than respiratory disease (e.g. brain or chest trauma, drug overdose).

Causes leading to impaired sO_2 are identical to those for pO_2, ctHb or ctO_2 (see pO_2).

Causes of decreased sO_2

Decreased sO_2 indicates that oxygen uptake is impaired and may be a result of [14]:

- Mechanical causes (e.g. airway obstruction, chest trauma)
- Neuromuscular diseases (e.g. Guillain-Barré syndrome, myasthenia gravis)
- Drugs that depress the respiratory center (e.g. opioids, heroin, morphine)
- Severe pneumonia
- Pulmonary embolism
- Pulmonary edema

- Acute asthma
- Acute respiratory distress syndrome (ARDS)
- Chronic obstructive pulmonary disease (COPD)
- Pulmonary disease (e.g. fibrosis)
- Pneumothorax
- Cyanotic congenital heart disease

Symptoms associated with decreased sO_2

Symptoms associated with hypoxemia and respiratory failure that might prompt sO_2 measurement include [14]:

- Breathlessness on minimal exertion
- Shortness of breath/difficulty breathing/respiratory distress (dyspnea)
- Increased respiratory rate (tachypnea)
- Cyanosis
- Nasal flaring
- Wheeze/crackles on auscultation
- Increased sweating (diaphoresis)
- Confusion, disorientation, somnolence
- Coma
- Increased red cell count (polycythemia) with prolonged chronic hypoxemia

Three ways of assessing sO_2 in critically ill patients

sO_2 can be determined in three ways: Pulse oximetry (SpO_2), calculated from pO_2, or measured directly on blood gas analyzers. For critically ill patients several studies have demonstrated the importance of using measured sO_2 rather than SpO_2 or calculated sO_2 to avoid mistreatment of these patients [26, 27, 28, 31]. Measured sO_2 is preferred in calculations, such as shunt and oxygen content to limit the critically important errors that may result from calculated sO_2 [29, 30]. Among intensive-care patients who are at high risk of tissue hypoxia, whether from pulmonary failure, cardiac decompensation, inadequate oxygen transport, or derangements at the cellular level, an accurate measurement of sO_2 is essential for planning therapy

[32, 33]. The Clinical and Laboratory Standards Institute (CLSI) guideline [24] state with regard to calculated sO_2 derived by blood gas analyzers:

"Clinically significant errors can result from incorporation of such an estimated value for sO_2 in further calculations such as shunt fraction, or by assuming that the value obtained is equivalent to fractional oxyhemoglobin."

Oxyhemoglobin – O_2Hb

FO_2Hb is the fraction of total hemoglobin (ctHb) that is present as oxyhemoglobin (O_2Hb). By convention the fraction is expressed as a percentage (%). Thus [24]:

$$O_2\text{Hb}\,\% = \frac{c\text{O}_2\text{Hb}}{c\text{O}_2\text{Hb} + c\text{HHb} + c\text{MetHb} + c\text{COHb}} \times 100$$

where $c$$O_2$Hb = concentration of oxyhemoglobin
cHHb = concentration of deoxyhemoglobin
cMetHb = concentration of methemoglobin
cCOHb = concentration of carboxyhemoglobin

The O_2Hb primarily reflects the potential oxygen transport capacity.

Reference interval O_2Hb – example

Adult (wb):	90 – 95 %

[4] wb: whole blood

What is O_2Hb?

The oxygen delivery process begins in the microvasculature of the lungs where the inspired oxygen diffuses into the erythrocytes and binds rapidly to hemoglobin; the product of this binding is O_2Hb. Blood, now oxygenated – with O_2Hb comprising practically all (~97 %) of the hemoglobin it contains – leaves the lungs. In the microvasculature of the tissues oxygen is released to the tissues from O_2Hb. In practice only around 25 % of the oxygen bound to hemoglobin in arterial blood is normally extracted by the tissues, so the returning venous blood from the tissues to the lungs contains both HHb and O_2Hb, although compared with arterial blood the amount of O_2Hb is decreased and the amount of HHb is increased.

Causes of decreased *F*O2Hb

In the absence of anemia, normal *F*O_2Hb is objective evidence of adequate blood oxygenation; it implies that oxygen in inspired air is diffusing at an adequate rate from lungs to blood. It is also evidence that the dyshemoglobins, COHb and MetHb, are present within normal reference intervals.

Decreased *F*O_2Hb occurs if the passage of oxygen from lungs to blood is impaired. The causes are identical to those leading to decreased *p*O_2 (see *p*O_2).

Decreased *F*O_2Hb may also result from increased dyshemoglobin levels. Elevated dyshemoglobin levels may lead to an effective anemia, despite no reduction in total hemoglobin concentration (see Hb). The causes may be:

- Carbon monoxide poisoning
- Drug-/chemical-induced methemoglobinemia
- Inherited methemoglobinemia
- Hemoglobin M disease

FO_2Hb versus oxygen saturation (*s*O_2)

FO_2Hb is a measure of how much of the arterial blood's oxygen transport capacity is being utilized and in this sense shares similarity with another parameter, *s*O_2 (see *s*O_2). The two parameters can be confused as *s*O_2 is sometimes erroneously referred to as *F*O_2Hb [34]. Therefore, it is important to emphasize the distinction between the two parameters. *s*O_2 is the ratio of O_2Hb concentration to concentration of functional hemoglobin (i.e. O_2Hb + HHb), whereas *F*O_2Hb is the ratio of O_2Hb concentration to total hemoglobin concentration, which includes both functional and non-functional hemoglobin species (i.e. O_2Hb + HHb + COHb + MetHb). Only in the absence of dyshemoglobins, COHb (see COHb) and MetHb (see MetHb), would *s*O_2 be equal to *F*O_2Hb. Since dyshemoglobins are normally present in small amounts and constitute less than 3 % of

total hemoglobin, sO_2 and FO_2Hb differ by only a small (clinically insignificant) degree in healthy individuals and most pathological conditions, with sO_2 being slightly (1–3 %) higher than FO_2Hb. Pathologies associated with increased COHb or MetHb will of course result in a marked reduction in FO_2Hb but leave sO_2 unchanged. Low sO_2 indicates that oxygen uptake can be improved, whereas low FO_2Hb, if accompanied by normal sO_2, warrants further investigation, e.g. of MetHb and COHb. Even FO_2Hb has a clear analytical definition, it lacks a clear physiological meaning and is a less specific parameter than sO_2 [34].

Oxygen content – ctO_2

Concentration of total oxygen (ctO_2) is the total amount of oxygen in the blood, and is the sum of the oxygen that is dissolved in blood plasma and that bound to hemoglobin. ctO_2 is also referred to as **oxygen content**. It is a calculated parameter derived from the oxygen partial pressure (pO_2), oxygen saturation (sO_2) and total hemoglobin in blood (ctHb) where the dyshemoglobins (carboxyhemoglobin (COHb) and methemoglobin (MetHb)) are subtracted to provide the concentration of functional hemoglobin. Thus [35]:

$$ctO_2 = \alpha O_2 \times pO_2 + (sO_2 \times ctHb \times (1 - FCOHb - FMetHb))$$

Where αO_2 (solubility coefficient of oxygen in blood) = 0.0105 mmol/L/kPa (0.00314 mL/dL/mmHg) [24]

Reference interval ctO_2 – examples

	mmol/L	mL/dL
Male (a):	8.4–9.9	18.8–22.2
Female (a):	7.1–8.9	15.9–19.9

[36] a: arterial

Delivery of oxygen to tissue cells

Critically ill patients are particularly susceptible to tissue hypoxia, and determining the adequacy of tissue oxygenation is of major importance in critical care [37, 38]. Oxygen delivery (DO_2) is defined as the product of cardiac output (Q) and ctO_2 of arterial blood, thus:

$$DO_2 = Q \times ctO_2$$

This relationship reflects the synergistic importance of both cardiac output and ctO_2 of arterial blood for the adequacy of tissue oxygenation, and highlights that oxygen delivery depends on the combined function of blood, heart and lungs (see oxygen status).

For example, a normal ctO_2 in arterial blood does in itself not guarantee adequate tissue oxygenation if cardiac output is decreased. Alternatively, the negative effect of a low ctO_2 on tissue oxygenation can be compensated by increased cardiac output. The relationship between ctO_2 and pO_2 at two ctHb levels is described graphically in Fig. 6.

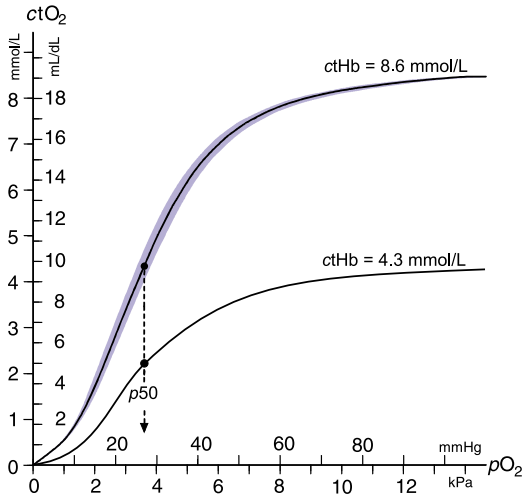


FIG. 6: The blood oxygen binding curve [39].
*p*50: value where hemoglobin is 50% satured with oxygen (see p50)

Why measure ctO_2?

ctO_2 is an expression of the amount of oxygen in blood and is used to determine the risk of tissue hypoxia, a major factor in the pathogenesis of organ dysfunction and failure, and thereby the necessity for supplemental-oxygen therapy and/or red cell transfusion.

Causes of decreased ctO_2

Decreased ctO_2 can result from:

- Reduction in pO_2
- Reduction in ctHb
- Reduction in sO_2

Decreased pO_2 and sO_2 result from decreased oxygenation of blood in the lungs, i.e. impairment of diffusion of oxygen from alveoli to blood across the alveolar membrane.

Causes associated with such impairment are identical to those for pO_2 (see pO_2), ctHb or sO_2.

*p*50

*p*50 is defined as the partial pressure of oxygen (pO_2) in blood at 50 % oxygen saturation (sO_2). It is a calculated parameter derived from pO_2 and sO_2 by extrapolation along the oxyhemoglobin dissociation curve (ODC) [40]. A distinction is made between "actual *in vivo*" *p*50 and "standard" *p*50 (*p*50(st)). *p*50(st) is "actual *in vivo*" *p*50, corrected to pH of 7.4 at a pCO_2 of 5.3 kPa (40 mmHg) and 37 °C (98.6 °F) [24]. *p*50(st) eliminates the effect of local pH, pCO_2 and temperature, so that an abnormal *p*50(st) can only be due to either an abnormal concentration of 2,3-DPG (Fig. 7) or a structural abnormality in hemoglobin that affects its affinity for oxygen. From the perspective of oxygen uptake and release, *in vivo p*50 is what matters [72]. Unless otherwise specified, in the text below the term *p*50 = *in vivo p*50.

Interpretation of *p*50 values

Increased *p*50 implies decreased hemoglobin affinity for oxygen, and thereby enhanced oxygen release to the tissues at the potential expense of decreased uptake of oxygen to hemoglobin in the lungs.

Decreased p50 implies increased hemoglobin affinity for oxygen, and therefore impaired oxygen release to the tissues.

	Increased p50	Decreased p50
Hemoglobin affinity for oxygen	↓	↑
Oxygen release to tissue	↑	↓

Reference interval *p*50 – examples

	kPa	mmHg
Male (a):	3.2–3.8	24–28
Female (a):	3.2–3.7	24–28
Male (v):	3.4–4.1	25–30
Female (v):	3.4–4.1	26–31
Newborn:	2.5–3.2	19–24

[36, 41] a: arterial; v: venous

The oxyhemoglobin dissociation curve (ODC) and concept of *p*50

The oxygen-delivery function of hemoglobin, that is its ability to "uptake" oxygen in the microvasculature of the lungs and "release" it in the microvasculature of the tissues is made possible in part by a reversible change in the structure of the hemoglobin molecule that alters its affinity for oxygen, and thereby its tendency to either bind or release oxygen. pO_2 (see pO_2) is one of a number of extraneous factors determining the relative affinity of hemoglobin for oxygen. Blood exposed to the oxygen-rich alveolar air has the highest pO_2 and it is this high pO_2 that drives the rapid binding of oxygen to hemoglobin, resulting in sO_2 close to 100 %. By contrast, tissue pO_2 is much lower facilitating oxygen release from hemoglobin to the tissues. The relationship between pO_2 and sO_2 is described graphically by the ODC (Fig. 7). *p*50 is defined by this curve, being the pO_2 at which hemoglobin is 50 % saturated with oxygen [42].

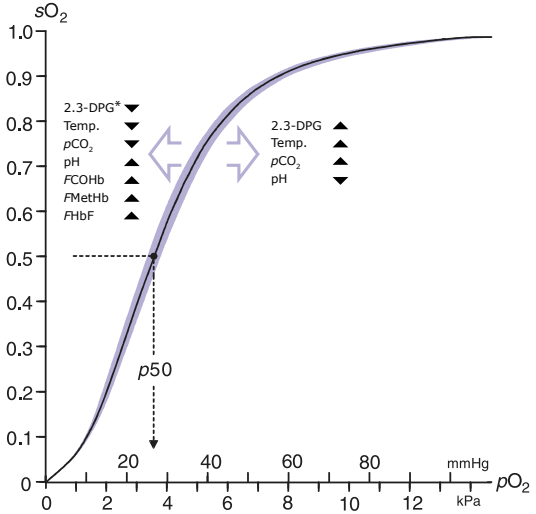


FIG. 7: Oxyhemoglobin dissociation curve, including extraneous factors determining left and right shift.

*2,3-diphosphoglycerate (2,3-DPG) is a highly anionic organic phosphate that is present in human erythrocytes at about the same molar ratio as hemoglobin. It binds to deoxyhemoglobin but not the oxyhemoglobin form, therefore diminishing the hemoglobin affinity for oxygen with a factor of up to 26. This is essential in enabling hemoglobin to release oxygen in tissue capillaries [43].

As a reference point on the ODC, *p*50 is an index of the hemoglobin affinity for oxygen and reflects the degree of right and left shift on the graph.

Factors influencing *p*50

The ODC, and therefore *p*50, is affected by local factors working in concert to either increase or decrease the hemoglobin affinity for oxygen [42]. The factors are listed in Fig. 7.

Decrease in *p*50

Under conditions of increased pH (alkalosis), decreased pCO_2, decreased temperature or decreased 2,3-DPG, the ODC curve is shifted to the left (Fig.7). In the lungs where some of these conditions prevail physiologically, the decreased *p*50 and the associated increased hemoglobin affinity for oxygen facilitates the binding of oxygen.

Increase in *p*50

In contrast, decreased pH (acidosis), increased pCO_2, increased temperature and increased 2,3-DPG, all cause the ODC curve to shift to the right (Fig. 7). In the tissues where some of these conditions prevail physiologically, the resulting increased *p*50 and the associated decreased hemoglobin affinity for oxygen facilitates oxygen release to tissue.

In critical illness, many factors affecting *p*50 may sometimes act simultaneously. Acidemia increases *p*50, but at the same time decreases 2,3-DPG production, decreasing *p*50. Alkalemia does the opposite. Prolonged hypoxemia increases 2,3-DPG concentrations and thus *p*50.

Reasons for determining *p*50

- Unexplained erythrocytosis (increased erythrocyte production)
- Unexplained cyanosis with or without anemia
- Helpful for the assessment of oxygen delivery to the tissues in critical illness
- Diagnosing tissue hypoxia caused by a high hemoglobin affinity for oxygen
- Elucidating the cause of apparent discordance between the two
- routinely used measures of blood oxygenation: pO_2 and sO_2

Causes of increased *p*50 [48]

- Acute acidosis
- Hypercapnia
- Fever
- Hemoglobinopathies (inheritance of a low-affinity hemoglobin variant)
- Sepsis

Causes of decreased *p*50 [48]

- Acute alkalosis
- Hypocapnia
- Hypothermia
- Presence of fetal hemoglobin (HbF)
- Carboxyhemoglobinemia (carbon monoxide poisoning)
- Methemoglobinemia
- Hemoglobinopathies
- Inherited deficiency of 2,3-DPG

Diagnostic value of *p*50 – examples

Critically ill patients may suffer reduced tissue oxygen delivery from varying combinations of low cardiac output states and anemia. Major vascular obstruction can cause severe regional ischemia. In these scenarios, increasing the *p*50 should improve oxygen extraction for a given venous oxygen tension.

Chronic anemia is associated with increased concentration of 2,3-DPG [17] and consequent a right shift in the ODC (increased *p*50). This is a protective, adaptive response that mitigates the potential deleterious effect of anemia on oxygen delivery; reduced hemoglobin affinity for oxygen, reflected in the increased *p*50, results in increased delivery of oxygen to tissue cells.

Fetal hemoglobin (HbF), i.e. the hemoglobin present during fetal development, persists for 3–6 months after birth. HbF has increased affinity for oxygen compared with hemoglobin A (HbA), evident as a left shift in the ODC curve of HbF compared with HbA. This is the reason why the *p*50 reference interval is lower for neonates [44].

*p*50(st) is valuable in the investigation of patients with erythrocytosis [45]. Among the many causes of erythrocytosis is inheritance of hemoglobin variants with an abnormally high affinity for oxygen. Close to 100 high-affinity hemoglobin variants have been described [46]; each is individually rare, but all are associated with a decreased *p*50.

The converse of the above, i.e. inheritance of a hemoglobin variant with an abnormally low affinity for oxygen is a rare cause of anemia and/or cyanosis [47]. Affected individuals release oxygen to the tissues far more efficiently than normal and as a result there is a reduced erythropoietic drive from the kidneys, leading to reduced erythrocyte numbers and reduced hemoglobin (anemia). Although arterial pO_2 is normal, reduced hemoglobin affinity for oxygen ensures that arterial sO_2 is reduced and deoxyhemoglobin (HHb) is increased, leading to cyanosis. Such low-affinity hemoglobin variants are associated with an increased *p*50(st) that is diagnostically useful [47].

Carboxyhemoglobin – COHb

*F*COHb is the fraction of total hemoglobin (*c*tHb) which is present as carboxyhemoglobin (COHb). By convention the fraction is expressed as a percentage (%). Thus [24]:

$$\mathrm{COHb}(\%) = \frac{c\mathrm{COHb}}{c\mathrm{tHb}} \times 100$$

In the range of 0–60 % COHb in arterial (COHb(a)) and venous blood (COHb(v)) is similar, i.e. either venous or arterial blood may be analyzed [49]. In most medical texts *F*COHb(a) is referred to as simply COHb, which also is used in the text below.

Reference interval COHb – examples

Reference interval depends on the level of exposure to carbon monoxide (CO) [8, 107]

Adult non-smoker:	0.5–1.5 %
Adult smoker:	1.5–5.0 %
Adult heavy smoker:	As high as 10 %
Newborn:	As high as 10–12 %, due to increased Hb turnover combined with a less developed respiratory system

What is COHb?

Carboxyhemoglobin is the product of CO binding to hemoglobin. CO crosses the alveolar membrane easily and binds to hemoglobin with a higher affinity (~250 times) than oxygen [50]. Since COHb is incapable of binding oxygen, it is categorized as a dyshemoglobin (see Hb).

The level of COHb in blood is determined by the amount of CO in blood. In healthy persons <2 % of total hemoglobin is present as COHb; this is the result of the small amount of CO produced endogenously during normal catabolism of heme to bilirubin [51] and the CO normally present in inspired air. The oxygen-carrying capacity of blood is reduced by the presence of COHb, and increased COHb is associated with risk of inadequate oxygen delivery and resulting tissue hypoxia.

When should COHb be measured?

The principal clinical utility of this test is in the diagnosis and monitoring of carbon monoxide poisoning [52]. Symptoms associated with carbon monoxide poisoning are indicated in Table II.

COHb (%)	Effect/Symptoms
10	No appreciable effect, except increased shortness of breath on vigorous exertion
20	Shortness of breath on moderate exertion, intermittent headache
30	Persistent headache, fatigue, dizziness, clouded judgment
40–50	Confusion, fainting, collapse
60–70	Convulsions, coma, respiratory failure; can be fatal
80	Immediately fatal

TABLE II: Hypoxic effect of increased COHb [56].

Cherry-red coloration of the skin is a well-known and more specific sign of carbon monoxide poisoning due to the color of COHb, but this sign is usually only evident post mortem [52].

Causes of increased COHb

Increased COHb is the result of increased CO in blood. The origins of this increased CO are either exogenous, endogenous or a combination of both; exogenous being more common than endogenous.

Exogenous causes of increased COHb, i.e. intentional or non-intentional carbon monoxide poisoning, can arise in a range of scenarios [52]; the most common include:

- Exposure to vehicle exhaust fumes
- Exposure to fumes produced during house fires/bonfires
- Exposure to fumes from faulty domestic gas heating systems
- Exposure to fumes from kerosene/paraffin heaters

The risk of carbon monoxide poisoning and raised COHb is increased if these exposures occur in a closed or poorly ventilated environment. COHb levels in cases of carbon monoxide poisoning are in general much higher than those associated with endogenous causes and are typically in the range of 15–30 %, but can be as high as 50–70 % if the carbon monoxide concentration of inspired air is particularly high [54].

Endogenous causes of increased COHb are confined to those pathological conditions associated with increased heme catabolism [53]. They are:

- Hemolytic anemias
- Severe inflammatory disease, critical illness, e.g. sepsis

These pathologies may increase COHb levels around 3–10 % [53].

Combined exogenous and endogenous cause

Methylene chloride (dichloromethane) toxicity is a rare cause of clinically significant increase in COHb. The increased level arises because methylene chloride metabolism in the liver is associated with increased endogenous production of CO [55]. In this case increased COHb has, uniquely, a combined exogenous and endogenous cause.

Interpretation of COHb in cases of delayed measurement

COHb has a half-life of 3–4 hours when room air is inspired; this is reduced to 30–90 minutes if 100 % oxygen is inspired [52]. The relatively short half-life of COHb means that measured COHb may provide the false impression of low CO exposure if there is a delay between patient removal from exposure and blood sampling. For example, peak measured COHb of 30 % at the site of exposure could theoretically be reduced to 7 % 6 hours later, if room air is inspired. A similar reduction could occur over a period of only 2 hours if the patient is being administered with 100 % oxygen. It is important when interpreting measured COHb results that this physiological aspect is taken into account. Whilst a raised measured COHb (>10 %) almost invariably indicates carbon monoxide poisoning, a normal measured COHb might not be sufficient to exclude the diagnosis if there has been delay in blood sampling, particularly if oxygen has been administered.

Blood oxygenation during carbon monoxide poisoning

The most significant effect of carbon monoxide poisoning and the resulting carboxyhemoglobinemia is reduced total oxygen (ctO_2) in blood, and consequent tissue hypoxia. Despite this, oxygenation status, as assessed by pulse oximetry (SpO_2) and blood gas parameters (pO_2 and sO_2), remains apparently normal. SpO_2 is falsely normal in the context of carbon monoxide poisoning as (most) pulse oximeters are unable to distinguish COHb and O_2Hb [57]. pO_2 is unaffected by carbon monoxide poisoning.

Methemoglobin – MetHb

*F*MetHb is the fraction of total hemoglobin (ctHb) that is present as methemoglobin (MetHb). By convention the fraction is expressed as a percentage (%). Thus [24]:

$$\text{MetHb (\%)} = \frac{c\text{MetHb}}{c\text{tHb}} \times 100$$

In most medical text boxes MetHb(a) is referred to as simply methemoglobin (MetHb), which will be used in the text below.

Reference interval MetHb – example

Adult (wb):	0.04 – 1.52 %

[4] wb: whole blood

What is MetHb?

The oxygen-binding property of hemoglobin depends on the four iron atoms contained within the structure of hemoglobin (see ctHb). If the iron ions are in the reduced ferrous (Fe^{2+}) state, a reversible bond with oxygen can be formed. The distinguishing feature of MetHb is that one or more of the four iron ions are in the oxidized ferric state (Fe^{3+}) rather than the ferrous state (Fe^{2+}) and MetHb is therefore incapable of binding oxygen [58], categorizing MetHb as a dyshemoglobin. Normal erythrocyte metabolism is associated with continuous production of MetHb from hemoglobin; however, erythrocytes also have mechanisms for the reversible process, conversion of MetHb back to hemoglobin. This ensures that in healthy persons no more than 1 – 2 % of total hemoglobin is present as MetHb. The reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}), necessary for the conversion of MetHb to hemoglobin, occurs due to the action of the enzyme cytochrome b5 reductase [9].

The oxygen-carrying capacity of blood is reduced by the presence of MetHb, and increased MetHb is associated with risk of inadequate oxygen delivery and tissue hypoxia. The severe hypoxia associated with marked increase in MetHb can have a fatal outcome.

Increased MetHb will have an impact on FO_2Hb values (see O_2Hb); however, the parameters pO_2, sO_2 and SpO_2 (pulse oximetry) are unaffected by increased MetHb.

When should MetHb be measured?

The most common reason for measuring MetHb is to diagnose patients with unexplained cyanosis, and patients suspected of suffering from the toxicological effects of a range of chemicals/prescribed drugs.

Causes of increased MetHb

Methemoglobinemia is an increase in the concentration of MetHb. It may be inherited or acquired. Acquired methemoglobinemia is far more common than inherited.

Acquired methemoglobinemia (acute) occurs as a result of exposure to oxidative and toxic chemicals/drugs (see list on next page) [59, 60, 61]. The oxidative potential of these substances results in an abnormally increased production of MetHb that exceeds the maximum rate at which MetHb can be converted back to hemoglobin.

Inherited methemoglobinemia (chronic) results from a deficiency of the enzyme cytochrome b5 reductase and therefore a reduced ability to convert MetHb to hemoglobin [62, 63]. Methemoglobinemia is also a feature of hemoglobin M disease, which is characterized by an inherited defect in the hemoglobin structure that inhibits conversion of MetHb to hemoglobin [64].

A non-exhaustive list of drugs/chemicals that have the capacity to induce methemoglobinemia [58, 65, 66]:

- Dapsone
- Amyl nitrate
- Aniline
- Benzocaine
- Chloroquinone
- Lidocaine
- Mafenide acetate
- Naphthalene
- Nitric oxide
- Nitrobenzene
- Nitroglycerine
- Paraquat
- Primaquine
- Sodium valproate
- Sulphonamides

Symptoms of methemoglobinemia

Symptoms depend on the severity of the increase and whether the condition is chronic or acute. In general, with inherited methemoglobinemia MetHb rarely exceeds 30 %; higher levels (up to 50–70 %) are seen among those with acquired methemoglobinemia [65, 66].

- **Mild increase (MetHb in the range of 2–10 %)** is usually asymptomatic
- **Moderate increase (10–30 %)** is almost always associated with some degree of cyanosis. Breathlessness on mild exertion may be experienced
- **More severe increase (30–50 %)** may cause cyanosis, headache, breathlessness, dizziness, lethargy and fatigue
- **Severe increase (50–70 %)** may cause cyanosis, cardiac dysrythmias, confusion, seizures, drowsiness, coma and lactic acidosis
- **MetHb >70 %** is associated with severe tissue hypoxia with resulting organ failure and is frequently fatal

Cyanosis in methemoglobinemia

Cyanosis is almost always present in patients with methemoglobinemia; it results from the dark blue/brown color of MetHb. Unlike the cyanosis that can occur in respiratory and cardiac disease, which results from increased concentrations of deoxyhemoglobin (HHb), the cyanosis of methemoglobinemia is not alleviated by administration of supplemental oxygen and is not associated with reduced pO2. Sampled venous blood containing increased concentrations of MetHb has a characteristic dark color, often described as "chocolate brown" [67].

Shunt

The shunt fraction (*F*Shunt) is the portion of blood that passes the lungs without being fully oxygenated in the alveoli. *F*Shunt is defined as the ratio between shunted cardiac output (Q_s) and total cardiac output (Q_t). It can be calculated as the ratio between alveolar arterial total oxygen content difference and the arterio-mixed venous total oxygen content difference. Thus [35]:

$$FShunt = \frac{Q_s}{Q_t} = \frac{ctO_2(A) - ctO_2(a)}{ctO_2(A) - ctO_2(v)}$$

where $ctO_2(A)$: total oxygen content in alveolar air
$ctO_2(a)$: total oxygen content in arterial blood
$ctO_2(v)$: oxygen content in mixed venous blood

Example: *F*Shunt of 0.1 indicates that 10 % of venous blood passes the lungs without being oxygenated, whereafter it is mixed with fully oxygenated blood.

*F*Shunt can be calculated with the input of simultaneously collected arterial and mixed venous samples. The mixed venous sample must be collected from the pulmonary artery. If pulmonary artery blood samples are not available, *F*Shunt can be estimated from a single arterial sample by using a fixed alveoli-mixed venous oxygen content ($ctO_2(A) - ctO_2(v)$) value of 2.3 mmol/L (5.1 mL/dL) [39].

Reference interval shunt – example

	%	Fraction
Adult	4–10	0.04–0.10

[39]

Ventilation/perfusion ratio, dead space and shunt

The relationship between the amount of air reaching the alveoli and the amount of blood reaching the alveoli is referred to as the ventilation-perfusion (V/Q) ratio. A V/Q ratio equal to 1 refers to normal ventilation and normal perfusion conditions (Fig. 8).

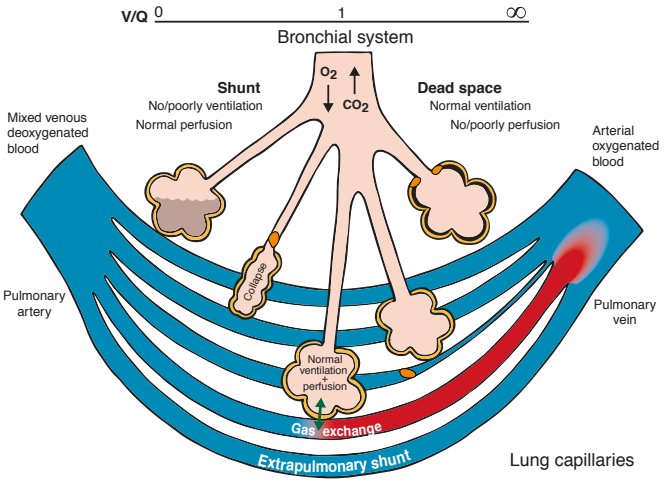


FIG. 8: Illustration of shunt and dead space, see description in the text below. Blue: deoxygenated blood; Red: oxygenated blood; Orange: obstruction

An increased V/Q ratio occurs in pulmonary embolism, where perfusion is impaired in relation to ventilation. In this case the alveolar ventilation will not be physiologically effective, due to the capillary supply being occluded and no perfusion taking place. This alteration of the V/Q ratio is referred to as dead space.

A decreased V/Q ratio is seen in most lung disorders, where the ventilation is impaired in relation to normal blood flow. Generally, when the V/Q ratio decreases, the arterial pO_2 decreases and the

arterial pCO_2 increases. Here the alveolar perfusion will not be physiologically effective, because the area is not ventilated. This is referred to as shunt.

A shunt can occur as a result of blood flowing right-to-left through a cardiac opening (extrapulmonary shunt) or in pulmonary arterio-venous malformations (intrapulmonary shunt). In extrapulmonary shunting a significant proportion of deoxygenated blood is allowed to completely bypass the pulmonary circulation. In intrapulmonary shunting no gas exchange takes place, as ventilation fails to reach the perfused area [218].

When the V/Q ratio is zero, the patient is said to have a true shunt, as the blood has had no opportunity for gas exchange, and in both extrapulmonary and intrapulmonary shunt deoxygenated blood is allowed to enter the left-sided systemic circulation. Patients with true shunt do not respond to supplemental-oxygen (100 %) therapy [218].

In the healthy individual, the normal right-to-left shunt is about 3 % of the cardiac output [220].

Conditions, when pulmonary capillary perfusion is in excess of alveolar ventilation, are referred to as relative shunt or shunt like effect. These conditions are readily corrected by oxygen therapy [220].

Why determine the *F*Shunt?

*F*Shunt is used as an indicator of adequate oxygen uptake in the lungs, highlighting the extent to which the pulmonary system contributes to hypoxemia. In critically ill patients, calculation of *F*Shunt seems to be a reliable index for evaluating and quantifying pulmonary oxygen transfer deficit [219].

When should *F*Shunt be determined?

Determination of *F*Shunt is useful in diagnosis, assessment and monitoring of critically ill patients with severe respiratory disease or respiratory failure due to conditions other than respiratory disease (e.g. cardiac diseases).

Interpretation guidelines for *F*Shunt in critically ill patients fitted with a pulmonary catheter

In critically ill patients fitted with a pulmonary catheter a *F*Shunt between 10–19 % would seldom require significant support. However a *F*Shunt between 20–29 % may be life-threatening in patients with limited cardiovascular function. If the *F*Shunt is greather than 30 % in this patient group, it usually requires significant cardiovascular support [219].

Causes of increased *F*Shunt

Intrapulmonary shunt [220]
- Acute respiratory distress syndrome (ARDS)
- Asthma
- Lung diseases with inflammation or edema that causes the membranes to thicken
- Pneumonia
- Cystic fibrosis
- Flail chest
- Pleural diseases
- Atelectasis
- Tuberculosis
- Smoke inhalation

Extrapulmonary congenital heart diseases [220]
- Pulmonary vascular tumors
- Intrapulmonary fistulas
- Cardiogenic pulmonary edema

Relative shunt [220]
- Hyperventilation
- Pneumoconiosis
- Chronic bronchitis
- Asthma
- Emphysema
- Cystic fibrosis
- Drugs which may cause an increase in cardiac output

Symptoms associated with increased *F*Shunt

Symptoms related to increased *F*Shunt are identical to the symptoms associated with decreased pO_2 (See pO_2).

Acid-base status

Normal cell function depends on maintaining pH of extracellular fluid at around 7.4. The physiological process responsible for this, called acid-base homeostasis (or acid-base balance) involves continuous regulation of carbon dioxide (CO_2) excretion by the lungs as well as regulation of non-carbonic acid excretion and bicarbonate (HCO_3^-) buffer regeneration by the kidneys. Disturbance of acid-base homeostasis is characterized by abnormality in one or more of the three following parameters: pH, pCO_2 and HCO_3^-. When these parameters are within their respective reference intervals, it may be assumed that the mechanisms involved in maintaining the pH of blood within healthy limits are functioning adequately and normal acid-base status is assured.

Imbalance of acid-base status may be caused by a primary disturbance of CO_2 excretion (respiratory) or primary disturbance of non-carbonic acid excretion/HCO_3^- regeneration (metabolic). Respiratory and metabolic disturbances may both be present (mixed cause). These abnormalities may be adjusted by the organism's acid-base regulatory mechanism (Fig. 9).

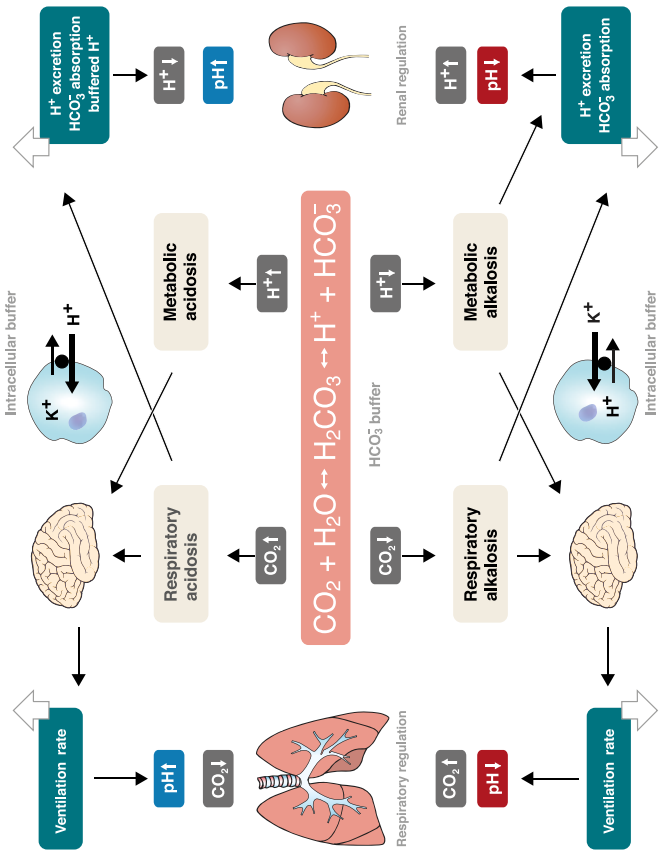


FIG.9: Mechanisms for regulation of extracellular pH (acid-base homeostasis).

The Siggaard-Andersen acid-base chart

The Siggaard-Andersen acid-base chart [68] (Fig. 10) and the acid-base flow chart (Fig. 11) is an aid in the description/interpretation of the acid-base status of blood.

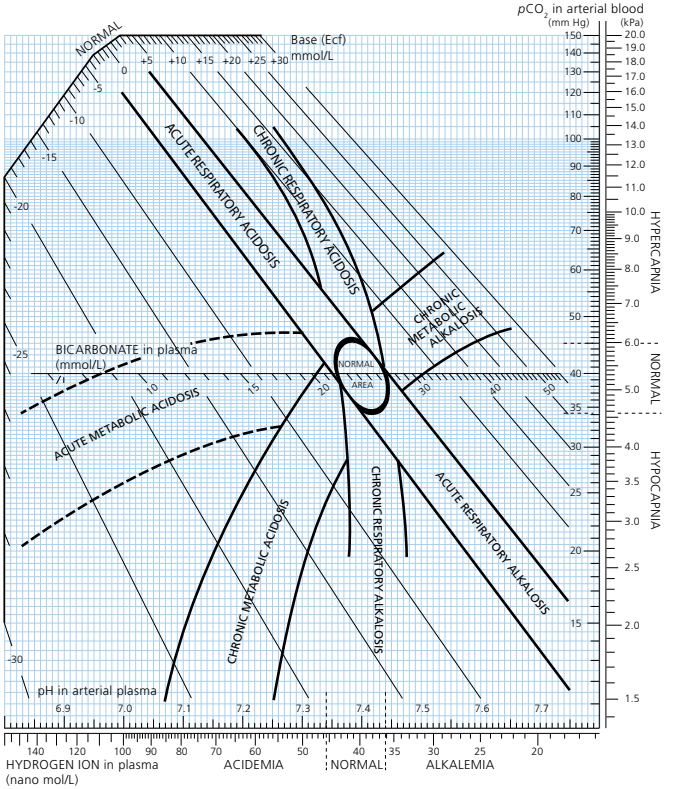


FIG. 10: The Siggaard-Andersen chart shows the normal values and values to be expected in typical acid-base disturbances, i.e. acute and chronic respiratory acidosis and alkalosis. Abscissa: pH, ordinate: pCO_2, oblique: cBase(Ecf). The HCO_3^- is indicated on the scale in the middle of the chart.

Defining terms used in interpretation of acid-base status [6, 23, 68, 69, 70]

Acidemia: Increased concentration of hydrogen ions (H^+) in blood, that is reduced blood pH; typically defined as pH <7.35.

Acidosis: Clinical term for the process that gives rise to acidemia. It is associated with pH <7.35 initially, but the process can include a compensatory response that might result in normalization of pH.

Alkalemia: Decreased concentration of H^+ in blood, that is increased blood pH; typically defined as pH >7.45.

Alkalosis: Clinical term for the process that gives rise to alkalemia. It is associated with pH >7.45 initially, but the process can include a compensatory response that might result in normalization of pH.

Metabolic acidosis: Acid-base disturbance that results from reduction in HCO_3^-. It is always associated with decreased pH. The normal physiological response to metabolic acidosis is increased ventilation and thereby reduced pCO_2. This respiratory compensatory mechanism increases pH towards normal, i.e. reduces the severity of the acidosis.

Metabolic alkalosis: Acid-base disturbance that results from increase in HCO_3^-. It is always associated with increased pH. The normal physiological response to metabolic alkalosis is decreased ventilation and thereby increased pCO_2. This respiratory compensatory mechanism decreases pH towards normal, i.e. reduces the severity of the alkalosis.

Hypercapnia: Increased pCO_2, i.e. >6.0 kPa (45 mmHg). A distinction is made between acute and chronic hypercapnia, the latter being a feature of chronic respiratory disease.

Hypocapnia: Decreased pCO_2, i.e. <4.5 kPa (34 mmHg).

Permissive hypercapnia: Pragmatic clinical decision applied to some patients with respiratory failure who require mechanical ventilation. Ventilation is adjusted to minimize the risk of ventilator-associated lung injury and these ventilator settings often result in hypercapnia. In this instance (mild) hypercapnia is the predicted consequence of a therapeutic objective.

CO_2 retention: Pathological accumulation of CO_2 in blood due to reduced CO_2 excretion by the lungs. It leads to hypercapnia and respiratory acidosis.

Respiratory acidosis: Acid-base disturbance that results from a primary increase in pCO_2. It is always associated with decreased pH (in the absence of metabolic compensation) and is almost invariably associated with alveolar hypoventilation. It is usually, though not necessarily, the result of respiratory disease.

Respiratory alkalosis: Acid-base disturbance that results from a primary decrease in pCO_2. It is always associated with increased pH (in the absence of metabolic compensation) and alveolar hyperventilation and can result from both respiratory and non-respiratory disease.

Respiratory compensation: Physiological response to a non-respiratory acid-base disturbance (metabolic acidosis and metabolic alkalosis) that aims to normalize pH. It involves adjustment in alveolar ventilation and a resulting change in pCO_2. Compensation for metabolic acidosis involves increased ventilation and a resulting reduced pCO_2, whereas compensation for metabolic alkalosis involves decreased ventilation and increased pCO_2.

Acid base flowchart

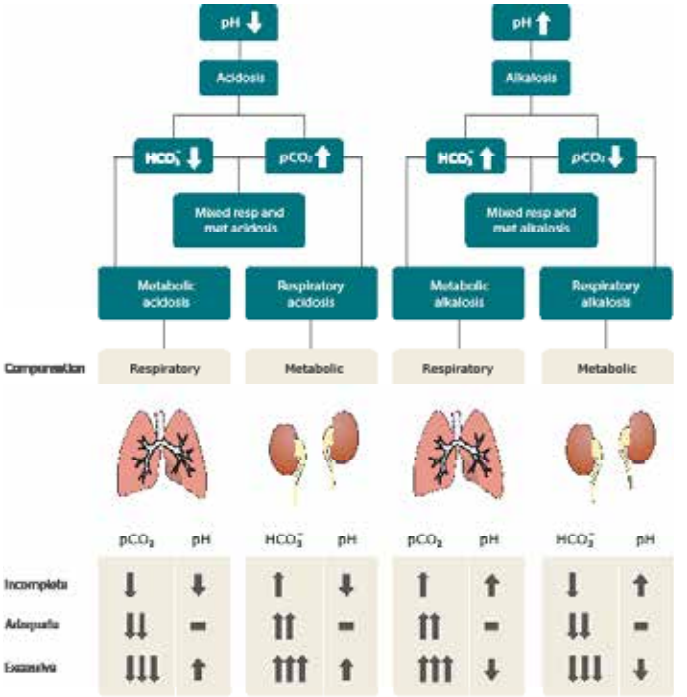


FIG. 11: Acid-base flowchart.

⬆ : Increasing value

⬇ : Decreasing value

= : Within normal reference interval

pH

The degree of acidity or alkalinity of any liquid (including blood) is a function of its hydrogen ion concentration [H^+], and pH is simply a way of expressing hydrogen ion activity. The relationship between pH and hydrogen ion concentration is described thus [24]:

$$\mathrm{pH} = -\log\ \mathrm{a}_{H^+}$$

where a_{H+} is hydrogen ion activity

Low pH is associated with acidosis and high pH with alkalosis.

Reference interval pH – examples

Adult, child (a):	7.35–7.45
Adult, child (v):	7.32–7.43
Adult 60–90 years (a):	7.31–7.42
Adult >90 years (a):	7.26–7.43
Newborn	
Premature 48 hours (a):	7.35–7.50
Full-term 24 hours (a):	7.29–7.45
Cord blood (a):	7.18–7.38
Cord blood (v):	7.25–7.45

[4] a: arterial; v: venous

Why measure pH?

Normal metabolism is associated with continuous production of H^+ and CO_2 that both tend to decrease pH. Despite this normal tendency towards acidosis, pH remains tightly controlled within very narrow limits (7.35–7.45). Even a small deviation outside this normal range can have numerous detrimental effects on cellular metabolism

that translates to tissue/organ dysfunction; and pH lower than 6.8 or higher than 7.8 is usually incompatible with life. Therefore, it is essential that abnormal pH is detected and the cause identified, to be able to make the necessary medical intervention. The maintenance of normal pH (acid-base homeostasis) is a complex synergy of actions involving lungs, kidneys, brain and chemical buffers present in blood (Fig. 9) [6]. pH provides evidence that these homeostatic mechanisms are either working normally or are disturbed in some way. Although measurement of pH is essential for the assessment of patient acid-base status (see acid-base), it is not sufficient of itself; two other parameters, pCO_2 and bicarbonate (HCO_3^-) are equally necessary (see pCO_2 and HCO_3^-) to accurately identify an acid-base disturbance and formulate effective therapy.

When should pH (pCO_2 and HCO_3^-) be measured?

pH (along with pCO_2 and HCO_3^-) is used both to diagnose and to monitor acid-base disturbance [73]. Given the complexity of acid-base homeostasis, involving as it does the function of several organ systems, measurement of pH (pCO_2 and HCO_3^-) has clinical value in the context of many severe acute or critical illnesses as well as significant injury (trauma). pH measurements are thus usually made in hospital settings, e.g. emergency room, operation room, critical care/intensive care setting, etc. In broad terms all acid-base disturbances can be attributed to one of three main causes:

- Disease of, or damage to, any one of the three organs, (lungs, kidneys, brain) whose function is necessary for keeping pH within normal healthy limits
- Disease or condition that results in increased production of metabolic acids (e.g. lactic acid, keto acids) such that the homeostatic mechanisms for the maintenance of normal pH are overwhelmed
- Medical intervention (mechanical ventilation as well as a number of drugs can cause or contribute to acid-base disturbance)

Causes of acid-base disturbances

A non-exhaustive list of diseases or conditions in which acid-base may be disturbed and pH (pCO_2 and HCO_3^-) measurement may be useful for diagnosis and/or monitoring includes [74]:

- Respiratory failure/distress (e.g. COPD, pneumonia, pulmonary edema, pulmonary embolism, asthma, acute respiratory distress syndrome, Guillain-Barré syndrome and traumatic chest injury)
- Acute/chronic renal failure
- Diabetic ketoacidosis
- Circulatory failure/shock (e.g. hemorrhage, burns, sepsis, cardiac arrest and other conditions with increased production of lactic acid)
- Liver failure (associated with decreased elimination of lactic acid)
- Traumatic brain injury, cerebral edema, brain tumor
- Fetal distress
- Drug overdose/toxic poisoning (e.g. salicylate, antacids, opiates, barbiturates, diuretics, methanol, ethanol and ethylene glycol)

Symptoms of acid-base disturbances

Symptoms that might indicate acid-base disturbance and prompt measurement of pH, pCO_2 and HCO_3^-:

- Reduced consciousness, drowsiness, confusion
- Convulsions/seizures
- Reduced blood pressure
- Reduced or increased respiratory rate
- Cardiac arrhythmia
- Anuria/polyuria
- Muscle spasm/tetany
- Electrolyte disturbance
- Hyperglycemia
- Anemia/hemorrhage
- Hypoxemia

Clinical interpretation

See acid-base status chapter, Fig. 9 and Fig. 11.

Although measurement of pH can identify an acid-base disorder, either acidosis or alkalosis, and provides an indication of its severity, it provides no indication of its cause. This depends on two further parameters: pCO_2 and HCO_3^-, which are related to pH thus:

$$\mathrm{pH} \propto \frac{[HCO_3^-]}{pCO_2}$$

This relationship allows distinction between acid-base disturbance caused by respiratory disease, in which pCO_2 is the primary abnormality, and acid-base disturbance caused by metabolic (non-respiratory) disease, in which HCO_3^- is the primary abnormality.

With these two additional parameters it is possible to classify the acid-base disturbance as one of four types [6]:

Respiratory acidosis: Characterized by decreased pH, increased pCO_2 and normal HCO_3^-

Respiratory alkalosis: Characterized by increased pH, decreased pCO_2 and normal HCO_3^-

Metabolic acidosis: Characterized by decreased pH, decreased HCO_3^- and normal pCO_2

Metabolic alkalosis: Characterized by increased pH, increased HCO_3^- and normal pCO_2

Because of the prime physiological importance of maintaining pH within normal limits, acid-base disturbances are associated with a compensatory response that aims to normalize pH (Fig. 11).

Metabolic disturbance, in which the primary determinant of abnormal pH is abnormal HCO_3^- concentration, is associated with a respiratory compensatory response that changes pCO_2, so that the ratio of HCO_3^- to pCO_2 and thereby pH is getting closer to normal. In a similar way respiratory disturbance provokes a compensatory response that changes HCO_3^-. In practice these compensatory responses move pH towards normality but do not usually achieve normality, although this can occur. It is important to be aware that pH within the reference intervals does not exclude an acid-base disturbance; it may simply reflect this compensatory response. Mixed acid-base disturbance (alkalosis and acidosis) is a common reason for a patient with acid-base disturbance to have a normal pH. In such cases the abnormally high pH associated with alkalosis is covered by the abnormally low pH associated with acidosis.

Fetal scalp pH and umbilical-cord pH

In obstetric/perinatal care fetal scalp pH is often used as a stand-alone parameter to make clinical decisions during labor in high-risk pregnancies, when electronic fetal monitoring (EFM) indicates risk of fetal hypoxia. Reduction in fetal pH (acidosis) is indicative of hypoxia, a condition that may significantly affect the function of various fetal organ systems, such as the central nervous system and the cardiovascular system [75]. Since low pH (acidosis) is associated with risk of birth asphyxia and consequent neurologic injury [76], it is an indication for urgent delivery by cesarean section. This is reflected in the guidance from NICE (National Institute for Clinical Excellence) [77] which states that fetal scalp pH should be used whenever possible to confirm fetal distress suggested by EFM, before undertaking cesarean section. A fetal pH equal to or higher than 7.25 is considered normal and reassuring of no fetal distress but a fetal pH lower than 7.20 is usually considered unequivocal evidence of acidosis and a distressed fetus that needs urgent delivery [78]. However, fetal scalp pH results need to be interpreted in the context of each individual labor.

Just as fetal scalp pH is used to detect fetal acidosis and associated hypoxia risk during labor, umbilical-cord arterial pH provides the same evidence in relation to babies at the time of birth. Severe acidemia at birth is indicative of hypoxia and risk of hypoxia-mediated serious long-term neurological deficit, up to and including that associated with cerebral palsy. Umbilical-cord pH is measured at birth if the baby is considered at risk of hypoxia either because of complications during labor, or there is evidence of fetal distress (e.g. decreased scalp pH) during labor. NICE recommends umbilical-cord pH measurement for all babies delivered by cesarean section because of fetal distress, to identify birth asphyxia and neurologic injury [77]. The use of cord blood testing on all neonates is discussed in a review article [332].

pH in pleural fluid

Measuring the pH of pleural fluid is sometimes of value in the assessment of patients with pleural effusion. Normal pleural fluid has a pH of 7.60–7.66. Probably the most common use is in the management of patients whose pleural effusion is the result of pneumonia. For these patients, a pleural fluid pH <7.2 is indicative of advanced disease and need for urgent drainage of the pleural cavity. Clinical utility of pleural fluid pH, which is not confined to this patient group, is fully discussed in a review article [79].

Carbon dioxide partial pressure – pCO_2

Carbon dioxide (CO_2) is an acidic gas; the amount of CO_2 in blood is largely controlled by the rate and depth of breathing or ventilation. pCO_2 is the partial pressure of CO_2 in blood. It is a measure of the pressure exerted by that small portion (~5 %) of total CO_2 that remains in the gaseous state, dissolved in the blood plasma [22]. pCO_2 is the respiratory component of acid-base balance and reflects the adequacy of pulmonary ventilation. The severity of ventilatory failure as well as the chronicity can be judged by the accompanying changes in acid-base status (see acid-base status).

Reference interval pCO_2 – examples

	kPa	mmHg
Adult female (a):	4.26–5.99	32–45
Adult male (a):	4.66–6.38	35–48
Infant (a):	3.59–5.45	27–41
Newborn (a):	3.59–5.32	27–40
Neonate (c):	3.80–6.50	29–49

[4, 80] a: arterial; c: capillary

Physiological significance of pCO_2

Cell metabolism results in continuous production of CO_2, which must be eliminated by the lungs in expired air. CO_2 is delivered to the lungs by venous blood. Most (90 %) of the CO_2 produced in the body is transported in blood in the form of bicarbonate (HCO_3^-) (see HCO_3^-). HCO_3^- is not included in the pCO_2 measurement. CO_2 diffuses from blood to alveolar air through the alveolar-capillary membrane, and the rate of alveolar ventilation determines how much CO_2 is expired.

The amount of CO_2 dissolved in blood (pCO_2) determines its pH according to the following relationship derived from the Henderson-Hasselbalch equation [6]:

$$\mathrm{pH} \propto \frac{[HCO_3^-]}{pCO_2}$$

In order to keep pH within normal healthy limits, the amount of CO_2 excreted by the lungs must be continuously adjusted to match the amount of CO_2 currently being produced by the tissues. This is achieved by regulation of alveolar ventilation [21]; CO_2 is central to this regulation. The aortic/carotid chemoreceptors react on CO_2 changes and transduce signals to the respiratory center of the brain, resulting in a compensatory ventilation change (Fig. 9).

Why measure pCO_2?

Measurement of pCO_2:

- Is essential together with pH and HCO_3^- for the diagnosis and monitoring of acid-base disturbances [73]. pCO_2 reflects the "respiratory" contribution to acid-base status
- Provides evidence of the adequacy of alveolar ventilation
- Provides the means for distinguishing type I and type II respiratory failure (see respiratory failure below)
- Is used to monitor the safety/efficacy of oxygen therapy and mechanical ventilation in patients with type II respiratory failure

When should pCO_2 (pH and HCO_3^-) be measured?

Given the complexity of acid-base homeostasis, which is a prerequisite for proper organ function, measurement of pCO_2, along with pH and HCO_3^-, is of major importance in the assessment of severe acute or critical illness as well as significant injury (trauma). pCO_2 along with pH and HCO_3^- measurements are often taken in the emergency room or critical care/intensive care setting.

In broad terms all acid-base disturbances can be attributed to one of three main causes:

- Disease or condition with impaired function of the lungs and kidneys
- Disease or condition with increased production of organic acids (e.g. lactic acid, keto acids) or accumulation of toxic acids (e.g. methanol)
- Medical intervention (e.g. mechanical ventilation, drugs)

Causes of increased pCO_2

Resulting in respiratory acidosis [74]

- COPD – emphysema and chronic bronchitis
- Severe asthma
- Pulmonary edema
- Suppression of the respiratory center in the brain stem by drugs (e.g. opiates, barbiturates)
- Traumatic brain injury/stroke
- Guillain-Barré syndrome
- Inadequate mechanical ventilation (but intended with permissive hypercapnia)
- Morbid obesity (can cause hypoventilation)
- Metabolic alkalosis (compensatory response to preserve normal pH)

Causes of decreased pCO_2

Resulting in respiratory alkalosis [74]

- Stress-related hyperventilation due to pain or anxiety
- Acute respiratory distress syndrome (ARDS)
- Pulmonary embolism
- Hypoxia, hypoxemia (can induce increased alveolar hyperventilation)
- Severe anemia
- Salicylate overdose (salicylate stimulates the respiratory center)
- Excessive mechanical ventilation
- Metabolic acidosis (compensatory response to preserve blood pH)

Symptoms related to pCO_2 imbalance

Symptoms are identical to the acid-base disturbances for pH (see pH) and HCO_3^-.

Abnormal pCO_2 has effect on cardiovascular and central nervous systems that results in the following symptoms that might prompt measurement of pCO_2.

Symptoms of increased and decreased pCO_2

Increased pCO_2 >6.5 kPa (49 mmHg)	Decreased pCO_2 <2.7 kPa (20 mmHg)
· Bounding pulse · Warm flushed skin · Sweating · Headache · Confusion · Flapping hand tremor (asterixis) · Cardiac arrhythmia · Depressed tendon reflexes · Seizure · Stupor, coma	· Dizziness/light-headedness · Muscle cramps · Parasthesia (tingling "pins and needles" numbness) in hands and feet · Tetany

[74, 81]

Clinical interpretation

See acid-base status and pH chapters

Respiratory failure type I and II

The respiratory system consists of the gas-exchanging organ (lungs) and the ventilator pump (respiratory muscles and thorax) (see oxygen status). Pulmonary gas exchange is the process by which oxygen is extracted from inspired air into the blood and simultaneously CO_2 is eliminated from the blood and expired. Impaired pulmonary gas exchange defines respiratory failure. There are two types of respiratory failure [74]:

Type I:

Respiratory failure is impaired oxygenation of blood with unchanged ventilation. It is defined as pO_2(a) <8.0 kPa (60 mmHg)) (hypoxemia) in association with normal pCO_2 or reduced pCO_2(a) due to increased ventilation invoked by hypoxia.

Type II:

Respiratory failure (hypercapnic respiratory failure) is impaired oxygenation of blood with inadequate ventilation and is defined as pO_2(a) <8.0 kPa (60 mmHg) (hypoxemia) in association with pCO_2(a) >6.6 kPa (50 mmHg) (hypercapnia).

Bicarbonate – HCO_3^-

Actual ($cHCO_3^-$(P)) and standard ($cHCO_3^-$(P,st)) HCO_3^-

Bicarbonate (HCO_3^-) is an indicator of the buffering capacity of blood. Plasma pH depends on the ratio of HCO_3^- concentration to partial pressure of carbon dioxide (pCO_2). The goal of the bicarbonate/carbonic acid buffering system is to "neutralize" excess acid or base and maintain plasma pH within limits (Fig. 9). HCO_3^- is referred to as the metabolic component (non-respiratory) of the acid-base balance. After chloride, HCO_3^- is the second-most abundant anion in plasma and so it is significant for maintaining the electrochemical neutrality of plasma.

The concentration of HCO_3^- in blood plasma is calculated from measured pCO_2 and pH, using this equation derived from the Henderson-Hasselbalch equation. Thus [24]:

$$c\mathrm{HCO_3^-(P)} = 0.23 \times p\mathrm{CO_2} \times 10^{(\mathrm{pH}-6.095)}$$

Two bicarbonate types: Actual bicarbonate ($cHCO_3^-$(P)) is the result obtained from the HCO_3^- equation described above, and standard bicarbonate ($cHCO_3^-$(P,st)) is the $cHCO_3^-$(P) in plasma from blood that is equilibrated with a gas mixture with pCO_2 = 5.3 kPa (40 mmHg) and pO_2 ≥ 13.3 kPa (100 mmHg) at 37 °C (98.6 °F) during measurement [85].

Reference interval HCO_3^- – examples

	mmol/L, meq/L
Adult male:	22.2–28.3
Adult female :	21.2–27.0
Neonate:	17.5–28.7
Neonate premature cord blood (a, c):	22–31

[82–84] a: arterial; c: capillary

Physiological significance of HCO_3^-

Cell metabolism is associated with continuous production of CO_2, which must be eliminated from the body in expired air. Around 70–80 % of CO_2 is transported from tissues to lungs in blood plasma as HCO_3^- [22]. CO_2 diffuses due to its partial pressure gradient from tissue cells, where it is produced, to the interstitial fluid and then to blood plasma in the tissue microvasculature. A small amount (~5 %) is dissolved in plasma, but most (~90 %) diffuses from plasma to erythrocytes. Here a small amount (2–5 %) combines with deoxyhemoglobin to form carbaminohemoglobin, but most is converted to HCO_3^-. This conversion involves first hydration of CO_2 to carbonic acid (H_2CO_3) by the enzyme carbonic anhydrase, followed by immediate, almost complete dissociation of carbonic acid to H^+ and HCO_3^-. Thus:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

The hydrogen ions (H^+) are buffered by hemoglobin and the HCO_3^- passes from erythrocytes to plasma in exchange for chloride ions (chloride shift). In the microvasculature of the alveoli the process is reversed, HCO_3^- passes from plasma to erythrocytes (in exchange for chloride ions), where it is converted back to CO_2. Thus:

$$H^+ + HCO_3^- \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

This CO_2 diffuses down a partial pressure gradient from erythrocytes to blood plasma and across the alveolar membrane to alveolar air, and is finally excreted from the body in expired air.

HCO_3^- is the principal chemical buffer system of blood. Maintenance of normal blood pH, despite continuous production of hydrogen ions derived from diet and other metabolic processes, is crucially dependent on maintaining an adequate amount of HCO_3^- in blood plasma. HCO_3^- concentration of blood determines its pH according to the following relationship defined by Henderson-Hasselbalch [6].

$$\mathrm{pH} \propto \frac{[\mathrm{HCO_3^-}]}{p\mathrm{CO_2}}$$

Renal regulation of HCO_3^- loss from the body in urine and HCO_3^- regeneration by renal tubule cells help ensure that plasma HCO_3^- concentration and therefore blood pH remains within normal reference intervals.

Why measure HCO_3^-?

Determination of HCO_3^-:

- Is essential together with pH and pCO_2 for the diagnosis and monitoring of acid-base disturbance [73]. HCO_3^- reflects the "non-respiratory" or "metabolic" contribution to acid-base status
- Is essential for the calculation of the anion gap (see AG); a parameter that has diagnostic utility principally for the investigation of patients with e.g. metabolic acidosis

When should HCO_3^- (pH and pCO_2) be measured?

HCO_3^- (along with pH and pCO_2) is used both to diagnose and monitor acid-base disturbance [73].

Given the complexity of acid-base homeostasis, involving as it does the function of several organ systems, determination of HCO_3^- (pH and

pCO_2) has clinical value in the context of many severe acute or critical illnesses as well as significant injury (trauma). HCO_3^- determinations are thus usually made in an emergency room or critical care/intensive care setting.

Clinical interpretation

See acid-base status and pH chapters

Causes of decreased HCO_3^-

Most causes of decreased HCO_3^- (resulting in metabolic acidoses) can be summarized under three headings [86]:

Consumption of HCO_3^- in buffering excess lactic acid, keto acids:

- Hemorrhagic shock associated with traumatic blood loss
- Cardiogenic shock/cardiac arrest
- Sepsis/septic shock
- Liver failure
- Diabetic ketoacidosis (DKA)
- Starvation
- Alcohol intoxication
- Salicylate overdose

Loss of HCO_3^- from the body:

- Prolonged diarrhea, pancreatic/intestinal fistulae (via the gastrointestinal tract)
- Renal failure (via urine)

Failure to regenerate HCO_3^- by the kidneys:

- Renal tubule acidosis

The normal physiological (compensatory) response to respiratory alkalosis involves decreased renal regeneration of HCO_3^- and consequent reduction in HCO_3^-. Thus, reduced HCO_3^- does not

necessarily indicate metabolic acidosis; it may reflect compensation for respiratory alkalosis. In this case pH will be increased rather than reduced [96].

Causes of increased HCO_3^-

Most causes of increased HCO_3^- (resulting in metabolic alkalosis) can be summarized under two headings [23]:

Excessive loss of H^+ and/or chloride ions

- Via the gastrointestinal tract by prolonged vomiting, gastric aspiration, pyloric stenosis
- Via urine by diuretic drugs, Cushing's disease, Conn's syndrome (hyperaldosteronism)
- Hypokalemia

Excessive administration/ingestion of HCO_3^-

- Excessive use of over-the-counter antacid drugs
- Excessive Intravenous HCO_3^-

The normal physiological (compensatory) response to respiratory acidosis involves increased renal regeneration of HCO_3^- and consequent increase in HCO_3^-. Thus, increased HCO_3^- does not necessarily indicate metabolic alkalosis; it may reflect compensation for respiratory acidosis. In this case pH will be reduced rather than increased.

Symptoms related to HCO_3^- imbalance

Symptoms related to HCO_3^- imbalance are identical to the acid-base disturbances for pH (see pH) and pCO_2

The distinction between actual and standard HCO_3^-

Traditional acid-base theory holds that "actual" and "standard" HCO_3^- are equal in all those with normal acid-base status. Among those with acid-base disturbance attributable solely to a metabolic (non-respiratory) cause, "actual" and "standard" HCO_3^- are equal but abnormal (both are theoretically raised to the same degree in patients with metabolic alkalosis and reduced to the same degree in metabolic acidosis). A difference between "actual" and "standard" HCO_3^- indicates that the respiratory component (pCO_2) is contributing to a disturbed acid-base status. Thus:

- An element of respiratory acidosis is indicated if actual HCO_3^- is *higher* than standard HCO_3^-
- An element of respiratory alkalosis is indicated if actual HCO_3^- is *lower* than standard HCO_3^-

Standard HCO_3^- is considered a more accurate measure of the metabolic (non-respiratory) component than actual HCO_3^- because the standardization process aims to eliminate any effect of the respiratory component on HCO_3^- [3].

Base Excess – BE

Actual Base Excess (*c*Base(B) or ABE)
Standard Base Excess (*c*Base (Ecf) or SBE)

The concept of BE

BE is the theoretical amount of acid that needs to be added to or subtracted from blood with a normal carbon dioxide partial pressure (pCO_2) to return it to a normal pH. Clearly, if pCO_2 and pH are both normal, then BE is zero, or at least within the reference interval. The clinical value of BE is that it provides the means for quantifying the metabolic (non-respiratory) component of acid-base balance [3]. It is by definition unaffected by the respiratory component, pCO_2. It provides essentially the same information as standard bicarbonate (HCO_3^-) (see HCO_3^-), the more widely used parameter for assessment of the metabolic (non-respiratory) component. The concept of BE is that although HCO_3^- is the principal buffer in blood plasma, there are others and each makes some contribution to the total buffer base and therefore the non-respiratory component of acid-base status. Since BE takes account of HCO_3^- as well as other non-carbonic acids and buffers that may affect the metabolic component, it is, theoretically at least, a more satisfactory parameter for the assessment of the metabolic component than HCO_3^-.

Reference interval *c*Base (Ecf) – examples

	mmol/L
Adult female:	–2.3 to 2.7
Adult male:	–3.2 to 1.8
Newborn (wb):	–10 to –2
Infant (wb):	–7 to –1
Child (wb):	–4 to 2

[82, 84] wb: whole blood; Ecf: extracellular fluid.

Actual base excess (*c*Base(B) or ABE)

Actual base excess is the concentration of titratable base when blood is titrated with a strong base or acid to a plasma pH of 7.40 at a pCO_2 of 5.3 kPa (40 mmHg) and 37 °C (98.6 °F) at actual oxygen saturation *in vitro* [87, 88]. It is calculated from three variables: pH, HCO_3^- and *c*tHb, using the Van Slyke equation [24]. Thus [89]:

$$c\text{Base(B)} = \{[HCO_3^-] - 24.8 + (1.43 \times c\text{tHb} + 7.7) \times (\text{pH} - 7.4)\} \times (1 - 0.014 \times c\text{tHb})$$

Standard Base Excess (*c*Base(Ecf) or SBE)

When pCO_2 increases, pH tends to decrease more in the poorly buffered interstitial fluid than in the well-buffered blood. H^+ therefore tends to diffuse from the interstitial fluid to the blood, resulting in a fall in BE of blood. Independence from pCO_2 is achieved by using the *in vivo* standard base excess extracellular fluid (Ecf) (*c*Base(Ecf)), of which blood represents approximately one third. The *c*Base(Ecf) value is calculated on the principle that blood hemoglobin effectively buffers the plasma as well as the much larger Ecf, i.e. the behavior is that of anemic blood (Hb = 3 mmol/L (5 g/dL)). The blood hemoglobin is diluted in the larger Ecf volume to *c*tHb/3 [90]. Thus [91]:

$$c\text{Base(Ecf)} = c\text{HCO}_3^- - 24.8 + 16.2 \times (\text{pH} - 7.4)$$

The *c*Base(Ecf) predicts the quantity of acid or base required to return the plasma *in vivo* to a normal pH under standard conditions. The buffering capacities differ in the extra-cellular compartments, which makes *c*Base(Ecf) more representative of *in vivo* BE compared with *c*Base(B) and a better indicator of non-respiratory acid-base disturbance [82].

Some [88, 92, 93] recommend the use of *c*Base(Ecf) over *c*Base(B), and *c*Base(Ecf) has become almost synonymous with BE [94]. In the text below, BE is used and it may denote both BE in blood and BE in extracellular fluid [91].

Why determine BE?

BE is useful for the elucidation of acid-base disturbances. It has particular value for assessing the severity of metabolic acidosis and metabolic alkalosis and identifying metabolic compensation in those with primary respiratory acid-base disturbance.

Clinical interpretation

BE can have a negative or positive value, depending on whether buffer base is increased or decreased. The magnitude of deviation from zero indicates the severity of the metabolic disturbance (Fig. 10). An abnormally negative value – sometimes referred to as base deficit – indicates decreased base (principally HCO_3^-) or relative increased non-carbonic acid, and a diagnosis of metabolic acidosis. An abnormally positive value indicates increased base (principally HCO_3^-) or decreased non-carbonic acid, and a diagnosis of metabolic alkalosis [82]. BE is normal in uncompensated respiratory acidosis and respiratory alkalosis. Abnormal BE in those with respiratory acidosis and respiratory alkalosis is evidence of renal (metabolic) compensation; it is abnormally positive (>+2) in compensated respiratory acidosis and abnormally negative (<–2) in compensated respiratory alkalosis. BE may be normal in complex acid-base disturbances involving both alkalosis and acidosis, the alkalinizing effect of one disturbance cancelling out the acidifying effect of the other.

BE should always be interpreted in relation to pCO_2 and pH. In general, there is good correlation between BE and HCO_3^-, with BE being abnormally negative in those with reduced HCO_3^- and abnormally positive in those with increased HCO_3^- [95].

Causes of abnormally negative BE

Most causes of negative BE (resulting in metabolic acidosis) can be summarized under three headings [23, 86]:

Consumption of HCO_3^- in buffering excess lactic acid, keto acids:

- Hemorrhagic shock associated with traumatic blood loss
- Hypoxia
- Cardiogenic shock/cardiac arrest
- Sepsis/septic shock
- Liver failure
- Diabetic ketoacidosis (DKA)
- Starvation
- Alcohol intoxication
- Salicylate overdose

Loss of HCO_3^- from the body:

- Prolonged diarrhea, pancreatic/intestinal fistulas (via the gastrointestinal tract)
- Renal failure (via urine)

Failure to regenerate HCO_3^- by the kidneys:

- Renal tubule acidosis

The normal physiological (compensatory) response to respiratory alkalosis involves decreased renal regeneration of HCO_3^- and consequent reduction in HCO_3^- and thereby abnormally negative BE. Thus negative BE does not necessarily indicate metabolic acidosis; it may reflect compensation for respiratory alkalosis. In this case pH will be increased rather than reduced [96].

Causes of abnormally positive BE

Most causes of abnormally positive BE (resulting in metabolic alkalosis) can be summarized under two headings [23]:

Increased generation of base (HCO_3^-) consequent on excessive loss of hydrogen ions and/or chloride ions

- Via the gastrointestinal tract by prolonged vomiting, gastric aspiration, pyloric stenosis
- Via urine by diuretic drugs, Cushing's disease, Conn's syndrome (hyperaldosteronism)
- Hypokalemia

Excessive administration/ingestion of HCO_3^-

- Excessive use of over-the-counter antacid drugs
- Excessive Intravenous HCO_3^-

The normal physiological (compensatory) response to respiratory acidosis involves increased renal regeneration of HCO_3^- and consequent increase in HCO_3^- and thereby abnormally positive BE. Thus abnormally positive BE does not necessarily indicate metabolic alkalosis; it may reflect compensation for respiratory acidosis. In this case pH will be decreased rather than increased [96].

Anion Gap – AG

Anion gap (AG) is a calculated parameter derived from bicarbonate (HCO_3^-) and two (or three) measured plasma/serum electrolyte concentrations: sodium (Na^+), potassium (K^+) and chloride (Cl^-) [97]. There are two AG equations; the most commonly used defines AG as the difference between the concentration of the principal plasma cation, Na^+ and the combined concentrations of the two principal plasma anions, Cl^- and HCO_3^-. The second equation includes the cation K^+. Thus:

Anion gap $= [Na^+] - ([Cl^-] + [HCO_3^-])$

Anion gap (K^+) $= ([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-])$

Reference interval AG – examples

	mmol/L, meq/L
Anion gap	8 – 16
Anion gap (K^+)	10 – 20

[98]

Irrespective of which equation is used, there is some variation in AG depending on the analyzer used to make the electrolyte determinations [99]. Local reference intervals should be used to accurately interpret patient AG values.

Concept and clinical significance of AG

The law of electrochemical neutrality requires that the total concentration of anions (measured and unmeasured) in plasma must equal the total concentration of cations (measured and unmeasured). AG reflects the fact that the concentration of the most abundant

cation (Na^+) in plasma exceeds the combined concentration of the two most abundant anions (Cl^- and HCO_3^-) in plasma. There are other unmeasured cations (Mg^{2+}, Ca^{2+}) and anions (proteins (e.g. albumin), organic acids (e.g. lactate), sulfates and phosphates) in plasma (Fig. 12). An increased AG indicates that there is a loss of HCO_3^- without a concurrent increase in Cl^-. Electroneutrality is maintained by the elevated levels of unmeasured anions, which are not included in the AG calculation and therefore a high anion gap result. In other terms, AG is the difference between unmeasured anions and unmeasured cations [97].

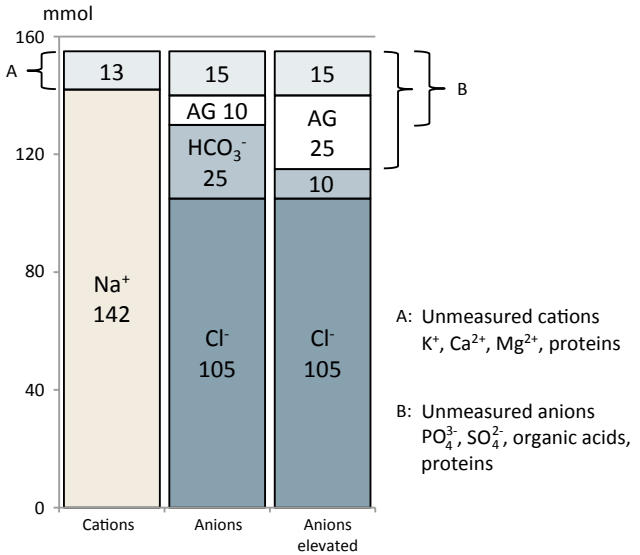


FIG. 12: Electrolyte composition of plasma [97].

Organic acids: e.g. lactate, pyruvate, ketone. Proteins: e.g. albumin, IgA, IgG

This definition of AG best explains its clinical utility because clinically important abnormality in AG is almost invariably due to an increase or decrease in unmeasured cations or unmeasured anions.

Increased AG, which is far more common than decreased AG, is usually the result of an increase in unmeasured anions, but can theoretically result from a decrease in unmeasured cations. Decreased AG, by contrast, results from an increase in unmeasured cations, or decrease in unmeasured anions.

Why determine AG?

The prime clinical utility of AG is the detection and analysis of acid-base disturbance, in particular metabolic acidosis. For those with a confirmed diagnosis of metabolic acidosis, AG can provide useful information about its cause. Abnormality of AG is not confined to those with acid-base disturbance so the test has limited wider diagnostic significance [100].

Metabolic acidosis and AG

Metabolic acidosis is typically associated with a marked increase in unmeasured anions, derived from dissociation of accumulating non-carbonic (volatile) metabolic acids. For example, lactic acidosis – the most common cause of metabolic acidosis – is associated with accumulation of the unmeasured anion lactate. This increase in unmeasured anion results in increased AG. Metabolic acidosis that is primarily due to abnormal accumulation of metabolic acid (the most common etiology) is associated with increased AG and is called "high-AG metabolic acidosis".

There are other mechanisms that give rise to metabolic acidosis and these are associated with a normal AG. In these cases there is no increase in unmeasured anions but there is abnormality in measured anions: plasma HCO_3^- is reduced (as it is in all cases of metabolic acidosis), and plasma Cl^- is increased to maintain electrochemical neutrality. The magnitude of the reduction in HCO_3^- matches the

magnitude of the increase in Cl^-, so the overall effect is a normal AG. The label "normal-AG metabolic acidosis" or "hyperchloremic metabolic acidosis" is applied in these cases. The most common cause of normal-AG metabolic acidosis is severe diarrhea, in which acidosis is due primarily to loss of HCO_3^- from the body. Less common causes include renal tubular acidosis in which normal-AG metabolic acidosis is due to the failure of renal tubular cells to reabsorb/regenerate HCO_3^- adequately (Table III).

If AG >30 mmol/L, the patient has a metabolic acidosis condition; however, only 2/3 of patients with an AG of 20–29 mmol/L will have metabolic acidosis [101].

Anion change	Effect on AG	Acid-base disorders
HCO_3^- ↓ Cl^- ↑	Minor	Diarrhea Hyperchloremic metabolic acidosis Renal tubular acidosis
HCO_3^- ↓ Cl^- ↓ Lactate ↑↑	↑↑	Lactate acidosis
HCO_3^- ↓ Cl^- ↓ Ketoacids ↑	↑↑	Ketoacidosis

TABLE III: Overview of changes in AG in some acid-base disorders.

Clinical interpretation

AG <20 mmol/L rarely indicates a significant acidosis and is most often secondary to changes in protein, phosphates or change equivalents.

AG >30 mmol/L is usually caused by easily identifiable organic acidosis (lactic acidosis or ketoacidosis) [102, 103].

Causes of increased AG

A – associated with metabolic acidosis:
(i.e. raised-AG metabolic acidosis) [23, 100]

Increased acid production:
- Lactic acidosis (most common)
- Diabetic ketoacidosis (DKA)
- Starvation ketoacidosis
- Alcoholic ketoacidosis

Decreased acid excretion:
- Renal failure (GFR <20 mL/min) (see creatinine)
- Pyroglutamic (5-oxoproline) acidosis

Increased acid addition (toxins):
- Aspirin (salicylic acid) overdose
- Methanol poisoning (methanol metabolized to formic acid)
- Ethylene glycol poisoning (ethylene glycol metabolized to glycolic acid)
- Toluene poisoning (toluene metabolized to hippuric acid)

The mnemonic KULT was devised to help recall the above principal causes of high-AG metabolic acidosis:
- **K**etoacidosis
- **U**remia
- **L**actic acidosis
- **T**oxins

B – not associated with metabolic acidosis [100]
Increased plasma albumin:
- Albumin is negatively charged and a significant contributor to total unmeasured anions in plasma. For every 10 g/L increase in plasma albumin, AG is increased by 2.5 mmol/L [104]

IgA myeloma:
- IgA paraprotein is negatively charged and because of its high concentration in the plasma of patients with IgA myeloma is a

significant contributor to total unmeasured anions in plasma
- Metabolic alkalosis – only slight increase (~5 mmol/L) and only in some cases

Causes of decreased AG

Less common than increased AG [23, 105]

Decreased serum albumin (most common cause):
- For every 10 g/L reduction in plasma albumin, AG is decreased by 2.5 mmol/L [104]. (Abnormality in serum albumin should be taken into account when interpreting patient AG. This is particularly important for critically ill patients, who commonly have decreased plasma albumin. The reduction in AG caused by hypoalbuminemia can mask evidence of metabolic acidosis in these patients [105]

IgG myeloma:
- IgG is positively charged, and because of its high concentration in the plasma of patients with IgG myeloma is a significant contributor to unmeasured cations in plasma
- Marked polyclonal IgG increase

Lithium overdose:
- Lithium is an unmeasured cation
- Severe hypercalcemia or severe hypermagnesemia. (Calcium and magnesium are unmeasured cations)

Potassium – K^+

Potassium (K^+) is the major cation in the intracellular fluid, where it has a 25–37-fold higher concentration (~150 mmol/L in tissue cells, ~105 mmol/L in erythrocytes) than in the extracellular fluid (~4 mmol/L) [4, 106]. K^+ has several vital functions in the body, e.g. regulation of neuromuscular excitability, regulation of heart rhythm, regulation of intracellular and extracellular volume and acid-base status.

Reference interval K^+ – examples

	mmol/L, meq/L
Adult male (P):	3.5–4.5
Adult female (P):	3.4–4.4
Adult (S):	3.5–5.1
Child (S):	3.4–4.7
Infant (S):	4.1–5.3
Newborn (S):	3.7–5.9
Newborn cord (S):	5.6–12.0
Premature, 48 hours (S):	3.0–6.0
Premature cord (S):	5.0–10.2

[4] P: plasma; S: serum

Serum samples return values slightly [3–5 %] higher than plasma samples [108].

Distribution and physiological significance of potassium

The human body contains around 3500 mmol (137 g) K^+, nearly all (98 %) of which is contained within cells; only 1.5 % is contained in the extracellular fluid at an approximate concentration of 4.0 mmol/L (Fig. 13). Intracellular K^+ concentration, by contrast, is close to 150 mmol/L [110]. The intracellular-to-extracellular ratio (150/4)

results in an electric potential gradient across the cellular membrane and plays a major role in establishing the resting cell membrane potential, particularly in cardiac and neuromuscular cells [112]. Even small changes in the extracellular K^+ concentration will have significant effects on the transmembrane potential gradient, and thereby the function of neuromuscular and cardiac tissues [112]. This large concentration gradient across cellular membranes is maintained by the Na^+/K^+-ATPase pump, located in the cellular membrane [113].

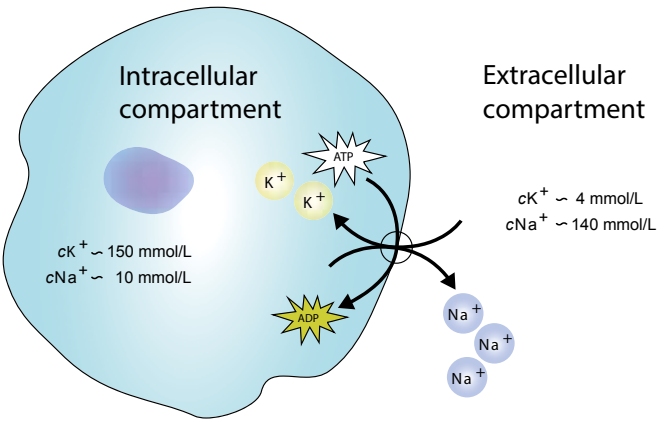


FIG. 13: The Na^+/K^+-ATPase pump in the cellular membrane [114].

This is an energy-consuming process of continuous "pumping" of two K^+ into cells in exchange for three Na^+ [115]. The K^+ concentration gradient is a determinant of the resting membrane potential and thereby the electrical properties of "excitable" cells, including their ability to transmit electrical signals.

Why measure potassium?

Disturbance of potassium handling and consequent abnormality in K^+ is a potential feature of a number of acute and chronic illnesses, some of which are relatively common. It is also a potential adverse effect of some commonly prescribed drugs [109]. An estimated 20–30 % of hospitalized patients have abnormal K^+ [110]. Diagnosing of K^+ disturbance is important because if it remains untreated, it can cause significant morbidity and in the most severe cases, sudden cardiac arrest [111]. All this accounts for K^+ being one of the most frequently requested/measured parameters of blood chemistry.

Physiological control of extracellular fluid potassium concentration

Extracellular fluid K^+ concentration (cK^+) represents the balance between K^+ intake and K^+ loss. A typical western diet ensures a K^+ intake of around 40–200 mmol/day [113]. Although a small amount (~5 mmol/day) is normally excreted via the gastrointestinal tract, the major route of excretion is urine, and K^+ balance depends largely on mechanisms that ensure renal regulation of K^+ loss in urine so that it matches K^+ intake [110]. Renal regulation of K^+ excretion depends on the adrenal hormone aldosterone; rising cK^+ stimulates its synthesis and release [116]. Aldosterone thus reduces cK^+ by increasing renal excretion of K^+.

Internal redistribution of K^+, i.e. the movement of K^+ into and out of cells, is an additional factor that can affect cK^+ without affecting whole body potassium [110]. Insulin stimulates the Na^+/K^+-ATPase pump and thereby the movement of K^+ into cells; insulin thus has the effect of reducing cK^+. Reciprocal movement of K^+ and hydrogen ions (H^+) across cellular membranes determines that cK^+ is affected by acid-base status (Fig. 9). Due to this high intracellular concentration of K^+, any pathology associated with marked cell destruction (lysis) results in massive efflux of K^+ from cells, and consequent increase in cK^+.

Maintaining potassium within reference intervals depends on:
- Adequate dietary intake of K^+
- Normal renal function
- Normal gastrointestinal tract function
- Normal production of aldosterone by adrenal glands
- Maintenance of normal acid-base balance
- Normal action of the Na^+/K^+-ATPase pump and integrity of cell membranes

Disturbance of any of the above can cause abnormality in K^+.

Reduced cK^+ (i.e. <3.5 mmol/L) is called **hypokalemia** [110].
Increased cK^+ (i.e. >5.0 mmol/L) is called **hyperkalemia** [110].

Of the two conditions, hypokalemia is the more common, affecting a broader range of patients, while hyperkalemia is potentially more serious and occurs almost exclusively in patients with some underlying renal abnormalities [112, 117].

Causes of hypokalemia

- Diuretic therapy – most common cause [110]. Confined to so-called "K^+-wasting diuretics" (thiazides and loop diuretics) that can cause inappropriate loss of K^+ in urine
- Severe or chronic diarrhea/vomiting (increased loss of K^+ via the gastrointestinal tract)
- Metabolic alkalosis (movement of K^+ into cells)
- Conn's syndrome/disease (increased aldosterone levels)
- Treatment of diabetic ketoacidosis (due to increased loss of K^+ in urine)
- Inadequate K^+ intake (starvation)
- Laxative abuse (increased loss of K^+ via the gastrointestinal tract)
- Liquorice abuse (liquorice contains a substance that causes effective increased aldosterone levels) [118]
- Beta blocker drug therapy (K^+ moves into cells)
- Insulin overdose (K^+ moves into cells) [111]

Symptoms of hypokalemia

Mild hypokalemia, i.e. cK^+ in the range of 3.0–3.5 mmol/L, is usually asymptomatic

Symptoms of moderate decrease cK^+ include [110]:
- Fatigue associated with muscular weakness
- Constipation due to impaired muscle tone of the gastrointestinal tract
- Characteristic ECG changes
- Hyporeflexia

Severe hypokalemia (cK^+ <2.5 mmol/L) may also cause:
- Flaccid paralysis
- Respiratory failure (if the respiratory musculature is affected) [120]
- Cardiac arrhythmias (including potentially fatal ventricular arrhythmia)
- Cardiac arrest

Causes of hyperkalemia [110, 116, 119]

- Chronic kidney disease – most common cause (reduced urinary excretion of K^+)
- Metabolic acidosis, including diabetic ketoacidosis (movement of K^+ out of cells)
- Severe tissue damage, e.g. rhabdomyolysis, trauma, major surgery (K^+ derived from damaged cells)
- Cytotoxic drug therapy for hematological malignancy (K^+ derived from drug-damaged cells)
- Addison's disease (reduced aldosterone levels)
- Excessive K^+ replacement therapy
- Some drugs (ACE inhibitors, spironolactone and other so-called "K^+-sparing diuretics")

Symptoms of hyperkalemia

Symptoms may be absent or relatively non-specific, emphasizing the clinical importance of measuring cK^+ among those at risk; when they do occur, symptoms include [110, 116, 121]:

- Muscle weakness/fatigue
- Diarrhea/abdominal pain
- Cardiac palpitations
- Characteristic ECG changes

The risk of potentially fatal ventricular arrhythmia and cardiac arrest increases as cK^+ rises above 6.5 mmol/L [110, 121].

Both severe hypokalemia [111] and severe hyperkalemia [110] are medical emergencies requiring prompt intervention.

Sodium – Na^+

Sodium (Na^+) is the dominant cation in the extracellular fluid, where it has a 14-fold higher concentration (~140 mmol/L) than in the intracellular fluid (~10 mmol/L). Na^+ is a major contributor of the osmolality of the extracellular fluid and its main function is largely in controlling and regulating water balance, and maintaining blood pressure. Na^+ is also important for transmitting nerve impulses and activating muscle concretion.

Reference interval Na^+ – examples

	mmol/L, meq/L
Adult:	136 – 145
Child:	138 – 145
Infant:	139 – 146
Newborn:	133 – 146
Newborn cord:	126 – 166
Premature 48 hours:	128 – 148
Premature cord:	116 – 140

[4]

Distribution and physiological significance of sodium

Around 30 % of the approximately 4000 mmol (92 g) of Na^+ present in the human body is in the form of complexes within bone; almost all of the remaining is in the extracellular fluid. As the most abundant extracellular fluid solute, Na^+ is the major determinant of its osmolality and thereby the principal determinant of water distribution between the intracellular and extracellular compartments (Fig. 13). This highlights the role of Na^+ in the maintenance of blood volume and thereby blood pressure. Dysnatremia can arise as a result of disturbance of water balance (most common), disturbance of Na^+

balance (less common), or a combination of both. If extracellular fluid Na^+ concentration (cNa^+) is too low (hyponatremia), water moves into the cells to balance the levels, causing the cells to swell. This is particular dangerous in brain cells, as their expansion increases intracranial pressure, causing cerebral edema [126].

Why measure sodium?

Disturbance of sodium and water metabolism and consequent abnormality in cNa^+ (called dysnatremia) is a potential feature of a number of acute and chronic illnesses, some of which are relatively common. It is also a potential adverse effect of some commonly prescribed drugs [122]. Reduced cNa^+ (hyponatremia) occurs in 15–20 % of hospitalized patients [123]; increased cNa^+ (hypernatremia) is less common, affecting around 1–2 % of hospitalized patients [124]. Identification of dysnatremia is important because if it remains uncorrected, it can, if sufficiently severe, cause significant morbidity and may be fatal. Dysnatremia has been shown to be an independent risk factor for death among the critically ill patients [125].

Measurement of cNa^+ is essential for calculating the anion gap, a parameter of high diagnostic utility particularly among those with acid-base disturbance (see AG).

Sodium balance

The body preserve cNa^+ within normal limits by continuously adjusting renal loss of water (urine volume) so that extracellular fluid water content is constant, despite varying water intake [127]. The thirst response and appropriate release of the antidiuretic hormone arginine vasopressin (AVP) is necessary for this control of extracellular fluid water content.

The preservation of normal cNa^+ also depends on total body sodium balance. A minimum of 10–20 mmol (0.23–0.46 g) of Na^+ is lost

from the body each day in urine, sweat and feces, and this must be replaced to remain in balance. In fact, a normal diet usually contains far in excess of this minimum. Daily Na^+ intake (predominantly in the form of salt flavoring) is usually around 150–170 mmol (3.4–3.9 g) but can range from less than 100 mmol (2.3 g) to more than 300 mmol (6.9 g) [128]. Excess Na^+ is excreted by the kidneys in urine, and Na^+ balance depends critically on the ability of the kidneys to regulate Na^+ excretion so that it matches intake. This renal process depends on the adrenal hormone aldosterone, and on an intact renin-angiotensin pathway for appropriate release of aldosterone [127]. Na^+ is a constituent of gastrointestinal secretions such as bile and pancreatic juice. In total, around 1500 mmol (34 g) of Na^+ is secreted into the gastrointestinal tract every day. Normally, nearly all of this Na^+ is reabsorbed and only 5–10 mmol (0.11–0.23 g) is excreted in feces, but increased loss of Na^+ via the gastrointestinal tract, e.g. in vomit or diarrhea, can lead to severe Na^+ imbalance (depletion).

In summary, the maintenance of Na^+ within normal limits depends on:

- Diet containing a minimum amount of Na^+ (at least 20 mmol (0.46 g)/day)
- Intact thirst response and free access to water
- Normal renal function
- Normal gastrointestinal function
- Appropriate release of the hormone arginine vasopressin by the pituitary gland
- Appropriate release of the hormone aldosterone by the adrenal gland
- Intact renin-angiotensin pathway

Disturbance of any of the above can cause abnormality in cNa^+.

Terms used in interpretation of sodium

Decreased cNa^+ (i.e. <135 mmol/L) is called **hyponatremia** [129]. Increased cNa^+ (i.e. >145 mmol/L) is called **hypernatremia** [130].

Since dysnatremia can be due to a variable combination of Na^+ and/or water depletion/retention, it is important that the extracellular volume (water) status of patients with hyponatremia and hypernatremia is assessed during clinical examination to one of three states:

- Hypovolemia (decreased circulating blood volume)
- Hypervolemia (increased circulating blood volume)
- Euvolemia (normal circulating blood volume)

This allows the important etiological/diagnostic distinction between the following conditions:

- Hypovolemic hyponatremia/hypernatremia
- Hypervolemic hyponatremia/hypernatremia
- Euvolemic hyponatremia/hypernatremia

Causes of hyponatremia [131]

- Heart failure
- Cirrhosis
- Hyperglycemia – diabetic ketoacidosis (DKA)
- Acute kidney disease
- Chronic kidney disease
- Syndrome of inappropriate antidiuretic hormone (SIADH) (a potential adverse effect of many commonly prescribed drugs and a complicating feature of some malignant diseases)
- Chronic vomiting/diarrhea
- Addison's disease (adrenal insufficiency)
- Diuretic therapy
- Fluid replacement therapy

Symptoms of hyponatremia

Mild hyponatremia (cNa^+ 130 – 135 mmol/L) is usually asymptomatic, although chronic mild hyponatremia is associated with increased risk of osteoporosis and bone fractures.

Moderate hyponatremia (cNa^+ 125 – 130 mmol/L) may cause [127]:
- Anorexia
- Nausea/vomiting
- Abdominal cramps

Severe hyponatremia (cNa^+ <125 mmol/L) can additionally cause any of the following neurological symptoms due to brain swelling (cerebral edema):
- Agitation
- Confusion
- Hallucinations
- Impaired mental function

The most severe hyponatremia (cNa^+ <115 mmol/L) can result in seizures, coma and death.

The neurological effect of hyponatremia is more severe if it has developed acutely (less than 48 hours).

Causes of hypernatremia [130, 132]

- Chronic kidney disease
- Inadequate water intake (common in the elderly)
- Failure of the thirst response due to unconsciousness, head injury
- Conn's syndrome/disease
- Cushing's syndrome/disease
- Diabetes insipidus
- Over-vigorous sodium replacement therapy
- Lithium therapy

Symptoms of hypernatremia

Mild hypernatremia (cNa^+ 145 – 150 mmol/L) is usually asymptomatic.

More severe hypernatremia may be associated with the following [130,132]:

- Anorexia
- Muscle weakness
- Nausea/vomiting

Severe hypernatremia (cNa^+ >160 mmol/L), particularly if with acute onset, is associated with dehydration of brain cells and the following resulting neurological symptoms:

- Lethargy
- Irritability
- Altered consciousness
- Coma

Acute-onset severe hypernatremia is potentially fatal.

A note on pseudohypo- and pseudohypernatremia

Falsely low or high cNa^+ (pseudohypo- or pseudohypernatremia) may be reported if plasma contains a particularly high or low, respectively, concentration of lipids or protein [133].

Both pseudohypo- and pseudohypernatremia reflect measurement artefacts that depend on the method used to determine the cNa^+ [134].

Chloride – Cl^-

Chloride (Cl^-) is the major anion in the extracellular fluid and one of the most important anions in blood. The main function of Cl^- is to maintain osmotic pressure, fluid balance, muscular activity, ionic neutrality in plasma, and help elucidate the cause of acid-base disturbances.

Reference interval Cl^- – examples

	mmol/L, meq/L
Adult > 90 years (P, S):	98 – 111
Adult (P, S):	98 – 107
0 – 30 days (P, S):	98 – 113
Premature (P, S):	95 – 110
Cord blood (P, S):	96 – 104

[4] P: plasma; S: serum

Distribution and physiological significance of chloride

In common with sodium (Na^+), most of the approximately 3200 mmol (113 g) of Cl^- present in the human body is contained within the extracellular fluid. Extracellular (plasma) concentration is around 100 mmol/L, whereas intracellular-fluid concentration is closer to 2 – 5 mmol/L. As the second most abundant extracellular fluid ion after Na^+, and the most abundant extracellular fluid anion, Cl^- is essential for the maintenance of normal plasma osmolarity, contributing 100 of the 300 mOsmol of extracellular tonicity [137]. In combination with Na^+, Cl^- determines water movement between extracellular and intracellular compartments and thereby regulation of blood volume and blood pressure. Cl^- is essential for maintaining the electrochemical neutrality of plasma, contributing 70 % of all negative charges in plasma, bicarbonate (HCO_3^-) contributing most of the remaining.

Cl^- ions are secreted from parietal cells of the stomach as hydrochloric acid, a constituent of gastric juice essential for many processes involved in the digestion and absorption of food, as well as the control of bacterial growth within the gastrointestinal tract.

Cl^- is present at high concentrations (~70 mmol/L) in erythrocytes compared with all other cells (2 – 5 mmol/L). This high concentration enables the movement of Cl^- between plasma and erythrocytes in exchange for bicarbonate (HCO_3^-) [137]. This so-called "chloride shift" is essential for the effective transport of carbon dioxide from tissues to lungs and the maintenance of normal blood pH (acid-base homeostasis).

Why measure chloride?

The clinical utility of measuring chloride concentration (cCl^-) is to help elucidate the cause of acid-base disturbances, as abnormal Cl^- levels alone usually signify a more serious underlying metabolic disorder, such as metabolic acidosis or alkalosis. It is also essential for the calculation of the anion gap (AG) [97] (see AG) that can be useful in the investigation of acid-base disturbance [135]. In the absence of acid-base disturbance, cCl^- almost invariably parallels sodium (cNa^+), so that cCl^- measurement is rarely of value in routine assessment of fluid and electrolyte balance; measurement of cNa^+ is sufficient and cCl^- provides no additional information [136].

Chloride balance

Maintaining Cl^- within normal limits principally depends on renal regulation of Cl^- loss in urine. Daily dietary intake of Cl^-, predominantly in the form of salt flavoring, ranges from 160 to 300 mmol (5.7 – 10.6 g) [137]. This is far in excess of what is required to replace normal obligatory physiological losses of Cl^- in urine, sweat and feces, so that most of this dietary intake must be excreted in urine to remain in balance. Renal regulation of Cl^- excretion, like that of sodium excretion, depends on the hormone aldosterone and the renin-

angiotensin pathway. Cl^- excretion is linked to HCO_3^- reabsorption/regeneration, an important mechanism for renal regulation of blood pH that has implication for the role of Cl^- in the pathogenesis of acid-base disturbance.

Cl^- balance also depends on the ability of the gastrointestinal tract to absorb almost all of the Cl^- present in gastrointestinal secretions, e.g. gastric juice, pancreatic juice, bile. Vomiting and/or diarrhea can lead to Cl^- depletion.

Terms used in interpretation of chloride

Decreased cCl^- (i.e. <98 mmol/L) is called **hypochloremia** [4].
Increased cCl^- (i.e. cCl^- >107 mmol/L) is called **hyperchloremia** [4].

Causes of hypochloremia and hyperchloremia

Since cCl^- almost invariably closely parallels cNa^+ in both health and disease, the causes of hypochloremia are identical with the causes of hyponatremia (see Na^+) and the causes of hyperchloremia are identical with the causes of hypernatremia (see Na^+)

The clinical value of measuring cCl^- is confined to acid-base disturbance when cCl^- may not parallel cNa^+.

The value of chloride in the investigation of acid-base disturbance

Measurement of cCl^- is useful in the investigation of patients with unexplained metabolic acidosis because it allows the distinction between "high-AG" metabolic acidosis and "normal-AG" metabolic acidosis (see AG) [138]. The first, which is the more common of the two, is characterized by abnormal increase in unmeasured (non-chloride) anions derived from a causative non-volatile acid (e.g. lactic acid, keto acid). The second is characterized by an increase in measured anion (Cl^-), so it is associated with hyperchloremia. In

general terms, "high-AG" metabolic acidosis is due to abnormal accumulation of an acid, such that the HCO_3^- buffer is consumed, and "normal-AG, hyperchloremic" metabolic acidosis is due to primary loss of HCO_3^- buffer from the body, either via the gastrointestinal tract or the kidneys. The resulting decreased extracellular fluid HCO_3^- induces hyperchloremia to correct the anion deficit and maintain the electrochemical neutrality of extracellular fluid. This is a condition in which cCl^- does not parallel cNa^+; whatever the sodium concentration there is relative hyperchloremia.

Causes of "high-AG" acidosis [86]

- Lactic acidosis (most common cause of metabolic acidosis)
- Diabetic ketoacidosis (DKA)
- Alcoholic ketoacidosis
- Starvation ketoacidosis
- Renal failure (acute and chronic)
- Toxins that metabolize to acids (e.g. ethylene glycol, methanol, salicylates)

Causes of "normal-AG hyperchloremic" acidosis [86]

- Renal tubule acidosis
- Severe diarrhea
- Drainage of pancreatic or biliary secretion
- Bowel fistula
- Carbonic anhydrase inhibitor drugs (e.g. acetazolamide)
- Excessive administration of HCl or NH_4Cl to correct metabolic alkalosis
- Fluid resuscitation (e.g. saline infusion)

Other acid-base disturbances associated with abnormal chloride

Hypochloremia can cause increased renal reabsorption of HCO_3^- and consequent metabolic alkalosis [139]. This hypochloremia is usually

the result of increased loss of Cl^- via the gastrointestinal tract in vomit but can be the result of renal losses, usually secondary to diuretic therapy. Symptoms of hypochloremia are due to the metabolic alkalosis that it induces.

Renal compensation for respiratory acidosis involves increased reabsorption of HCO_3^- in exchange for Cl^-, so that chronic respiratory acidosis is associated with increased loss of Cl^- in urine and consequent hypochloremia.

Ionized calcium – Ca^{2+}

The calcium ion (Ca^{2+}) is one of the most prevalent cations in the body, where approximately 1 % is present in the extracellular fluid of blood. Ca^{2+} plays a vital role for bone mineralization and many cellular processes, e.g. contractility of the heart and the skeletal musculature, neuromuscular transmission, hormone secretion and action in various enzymatic reactions such as, e.g. blood coagulation.

Reference interval Ca^{2+} – example

	mmol/L	mg/dL
Adult:	1.15 – 1.33	4.6 – 5.3

[4]

Distribution and physiological significance of calcium

Practically all (99 %) of the approximately 1 kg of calcium present in the human body is contained within bones and teeth. The remaining 1 % is distributed between intracellular fluid of all cells and extracellular fluid. Only 8.7 mmol (350 mg) calcium circulates in blood plasma at a total concentration of ~2.5 mmol/L (10 mg/dL). Of these 350 mg, around 40 %, is bound to protein (mostly albumin) and 10 % is complexed with a range of anions (bicarbonate, lactate, phosphate, etc.). The remaining 50 % circulates as "free" ionized calcium (Ca^{2+}) at a concentration of ~1.25 mmol/L (5 mg/dL). The three fractions of calcium present in blood plasma are in equilibrium, but only the Ca^{2+} fraction is physiologically active [142]. A very small proportion of the calcium in bone is exchangeable with that in plasma; this is important for the regulation of cCa^{2+} (Fig. 14). The maintenance of cCa^{2+} within normal limits is not only important for the structural integrity of bones but for a range of physiological functions, including:

- Hemostasis (calcium is an essential co-factor in the blood coagulation cascade)
- Cardiac and skeletal muscle cell contraction
- Neuromuscular transmission
- Action of many hormones (calcium-signaling)

Why measure calcium?

Disturbance of calcium metabolism and resulting abnormal calcium concentration (cCa^{2+}) is common among hospitalized patients, particularly the critically ill patients in whom prevalence has been estimated to be as high as 85 % [140]. Both increased and decreased cCa^{2+} have significant symptomatic effects, and if severe, they are both potentially life-threatening conditions. Even mild abnormality, if not identified and treated, has the potential for detrimental impact on health in the long term. "In patients undergoing massive transfusion, it is critically important to frequently monitor Ca^{2+} levels and keep them within the normal range" [141].

Regulation of calcium

In broad terms cCa^{2+} reflects the balance between dietary-derived calcium absorbed via the gastrointestinal tract and that lost from the body in urine. In addition, as outlined above, calcium can move between plasma and bone. Urinary excretion and the movement of calcium from bone is regulated by the parathyroid hormone (PTH), and absorption of dietary calcium from the gastrointestinal tract is regulated by the vitamin D-derived hormone calcitriol (1,25-dihydroxy cholecalciferol). PTH is released from the parathyroid glands in response to reducing Ca^{2+} (Fig. 14); it has effect on the kidneys, where it decreases renal excretion of calcium, and on bone, where it promotes release of calcium from bone to plasma. PTH also promotes release of calcitriol secretion from the kidneys, which in turn promotes increased absorption of dietary calcium. The net effect of both PTH and calcitriol is to raise cCa^{2+} to a level that halts by feedback mechanism PTH secretion and thereby calcitriol secretion.

By the integrated action of PTH and calcitriol, cCa^{2+} is maintained within normal limits [143].

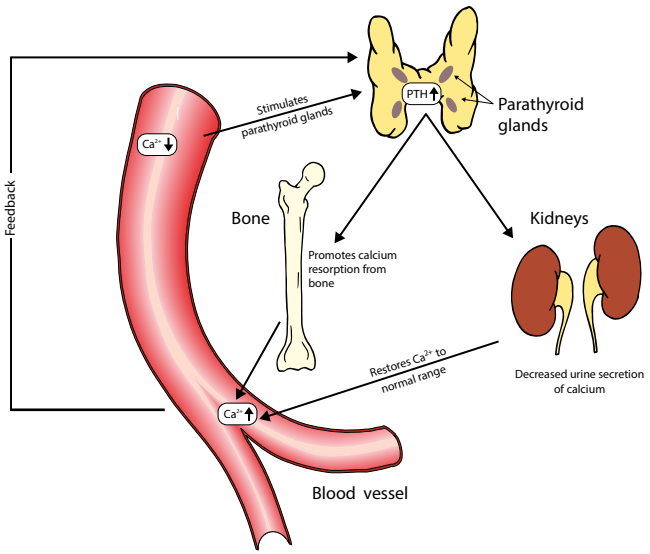


FIG. 14: Regulation of plasma Ca^{2+} level. Modified from [144].
PTH: Parathyroid hormone

To summarize, maintaining cCa^{2+} within reference intervals depends on:

- Normal diet containing adequate amounts of calcium and vitamin D
- Normal gastrointestinal function for dietary absorption of both
- Exposure to sunlight for adequate endogenous production of vitamin D
- Normal parathyroid function for appropriate secretion of PTH
- Normal liver and renal function for conversion of vitamin D to calcitriol
- Normal renal function for secretion of calcitriol and appropriate adjustment of calcium loss in urine

- Normal bone metabolism for appropriate movement of calcium between blood and bone

Disturbance of any of the above can potentially lead to abnormal cCa^{2+}.

Terms used in interpretation of calcium

Reduced plasma cCa^{2+} (i.e. <1.15 mmol/L (4.6 mg/dL)) is called **hypocalcemia**. Clinically, hypocalcemia is never a singular finding; it may occur in the context of coexisting acidosis, hypothermia and dilution [145].

Increased plasma cCa^{2+} (i.e. >1.30 mmol/L (5.2 mg/dL)) is called **hypercalcemia**.

Causes of hypocalcemia [146]

- Hypoparathyroidism (reduced PTH due to disease/damage of the parathyroid glands)
- Vitamin D deficiency (reduced production, dietary deficiency, malabsorption)
- Chronic kidney disease
- Chronic liver disease
- Critical illness, e.g.:
  - Sepsis
  - Acute kidney injury (AKI)
  - Acute pancreatitis
  - Rhabdomyolysis
  - Severe burns
  - Massive red cell transfusion
- Neonatal prematurity (e.g. immature parathyroid glands)

Preserving normal plasma $\boldsymbol{c}Ca^{2+}$ is more important for survival than preserving normal amounts of calcium in bone, and if calcium is in short supply, the body sacrifices bone mineralization in order to maintain plasma cCa^{2+}.

Symptoms of hypocalcemia

Mild hypocalcemia may be associated with no symptoms

Symptoms reflect the role of calcium in neural signaling and neuromuscular transmission include [146]:
- Muscle twitching
- Carpopedal spasm – positive Trousseau's sign
- Parasthesia (tingling, numbness)

Severe hypocalcemia can cause:
- Tetany with laryngeal spasm and breathing difficulty
- Convulsions, seizures, fits
- Cardiac arrhythmia with characteristic ECG changes

In the long term chronic hypocalcemia can cause:
- Neuropsychiatric symptoms
- Cataracts
- Heart failure

Causes of hypercalcemia

The three most common causes, accounting for 90 % of cases, are [147]:
- Primary hyperparathyroidism (excessive uncontrolled secretion of PTH)
- Malignant disease: most cancer types can be associated with hypercalcemia, especially such diseases as lung, breast and esophagus cancer with excessive production of PTH-similar peptide
- Drugs (e.g. thiazide diuretics, lithium, excessive use of antacids, excessive vitamin D)

Rare causes include:
- Tuberculosis
- Sarcoidosis
- Hyperthyroidism
- Inherited hypercalcemia

Symptoms of hypercalcemia [147]

Mild hypercalcemia can occur without symptoms

- Abdominal pain
- Nausea
- Muscle weakness
- Constipation
- Thirst and polyuria
- Tiredness, fatigue, depression
- Palpitations – ECG changes
- Renal (calcium) stones
- Severe hypercalcemia can cause convulsions and coma
- Chronic (long-standing) hypercalcemia can cause irreversible chronic kidney disease

Glucose

Glucose, the most abundant carbohydrate in human metabolism, serves as the major intracellular energy source (see lactate). Glucose is derived principally from dietary carbohydrate, but it is also produced – primarily in the liver and kidneys – via the anabolic process of gluconeogenesis, and from the breakdown of glycogen (glycogenolysis). This endogenously produced glucose helps keep blood glucose concentration within normal limits, when dietary-derived glucose is not available, e.g. between meals or during periods of starvation.

Reference interval glucose – examples

	mmol/L	mg/dL
>90 years, (S fasting):	4.2–6.7	75–121
>60 years, (S fasting):	4.6–6.4	82–115
Adult (wb):	3.6–5.3	65–95
Adult, (S fasting):	4.1–5.6	74–100
Child, (S fasting):	3.3–5.6	60–100
Newborn >1 day, (S fasting):	2.8–4.5	50–80
Newborn 1 day, (S fasting):	2.2–3.3	40–60
Neonate, (S fasting):	1.7–3.3	31–60
Premature, (S fasting):	1.1–3.3	20–60
Cord, (S fasting):	2.5–5.3	45–96

[4] S: serum; wb: whole blood

Physiological significance of glucose and blood glucose regulation

The body can only utilize glucose within cells, where it is the major source of energy. In every cell of the body this energy is released by the oxidation of glucose to carbon dioxide and water in two

sequential metabolic pathways: the glycolytic pathway and the citric acid cycle. During this oxidative process the energy-rich compound adenosine triphosphate (ATP) is formed, and this, in turn, drives the multiplicity of chemical reactions required for tissue cells to remain viable and fulfill their function. Oxidation of one molecule of glucose by this process yields 36 molecules of energy-rich ATP (Fig. 15).

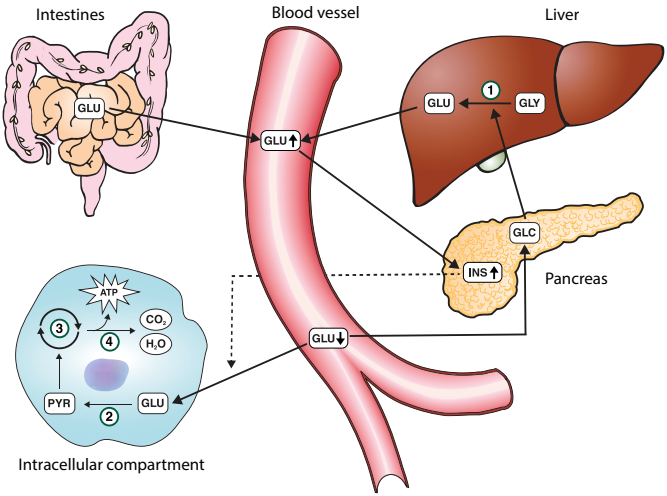


FIG. 15: The glucose pathway.

1: Glycogenolysis; 2: Glycolysis; 3: Citric acid cycle; 4: Oxidative phosphorylation; GLU: Glucose; GLY: Glycogen; GLC: Glucagon; INS: Insulin; PYR: Pyruvate; ATP: Adenosine triphosphate

For this energy-generating function to proceed, glucose must be transported from the intestines (food) or liver (gluconeogenesis, glycogenesis) to body cells via the blood circulation, and enter the tissue cells. Glucose entry to cells from blood is dependent on insulin. This, in part, explains why hyperglycemia is a defining feature of diabetes and highlights the role of insulin in regulating blood glucose concentration. The maintenance of blood glucose

concentration within normal limits is in fact dependent on two pancreatic hormones: insulin and glucagon. Insulin is secreted from the pancreas in response to rising blood glucose, and has the effect of reducing blood glucose; whereas glucagon is secreted from the pancreas in response to falling blood glucose and has the effect of increasing blood glucose. By the synergistic opposing action of these two hormones, blood glucose concentration remains within normal limits.

Insulin reduces blood glucose by:

- Enabling entry of glucose to cells from blood
- Promoting cell metabolism (oxidation) of glucose via the glycolytic pathway
- Promoting formation of glycogen from glucose in the liver and muscle cells
- Inhibiting liver/kidney production of glucose via gluconeogenesis

Glucagon increases blood glucose by:

- Promoting liver/muscle production of glucose from glycogen (glycogenolysis)
- Promoting liver/kidney production of glucose from non-carbohydrate sources (gluconeogenesis)

In situations where there is reduced carbohydrate supply from the intestines, gluconeogenesis becomes particularly important for maintaining a normal blood glucose level and thereby the supply of glucose to all tissues [151].

The non-carbohydrate substrates from which glucose is formed during gluconeogenesis include:

- Proteins
- Glycerol
- Glucose metabolism intermediaries like lactate and pyruvate[152]

Despite widely variable intervals between meals or the occasional consumption of meals with a substantial carbohydrate load, blood glucose concentration does not usually rise above around

8.0–9.0 mmol/L (144–162 mg/dL) nor fall below around 3.5 mmol/L (63 mg/dL) in healthy individuals [153]. The highest levels (8.0–9.0 mmol/L) occur 1–1.5 hours after eating carbohydrate-containing food and the lowest levels occur before food in the morning (i.e. after an overnight fast).

Why measure blood/plasma glucose?

The principal reason for measuring circulating glucose concentration is to diagnose and monitor diabetes mellitus, a very common chronic metabolic condition characterized by increased blood glucose concentration (hyperglycemia), due to an absolute or relative deficiency of the pancreatic hormone insulin [148]. The two main types of diabetes are referred to as type 1 (insulin-dependent) and type 2 (insulin-resistant). Diabetes treatment, which is aimed at normalizing blood glucose concentration, is associated with constant risk of reduced blood glucose (hypoglycemia), which can lead to impaired cerebral function, impaired cardiac performance, muscle weakness, and is associated with glycogen depletion and diminished glucose production.

Abnormality in blood glucose concentration is not confined to those with diabetes. Transient (stress-related) hyperglycemia is a common acute effect of critical illness, whatever its cause. Identification and effective treatment of hyperglycemia (i.e. normalizing blood glucose) improves the chances of surviving critical illness for both diabetic and non-diabetic patients [149].

Neonates, particularly those born prematurely, are at high risk of reduced blood glucose (hypoglycemia). According to [150] the key to prevent complications from glucose deficiency "is to identify infants at risk, promote early and frequent feedings, normalize glucose homeostasis, measure glucose concentrations early and frequently in infants at risk, and treat promptly when glucose deficiency is marked and symptomatic" [150].

When should glucose be measured?

When there are signs and symptoms of hypoglycemia, suspicion of diabetes (hyperglycemia), or hyperglycemia as result of stress in critically ill patients [154].

Hyperglycemia and diabetes

In the absence of critical illness diabetes is confirmed if fasting plasma glucose is ≥7.0 mmol/L (126 mg/dL) or random plasma glucose is consistently ≥11 mmol/L (>198 mg/dL). Those with fasting plasma glucose in the range of 5.6–6.9 mmol/L (101–124 mg/dL) have fasting hyperglycemia, but it is not sufficiently severe to make the diagnosis of diabetes. The label "impaired fasting glucose" is applied to these individuals, who are at much greater-than-normal risk of developing diabetes at some time in the future. The acute and chronic long-term complications of diabetes are avoided by normalization of blood glucose concentration using exogenous insulin and/or other blood glucose-lowering drugs. Recommended targets are for preprandial (fasting) plasma glucose to be maintained in the range of 3.9–7.2 mmol/L (70–130 mg/dL), and peak (1–2 hours postprandial) plasma glucose should not exceed 10.0 mmol/L (180 mg/dL) [155].

Hyperglycemia and the critically ill patients

Hyperglycemia occurs frequently, whether secondary to diabetes or stress-induced (in the non-diabetic), in the critically ill patient [156]. The body increases glucose production and can become resistant to the effects of insulin, with resulting hyperglycemia. In one study, intensive insulin therapy targeting arterial glucose levels of 4.4–6.1 mmol/L (79–110 mg/dL) in a primarily surgical ICU patient population resulted in a significant decrease on morbidity and mortality [154]. However, aggressive intensive insulin therapy can lead to hypoglycemia [157]. The American Diabetes Association (ADA) [155] recommends that in the majority of critically ill patients

in the ICU, insulin infusion should be used to control hyperglycemia if blood glucose exceeds 10 mmol/L (180 mg/dL). The aim of such therapy is to maintain glucose in the range of 7.8–10 mmol/L (141–180 mg/dL). For selected patients more stringent goals, such as 6.1–7.8 mmol/L (110–141 mg/dL), may be appropriate as long as it does not lead to significant hypoglycemia. According to ADA a target <6.1 mmol/L (110 mg/dL) is not recommended [155].

Causes of hyperglycemia [23]

- Trauma
- Stroke/myocardial infarction
- Surgery
- Diabetes mellitus
- Acute pancreatitis
- Endocrine hyperfunction
- Hemochromatosis
- Impaired glucose tolerance/impaired fasting glucose
- Drugs

Symptoms of hyperglycemia

- Headaches
- Dehydration
- Palpitations
- Respiratory abnormalities
- Frequent urination
- Fatigue
- Weight loss
- Thirst
- Gastrointestinal disturbances
- Altered mental status and/or sympathetic nervous system stimulation
- Ketonemia/-uria
- Pseudohyponatremia
- Metabolic acidosis

Critically ill patients can experience rise in blood glucose concentration as a result of:

- The initial trauma
- Surgery
- Inhaled anesthesia
- Medications, particularly corticosteroids
- Intravenous solutions used for drug and fluid administration
- Dialysis solutions
- Infections, particularly sepsis

Hypoglycemia

Hypoglycemia is defined as decreased blood glucose concentration. The glucose level at which an individual becomes symptomatic is highly variable; therefore a single blood glucose concentration that categorically defines hypoglycemia is not established [158]. In some intensive care settings hypoglycemia is defined as blood glucose <2.2 mmol/L (40 mg/dL) [156].

Causes of hypoglycemia [23]

- Diabetes treatment (most common cause)
- Insulinoma, liver disease
- Postgastrectomy
- Insulin abuse

Symptoms of hypoglycemia

Hypoglycemia symptoms can either result from adrenergic discharge or from neuroglycopenia.

Adrenergic discharge	Neuroglycopenia
· Anxiety	· Headache
· Nervousness	· Weakness
· Tremulousness	· Tiredness
· Sweating	· Confusion
· Hunger	· Dizziness
· Palpitations	· Feeling cool
· Irritability	· Clumsy or jerky movements
· Nausea	· Senile dementia
· Flushing	· Amnesia
· Angina	· Abnormal mentation
	· Sympathetic nervous system stimulation
	· Seizures
	· Coma

Hypoglycemia and neonates

In neonates, hypoglycemia is a common metabolic issue. However, there is no consensus on a single blood glucose concentration that defines hypoglycemia in this population. Experts agree that the neurological disabilities associated with neonatal hypoglycemia depend on gestational and chronological age and associated risk factors such as hypoxic-ischemic encephalopathy and that they frequently result after situations of persistent and severe hypoglycemia [159,160].

Although there is no consensus, most expert authors support the cut-off value of 2 mmol/L (36 mg/dL) for asymptomatic healthy

newborns. Values down to 1.7 mmol/L (31 mg/dL) have been suggested in an otherwise healthy term infant [161]. Operational thresholds <2.2 mmol/L (<40 mg/dL) during the first 24 hours and <2.8 mmol/L (<50 mg/dL) thereafter are also suggested [162]. Many neonate units aim to maintain blood glucose levels above 2–3 mmol/L (26–54 mg/dL) and below 10–15 mmol/L (180–270 mg/dL) in low-birth-weight or sick babies [163].

Causes of hypoglycemia in neonates include [164]:

- Inappropriate changes in hormone secretion
- Inadequate substrate reserve in the form of hepatic glycogen
- Inadequate muscle stores as a source of amino acids for gluconeogenesis
- Inadequate lipid stores for the release of fatty acids

Lactate

Lactate, the anion that results from dissociation of lactic acid, is an intracellular metabolite of glucose. It is produced by skeletal muscle cells, red blood cells (erythrocytes), the brain, and other tissues during anaerobic energy production (glycolysis). Lactate is formed in the intracellular fluid from pyruvate; the reaction is catalyzed by the enzyme lactate dehydrogenase (LDH) [165].

Reference interval lactate – examples

	mmol/L	mg/dL
Adult (bed rest, wb, a):	0.36–0.75	3–7
Adult (bed rest, wb, v):	0.56–1.39	5–12
Child 7–15 years:	0.6–0.9	5–8
Child 1–7 years:	0.8–1.5	4–17
Infant 1–12 months:	1.1–2.3	10–21

[4, 84] Wb: whole blood, a: arterial, v: venous

The normal blood lactate concentration in unstressed patients is 1–1.5 mmol/L (9–14 mg/dL). Patients with critical illness is usually considered to have normal lactate concentrations <2 mmol/L (<18 mg/dL).

Physiological significance of lactate

Conversion of glucose to pyruvate is a sequence of 13 enzymatic reactions, called the glycolytic pathway. In well-oxygenated tissue cells that contain mitochondria, pyruvate diffuses into the mitochondria and is metabolized via the citric acid cycle and oxidative phosphorylation to carbon dioxide (CO_2), water and adenosine triphosphate (ATP), the body's primary energy source. The body turns to the less efficient anaerobic glycolysis, whenever cellular oxygen

levels decrease and/or the mitochondria are not functioning properly, to metabolize glucose and produce ATP. The primary byproduct in this process is lactate, which can build up faster than the liver can break it down. In aerobic glycolysis the conversion of one molecule of glucose to CO_2 and water has a high energy yield of 36 ATP molecules, while in anaerobic glycolysis conversion of one molecule of glucose to lactate yields only two molecules of ATP (Fig. 16) [167].

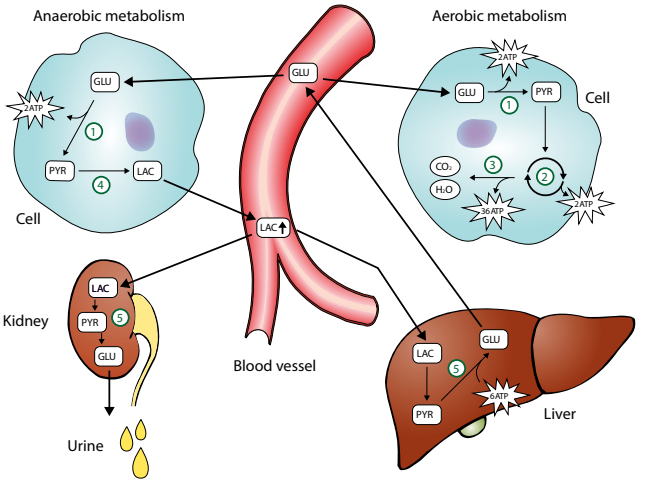


FIG. 16: The lactate pathway.

1: Glycolysis; 2: Citric acid cycle; 3: Oxidative phosphorylation;

4: Lactic acid fermentation; 5: Gluconeogenese

GLU: Glucose; LAC: Lactate; PYR: Pyruvate; ATP: Adenosine triphosphate

Erythrocytes contain no mitochondria so the lactate produced within them cannot be metabolized further and is released to circulation. In some tissues (e.g. skeletal muscle) lactate may be produced at a faster rate than it can be metabolized and in these circumstances lactate will be released to circulation. Normal daily lactate production from these two sources is approximately 1300–1500 mmol (117–135 g) and so

long as a normal rate of metabolic disposal by the liver and kidneys is maintained, blood lactate remains within normal limits. Blood lactate concentration thus reflects the balance between the rate of lactate released to blood from erythrocytes and other tissue cells (principally exercising muscle cells) and the rate of lactate clearance from blood.

Less than 2 % of lactate is eliminated unchanged in urine but the main route of lactate clearance from blood is uptake by the liver and kidneys and conversion to pyruvate in the intracellular fluid of these tissue cells (Fig. 16). The pyruvate has two possible fates: aerobic metabolism to CO_2 and ATP in the mitochondria, or conversion to glucose in the intracellular fluid, a process called gluconeogenesis [168] (see glucose). The ability of the liver to consume lactate and metabolize it to glucose is concentration-dependent and progressively decreases as the level of blood lactate increases. Lactate uptake by the liver is impaired by several other factors, including acidosis, hypoperfusion and hypoxia.

Why measure lactate?

Increase in lactate levels is an early sensitive indicator of imbalance between tissue oxygen demand and oxygen supply [23]. It is used as

- A prognostic indicator for patient outcome
- A marker of tissue hypoperfusion in patients with circulatory shock
- An index of adequacy of resuscitation after shock
- A marker for monitoring resuscitation therapies

The clinical benefits offered by lactate testing depend on the clinical setting [166]. Lactate measurement is especially useful in monitoring the effect of treatment in critically ill patients, as the presence of an elevated lactate level in this population is strongly associated with morbidity and mortality [1].

When should lactate be measured?

When there are signs and symptoms such as rapid breathing, nausea, hypotension, hypovolemia and sweating that suggest the possibility of reduced tissue oxygenation or an acid/base imbalance, as well as when there is suspicion of inherited metabolic or mitochondrial disorder.

Clinical interpretation

As lactate concentration increases beyond 3 – 4 mmol/L (27 – 36 mg/dL) [71], there is increasing risk of associated acidosis (see acid-base status and AG). The combination of hyperlactatemia and acidosis is called lactic acidosis, which is a disruption of acid-base balance. Lactic acidosis occurs in ~1 % of hospital admissions, and mortality rate may be >60 % especially in combination with hypotension [4]. Lactic acidosis can cause symptoms such as muscular weakness, rapid breathing, nausea, vomiting, sweating and even coma.

Hyperlactatemia

Hyperlactatemia is typically defined as increased blood lactate (>2.0 mmol/L (>18 mg/dL)). Hyperlactatemia appears when the rate at which lactate is released from peripheral tissue cells to circulation exceeds the rate at which it is removed from circulation by the liver and kidneys.

Lactic acidosis

There is no universal agreement on a definition of lactic acidosis, but lactic acidosis is characterized by persistent hyperlactatemia (usually >5 mmol/L (45 mg/dL)) in association with reduced blood pH (<7.25) [171]. Lactic acidosis is the most common cause of metabolic acidosis [172].

The development of lactic acidosis depends on the magnitude of hyperlactatemia, the buffering capacity of the body, and the

coexistence of other conditions that produce tachypnea and alkalosis (e.g. liver disease, sepsis).

Lactic acidosis can be classified as:
Type A (hypoxic) – may be due to inadequate oxygen uptake in the lungs and/or to reduced blood flow, resulting in decreased transport of oxygen to the tissues.

Type B (metabolic) – is caused by conditions that increase the amount of lactate in the blood but are not related to a decreased availability of oxygen (normal tissue perfusion and adequate tissue oxygenation).

Causes of type A: Inadequate tissue oxygenation [173, 174, 175]:
- Shock from blood loss/sepsis
- Myocardial infarction/cardiac arrest
- Congestive heart failure
- Pulmonary edema
- Severe anemia
- Severe hypoxemia
- Carbon monoxide poisoning

Causes of type B: Metabolic derangements [176–181]:
- Liver disease
- Kidney disease
- Diabetic ketoacidosis
- Leukemia
- HIV
- Glycogen storage diseases (e.g. glucose-6-phosphatase deficiency)
- Severe infections – both systemic sepsis and meningitis
- Congenital lactic acidosis: A variety of inherited metabolic and mitochondrial diseases that are forms of muscular dystrophy and that affect normal ATP production
- Drugs and toxins represent by far the most common cause of type B lactic acidosis (Table IV)
- Strenuous exercise

· Biguanides (e.g. metformin)	· Simvastatin
· Ethanol	· Salicylates
· Methanol	· Paracetamol (acetaminophen)
· Antiretroviral drugs	· Lactulose
· Cyanide	· Propylene glycol
· Theophylline	· Epinephrine, norepinephrine
· Cocaine	

TABLE IV: Examples of drugs and toxins that can cause lactic acidosis.

Elevated lactate levels are associated with mortality in both emergency departments and hospitalized patients [1, 182, 183, 184]. The Surviving Sepsis Campaign recommends, among others, to measure lactate within 3 hours of admission. If the initial lactate is elevated, a second lactate measurement should be completed within 6 hours [185].

L- and D-lactate

Lactic acid exists in two optical isomeric forms, L-lactate and D-lactate. Practically all the lactate produced in the human body is L-lactate, and it is this form that is pathophysiologically the most significant [169]. D-lactate is a byproduct of bacterial metabolism and may accumulate in patients with short-gut syndrome or in those with a history of gastric bypass or small-bowel resection, resulting in D-lactic acidosis [170].

Bilirubin

Bilirubin is the yellow breakdown product of the degradation of the heme group of hemoglobin. It is transported in blood from its site of production – the reticuloendothelial system – to the liver, where it is biotransformed before excretion in bile. Jaundice, the pathological yellow discoloration of skin, is due to abnormal accumulation of bilirubin in the tissues, and is always associated with elevated blood concentration of bilirubin (hyperbilirubinemia).

Reference interval bilirubin – examples

	µmol/L	mg/dL
Adult:	0–34	0–2.0
3–5 days (fb):	68–137	4.0–8.0
3–5 days (pm):	171–240	10.0–14
1–2 days (fb):	103–171	6.0–10
1–2 days (pm):	103–205	6.0–12
0–1 day (fb):	34–103	2.0–6.0
0–1day (pm):	17–137	1.0–8.0
Cord blood (fb):	<34	<2.0
Cord blood (pm):	<34	<2.0

[4] fb: full-born; pm: premature

Bilirubin metabolism

Most bilirubin is the product of heme catabolism from hemoglobin in aged erythrocytes removed from the circulation to reticuloendothelial sites in the spleen, liver and bone marrow (Fig. 17). The remainder is derived from inefficient erythropoiesis and from catabolism of other heme-containing proteins, such as myoglobin, catalases and the cytochromes. This unconjugated bilirubin is released into the circulation where it is rapidly bound to albumin for transport to the liver. After release from albumin, unconjugated bilirubin is

transported into hepatocytes where it combines enzymatically with glucuronic acid, producing bilirubin glucuronides (i.e. conjugated bilirubin), which are excreted in bile to the intestines. The action of colonic bacteria deconjugates bilirubin and converts the resulting unconjugated bilirubin to the final excretory product, urobilinogen, that passes from the body in feces. A small proportion of urobilinogen is reabsorbed into the blood circulation and back to the liver for re-excretion (enterohepatic circulation of bilirubin) or to be excreted in urine by the kidneys.

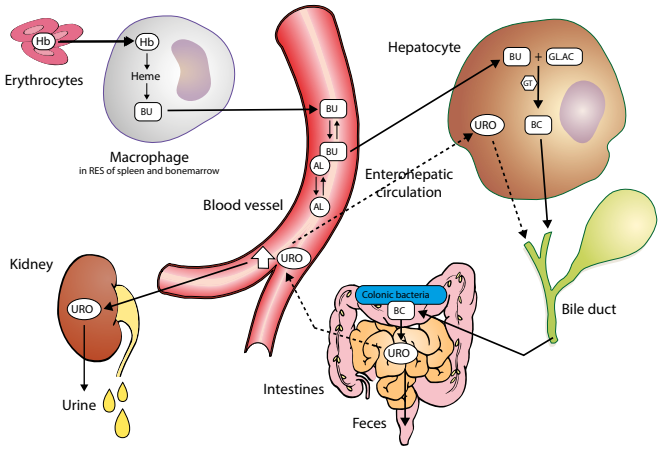


FIG. 17: The bilirubin pathway.

BC: Conjugated bilirubin; BU: Unconjugated bilirubin; Hb: Hemoglobin; RES: Reticuloendothelial system; AL: Albumin; GL.AC: Glucoronic acid; GT: Glucoronosyltransferase; URO: Urobilirubin

Several factors peculiar to neonatal physiology contribute to jaundice at this age [188]:

- Increased red cell destruction and therefore increased bilirubin production
- Immature liver and therefore reduced ability to conjugate bilirubin
- Increased enterohepatic circulation of bilirubin
- Lack of intestinal bacterial flora to convert bilirubin to excretion products

In neonates, much of the conjugated bilirubin in the intestines is hydrolyzed back to unconjugated bilirubin. This unconjugated bilirubin is reabsorbed into the blood stream by way of the enterohepatic circulation, adding an additional bilirubin load to the already overstressed liver [188].

Types of bilirubin found in plasma

- Unconjugated (indirect) bilirubin (bound to albumin). It is water-soluble and non-toxic. This fraction normally comprises around 90 % of total bilirubin
- Unconjugated (indirect) free bilirubin (i.e. not bound to albumin). It is poorly soluble in water and is potentially toxic as it can pass the lipid membranes and cause kernicterus. This fraction normally comprises <0.1 % of all unconjugated bilirubin
- Conjugated (direct) bilirubin (bound to glucuronic acid) is water-soluble and non-toxic. This fraction normally comprises around 10 % of total bilirubin
- Delta (δ) bilirubin is covalently bound to plasma proteins to form a conjugated bilirubin-protein complex. It is water-soluble, non-toxic and appears in serum when hepatic excretion of conjugated bilirubin is impaired in patients with hepatobiliary disease [187]

Total bilirubin is typically defined as unconjugated plus conjugated. As δ-bilirubin is usually low (0–2 % of total bilirubin) and difficult to measure, it is most often not included in the total bilirubin calculation.

Why measure bilirubin?

Monitoring neonatal jaundice is one of the most common reasons for measuring bilirubin concentration. Most newborns have some degree of jaundice, although it is usually mild, benign and resolves without treatment during the second week of life. The potential neural toxicity of bilirubin requires that jaundiced newborns be monitored to identify those who might, without treatment, develop severe hyperbilirubinemia, which is associated with risk of acute bilirubin encephalopathy or kernicterus (See note at the end of this chapter) [186].

In older infants, children and adults bilirubin should be measured when there is a suspicion of jaundice and/or liver or biliary-tract disease.

When should bilirubin be measured?

In newborns when there are signs and symptoms of hyperbilirubinemia [23, 186]:

- Yellow staining of the skin (jaundice)
- Vomiting
- Dark urine
- Poor sucking/feeding
- Lethargy
- Hypo/hypertonia
- Distinctive high-pitched cry
- Irritability
- Fever

In adults when there is suspicion of e.g. liver or biliary-tract disease, hemolytic anemia or jaundice.

Interpretation of bilirubin values

Increased bilirubin and the resulting jaundice can be attributed to disturbances at any of the steps along the metabolic pathway outlined above. It is helpful in the differential diagnosis of jaundice to know if the increase in total bilirubin is due to a predominant increase in conjugated or unconjugated bilirubin.

For example, increase in unconjugated bilirubin suggests that jaundice is due to either increased bilirubin production from heme or reduced ability of liver cells to conjugate bilirubin. Both of these mechanisms operate in physiological neonatal jaundice. Increased bilirubin production also explains the jaundice that can occur in those with any form of hemolytic anemia (called hemolytic jaundice). Reduced ability of liver cells to conjugate bilirubin explains the jaundice that

occurs in Gilbert's syndrome, a genetic disorder characterized by an inherited deficiency of the enzyme required for bilirubin conjugation [189].

Increase in conjugated bilirubin, on the other hand, implies that conjugated bilirubin is not being excreted in bile as efficiently as normal, and instead is spilling into blood. This can be due to impairment of conjugated bilirubin delivery from hepatocyte to bile canaliculi or reduced bile flow (cholestasis) within the liver (called hepatocellular jaundice or cholestatic jaundice); or obstruction of bile flow through the biliary tract (called obstructive jaundice).

Physiological classification of jaundice

Unconjugated hyperbilirubinemia in newborns [4, 23]

Increased production of unconjugated bilirubin from heme:

- Hemolytic disease:
  - Red cell incompatibility
  - Due to Rhesus (Rh) incompatibility; for example, Rh(–) mother, Rh(+) fetus and blood type ABO incompatibility, mother (O) and infant (A or B)
- Breast-feeding jaundice:
  - Due to α-glucuronidase in breast milk, which hydrolyzes conjugated to unconjugated bilirubin in the intestines
- Ineffective erythropoiesis:
  - Rapid turnover of increased red blood cell mass in neonates

Decreased uptake of unconjugated bilirubin across the hepatocyte membrane:

- Competitive inhibition
- Drugs
- Gilbert's syndrome
- Sepsis, fasting

Decreased biotransformation (conjugation):
- Physiological jaundice
- Inhibition (drugs)
- Hereditary (Crigler-Najjar syndrome)
- Hepatocellular dysfunction
- Gilbert's syndrome

Conjugated hyperbilirubinemia (cholestasis) [4, 23]
Decreased secretion of conjugated bilirubin into canaliculi:
- Hepatocellular disease:
  - Hepatitis
  - Cholestasis (intrahepatic)
  - Extrahepatic obstruction
- Dubin-Johnson syndrome
- Roter syndrome
- Drugs

Decreased drainage:
- Extrahepatic obstruction:
  - Stones
  - Carcinoma
  - Stricture
  - Atresia

- Intrahepatic obstruction:
  - Drugs:
    - Granulomas
    - Primary biliary cirrhosis
    - Bile-duct paucity
  - Tumors

Physiological jaundice of newborns

Neonates are physiologically predisposed to jaundice, due to increased breakdown of hemoglobin and limited hepatic function. Low concentration of albumin increases the risk of rising free

unconjugated bilirubin, which in turn increases the risk of neurotoxicity and resulting acute bilirubin encephalopathy (kernicterus) [186, 188]. (See note at the end of this chapter).

In full-term infants jaundice usually develops after 24 hours of life and peaks on day 3 or 4 [23]. It may be noticeable as yellow discoloration of the sclera at levels of about 34–51 µmol/L (2–3 mg/dL) [190] and of the skin at higher levels (jaundice).

Premature babies are more likely to develop jaundice than full-term babies. In preterm infants it usually begins 48 hours after birth, peaks on day 5 and may last 2 weeks [23]. Neonates need treatment if the total bilirubin level is too high or is rising too quickly.

Physiological jaundice is generally mild and not harmful, but bilirubin concentrations above 170 µmol/L (10 mg/dL), coupled with prematurity, low serum albumin, acidosis, and substances that compete for the binding sites of albumin (e.g. aspirin) may increase the risk of kernicterus [4].

Treatment for hyperbilirubinemia/jaundice in newborns

- Phototherapy to convert bilirubin to products that can bypass the liver's conjugating system and be excreted in bile or in urine without further metabolism [191]
- Exchange transfusion to remove bilirubin mechanically
- Pharmacological agents given to interfere with heme degradation and bilirubin production, accelerate the normal metabolic pathways for bilirubin appearance, or inhibit the enterohepatic circulation of bilirubin

Action limits for treatment of newborns with jaundice?

Guidelines for the use of phototherapy and exchange transfusion in term and near-term infants with a gestational age of 35 weeks or more are provided by the American Academy of Pediatrics [186].

These guidelines, however, are not evidence-based but primarily the product of expert opinion. The use of phototherapy in infants with low birth weight is prophylactic and based on either birth weight or gestational age [192]. The time-honored bilirubin concentration of 340 µmol/L (20 mg/dL), which was considered critical and required action (intensive phototherapy, exchange transfusion), is now being abandoned and replaced [4]. It is now recommended to monitor the increase in total bilirubin concentration from time of birth until the time of discharge from hospital (36–73 hours). A plot of total bilirubin concentration versus time (hours) should be made and compared with the similar plot found in the guideline from the American Academy of Pediatrics, Subcommittee of Hyperbilirubinemia [186] to guide implementation of phototherapy.

Note: The American Academy of Pediatrics, Subcommittee of Hyperbilirubinemia [186] recommends that in infants the term "acute bilirubin encephalopathy" be used to describe the acute manifestations of bilirubin toxicity seen in the first weeks after birth and that the term "kernicterus" be reserved for the chronic and permanent clinical sequelae of bilirubin toxicity.

Creatinine

Creatinine is an endogenous waste product of muscle metabolism, derived from creatine, a molecule of major importance for energy production within muscle cells. Creatinine is removed from the body in urine and its concentration in blood reflects glomerular filtration and thereby kidney function.

Reference interval creatinine – examples

	µmol/L	mg/dL
Adult male (S):	55–96	0.62–1.10
Adult female (S):	40–66	0.45–0.75
10 years (S):	19–52	0.22–0.59
6–9 years (S):	18–46	0.20–0.52
2–5 years (S):	4–40	0.04–0.45
0–1 year (S):	4–29	0.04–0.33

[4] S: serum

Serum (S) and plasma (P) creatinine values are considered equivalent [193].

Creatinine biochemistry and physiology

Creatinine is a waste product of creatine metabolism; a small amount is derived from dietary sources, principally cooked meat [195]. Creatine is synthesized in the kidneys, liver and pancreas by two enzymatically mediated reactions and transported in blood to muscle (Fig.18). Here it is phosphorylated to phosphocreatine, the phosphate being donated by ATP. Interconversion of phosphocreatine and creatine and concomitant interconversion of ADP and ATP provide the energy source for muscle contraction. Daily, around 1–2 % of creatine in muscle is converted to the waste product, creatinine,

which is then released from muscle cells to the circulation. The amount of creatinine produced depends on total muscle mass and therefore varies considerably between individuals; it is of the order of 0.5 g/day in children, 1.5 g/day for adult females and 2.0 g/day for adult males [196]. Although there is variability between individuals, for a given individual the daily creatinine production remains pretty well constant so long as total muscle mass is unchanged. Creatinine is cleared from blood by the kidneys, principally during glomerular filtration; a small proportion (7–10 %) is cleared by tubular secretion [197].

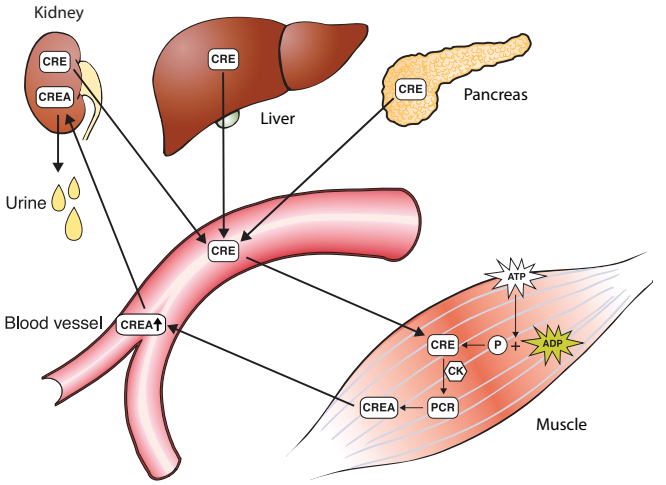


FIG. 18: The creatinine pathway.

CRE: Creatine; PCR: Phosphocreatine; CREA: Creatinine; CK: Creatine kinase; P: Phosphate; ATP: Adenosine triphosphate; ADP: Adenosine diphosphate

Why measure creatinine?

Creatinine is measured to assess kidney dysfunction, i.e. to detect and monitor chronic kidney disease (CKD) and/or acute kidney injury (AKI). Measuring creatinine helps identify patients with inadequate kidney

function before they undergo diagnostic investigation with potential nephrotoxic image-enhancing contrast media.

The principal function of the kidneys is formation of urine from filtered blood. Urine is the vehicle for excretion of many toxic or waste products of metabolism – including creatinine – as well as substances (water, sodium, potassium etc.) that are essential for life but present in quantities larger than the body's immediate needs. By their ability to continuously vary the volume, chemical composition and pH of urine over wide limits, the kidneys serve a major role in preserving normal fluid, electrolyte and acid-base balance.

The process of urine formation begins with filtration of blood. The parameter glomerular filtration rate (GFR) reflects the rate at which blood is filtered in the kidneys and thus of major clinical significance. Kidney disease/dysfunction is associated with reduction in GFR, and that is inversely correlated with the severity of the underlying condition.

The value of creatinine as a marker of kidney function is based on the constancy of endogenous creatinine production and the observation that creatinine is cleared from blood to urine almost entirely by glomerular filtration. These factors determine that creatinine concentration broadly reflects GFR and can be used to estimate its value. The National Kidney Disease Education Program (NKDEP) encourages to routinely estimate GFR with every measurement of creatinine in patients aged 18 and older, as mild and moderate kidney injury is poorly inferred from creatinine alone [194].

When should creatinine be measured?

- With clinical evidence or history of kidney disease/dysfunction
- Acute/critical illness, i.e. patients assumed to be at risk of AKI
- In chronic conditions e.g. diabetes, associated with risk of renal impairment. Here creatinine is monitored at regular intervals
- Before and after administration of nephrotoxic contrast agents,

e.g. with computed tomography (CT) or magnetic resonance imaging (MRI)
- Before and after prescription of any potentially nephrotoxic drug
- Before and at intervals during prescription of drugs whose principal route of elimination is via the kidneys

Clinical interpretation

Irrespective of its cause, reduction in kidney function is associated with increased creatinine concentration, although creatinine concentration is an insensitive marker of early asymptomatic CKD. The principal distinction between CKD and AKI is the speed of progression, and therefore the rate of decline in GFR. AKI progresses rapidly over a period of hours and days and is potentially reversible, whereas CKD progresses slowly over a period of months, years or even decades and is irreversible, although medical intervention can slow progression. The way creatinine concentration is used to detect and monitor AKI is slightly different from the way it is used to detect and monitor CKD (Table V).

Both AKI and CKD can progress to end-stage kidney disease. When kidney replacement therapy (either dialysis or transplantation) is required for survival, creatinine typically exceeds 600 µmol/L (6.8 mg/dL) and may be as high as 1000 µmol/L (11.3 mg/dL) [198].

Creatinine levels higher than normal levels may be due to [199]:
- Acute tubular necrosis
- Dehydration
- Diabetic nephropathy
- Glomerulonephritis
- Kidney failure
- Muscular dystrophy
- Preeclampsia (pregnancy-induced hypertension)
- Pyelonephritis
- Reduced kidney blood flow (shock, congestive heart failure)
- Rhabdomyolysis

· Urinary-tract obstruction

Creatinine levels lower than normal levels may be due to [199]:

· Muscular dystrophy (late stage)
· Myasthenia gravis

How is creatinine used to diagnose and stage AKI?

AKI is a sudden decline in kidney functions, and can be potentially life-threatening. It is defined by KDIGO (International Kidney Disease: Improving Global Outcomes), when one of the following criteria is met [200]:

"Increase in S_{cr} by ≥26 μmol/l(≥0.3 mg/dl) within 48 hours; or Increase in S_{cr} to ≥1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or Urine volume <0.5 ml/kg/h for 6 hours."

"The reference serum creatinine (S_{cr}) should be the lowest creatinine value recorded within 3 months of the event"

Furthermore, the KDIGO recommends that the following staging classification* of AKI be adopted [200].

Stage	Serum creatinine (S_{cr}) criteria	Urine-out criteria
1	increase ≥26 μmol/L (0.3 mg/dL) within 48 hours or increase ≥1.5 to 1.9 × reference S_{cr}	<0.5 mL/kg/h for >6 consecutive hours
2	increase ≥2.0 to 2.9 × reference S_{cr}	<0.5 mL/kg/h for ≥12 hours
3	increase ≥3.0 × reference S_{cr} or increase ≥354 μmol/L (4 mg/dL) or commenced on renal replacement therapy (RRT) irrespective of stage	<0.3 mL/kg/h for ≥24 hours or anuria for ≥12 hours

TABLE V: Classification of AKI

*Must have met initial criteria for definition of AKI

In AKI the progress from normal kidney function to end-stage kidney disease can occur over a period of days or weeks. The loss of function is so rapid that creatinine is almost invariably raised to some extent, and a consistently normal creatinine concentration excludes a diagnosis of AKI. AKI is especially common in critically ill patients, with prevalence of >40 %, at admission to the intensive care unit (ICU) if sepsis is present [201]. During ICU admission, the AKI prevalence can be >60 % [202, 203]. Nephrotoxic drugs contribute to AKI in approximately 20 % of patients, especially in the critically ill patient population [204, 205].

How is creatinine/GFR used to diagnose and stage CKD?

CKD is a worldwide problem that carries a substantial risk of cardiovascular morbidity and death [206]. It is defined as [207, 208]:

"Kidney damage for three or more months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifested by either pathologic abnormalities or markers of kidney damage, including abnormalities in the composition of the blood or urine or abnormalities in imaging tests."

"GFR <60 mL per minute per 1.73 m^2 for three months or more, with or without kidney damage."

Creatinine can remain within the reference range in the early asymptomatic stages of CKD, implying incorrectly no loss of kidney function. The preferred method of diagnosing and staging CKD (which has proved more sensitive than creatinine concentration) is to estimate GFR (e-GFR) from the creatinine concentration (Table VI).

Symptoms of CKD

Changes in urination; swelling of the feet, ankles, hands or face; fatigue or weakness; shortness of breath; ammonia breath or an ammonia or metal taste in the mouth; back or flank pain; itching; loss of appetite; nausea and vomiting; and more hypoglycemic episodes if diabetic.

Causes of CKD

- Diabetes
- Hypertension
- Glomerular disease (autoimmune, infections)
- Inherited and congenital kidney disease
- Poisons
- Trauma
- Medications

CKD stage	Description	e-GFR mL/min/1.73 m^2
1	Kidney damage with normal or increased GFR (e.g. proteinuria)	≥90
2	Kidney damage with mild decrease in GFR (e.g. proteinuria)	60–89
3	Moderate decrease in GFR (e.g. chronic/early kidney insufficiency)	30–59
4	Severe decrease in GFR (e.g. chronic/late kidney insufficiency, pre-end-stage kidney disease)	15–29
5	End-stage kidney disease (kidney failure). Patients require kidney replacement therapy (either dialysis or transplantation)	<15

TABLE VI: Classification of chronic kidney disease using e-GFR [207].
CKD may be stable or progressive. Progress is defined as e-GFR of >5 mL/min/1.73 m^2 in 1 year or >10 mL/min/1.73 m^2 in 5 years

Nephrotoxic drugs

Radiographic contrast media used for X-ray imaging, CT and MRI can be nephrotoxic.

Measuring creatinine and calculating e-GFR can help identify patients with inadequate kidney function before they undergo diagnostic investigation with image-enhancing contrast media before X-ray imaging, CT or MRI to prevent nephrogenic systemic fibrosis (NSF) and contrast-induced nephropathy (CIN).

NSF

Contrast media containing gadolinium can cause nephrogenic systemic fibrosis (NSF) in patients with renal impairment, particularly those with stage-5 CKD (e-GFR <15 mL/min/1.73 m^2) receiving dialysis. The factors that determine susceptibility to NSF among those with severe kidney disease are unknown so all these patients must be considered at equal risk of NSF [198].

CIN

Iodinated contrast agents (ICA) are responsible for contrast-induced nephropathy (CIN), mainly in patients with CKD and diabetes. CIN is not well understood; however, the adverse effect is AKI-evidenced by a transitory increase in creatinine (fall in e-GFR) in the days following administration [209]. The clinical course of CIN is characterized by increased creatinine within 24 hours of contrast administering that peaks within 3–7 days and returns to baseline within 14 days [210]. CIN is associated with increased risk of morbidity and mortality. In patients undergoing cardiovascular angiographic procedures it is strongly recommended to routinely use e-GFR to identify patients at risk of CIN [211].

Patients at risk of CIN [209]

- Patients with CKD
- Patients with diabetes
- Patients prescribed potentially nephrotoxic drugs or with a history of prescribed chemotherapy
- Patients in shock/hypotension (volume depletion)
- Patients at an advanced age (>75 years)
- Patients with advanced congestive heart failure
- The acutely/critically ill (e.g. sepsis)
- Recipients of kidney transplant

Estimating glomerular filtration rate

Creatinine concentration can be used to estimate the glomerular filtration rate (GFR) [212]. GFR is the parameter that best defines kidney function and it is a sensitive indicator of early chronic kidney disease (CKD) (Table VI). Estimated GFR (e-GFR) based on creatinine concentration, age, gender and ethnicity has emerged in recent years as the internationally recommended means of assessing renal function and identifying those with CKD [8–10]. More than 25 different equations for calculating e-GFR in both adults and children, using creatinine corrected for some or all of gender, body size, race and age, are described in the literature [208, 213].

GFR is defined as the volume of blood filtered through the kidneys per minute and is expressed in mL/min. As normal GFR increases with increasing body size, a correction factor using body surface area (BSA) typically is applied. An accepted average adult BSA is 1.73 m^2. Adjusted GFR results are then expressed as mL/min/1.73 m^2.

Reference interval mean e-GFR versus age – examples [194]

Years	mL/min/1.73 m^2
20–29	116
30–39	107
40–49	99
50–59	93
60–69	85
70+	75

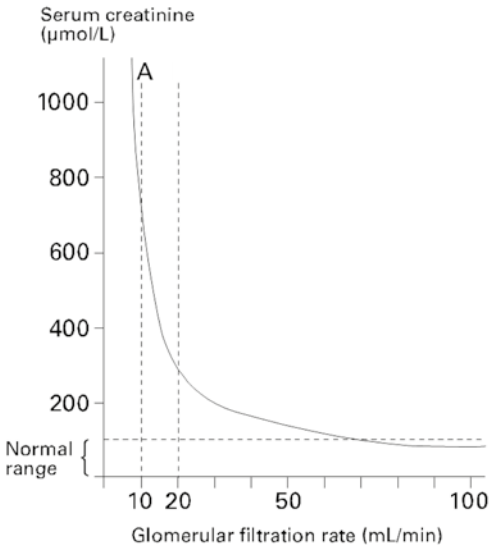


FIG. 19: Relationship between creatinine and GFR. By the time creatinine is above the normal range (dashed horizontal line), the GFR value may have decreased to half its normal value. (Modified from [212].

Estimating GFR equations recommended by NKDEP

MDRD study equation for adult patient age ≥18 years

The Modification of Diet in Renal Disease (MDRD) study equation [212] can be used to detect CKD among patients with risk factors – diabetes, hypertension, cardiovascular disease, family history of kidney disease or patients already diagnosed with CKD.

The IDMS (isotope dilution mass spectrometry)-traceable MDRD study equation does not require weight or height variables because the results are reported normalized to 1.73 m^2 body surface area.

GFR (mL/min/1.73 m^2) = 175 × (S_{cr}/88.4)$^{-1.154}$ × (Age)$^{-0.203}$ × (0.742 if female) × (1.212 if African American) (SI units)

S_{cr} indicates serum creatinine measured in µmol/L.

A calculator is available from NKDEP [214]

The CKD-EPI equation (Chronic Kidney Disease Epidemiology Collaboration) for patient age ≥18 years

The CKD-EPI equation is based on the same four variables as the MDRD study equation but uses a 2-slope "spline" to model the relationship between GFR and serum creatinine, age, sex and race. The equation was developed especially to create a formula more accurate than the MDRD formula at actual GFR >60 mL/min per 1.73 m^2 [215].

The CKD-EPI equation, expressed as a single equation, is:

GFR = 141 × min(S_{cr}/κ,1)$^{\alpha}$ × max(S_{cr} /κ,1)$^{-1.209}$ × 0.993^{Age} × 1.018 (if female) × 1.159 (if African American)

where S_{cr} is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is –0.329 for females and –0.411 for males, min indicates

the minimum of S_{cr}/κ or 1, and max indicates the maximum of S_{cr}/κ or 1 [215, 217].

"The National Kidney Disease Education Program (NKDEP) has not made a recommendation on general implementation of this equation. The equation is still being validated and, while offering some improvement for e-GFR between 60 and 120 mL/min/1.73 m^2, it is not clear that implementing CKD-EPI in place of the MDRD equation would alter clinical detection or management of patients with CKD" [194].

A calculator is available from the National Kidney Foundation [216]

The Bedside Schwartz equation for patient age <18 years

NKDEP recommends that the Bedside IDMS-traceable Schwartz equation [193] be used to estimate GFR for infants, toddlers, children, and teens under the age of 18 [194].

GFR (mL/min/1.73 m^2) = (36.2 × height in cm) / creatinine in µmol/L

A calculator is available from NKDEP [214]

Cardiac troponins – cTnI and cTnT

Cardiac troponin I (cTnI) and cardiac troponin T (cTnT) – are proteins of heart muscle cells (cardiac myocytes) normally only barely detectable or undetectable in blood. However, damage to these cells, called myocardial necrosis, results in the leakage of cell contents – including cTnI and cTnT – to the circulation, and rising concentration in blood. cTnI and cTnT are thus sensitive and specific blood markers of myocardial necrosis. A number of cardiac and some non-cardiac diseases are associated with some degree of myocardial necrosis but myocardial infarction (heart attack) is the most common cause of extensive myocardial necrosis. The primary clinical utility of both the cTnI test and the cTnT test is indicated for use as an aid in the diagnosis of myocardial infarction (MI) and in the risk stratification of patients with acute coronary syndromes (ACS) with respect to their relative risk of mortality. Both tests are equally useful in their ability to detect myocardial necrosis [221].

Physiological significance of troponin

The troponin complex is a protein constituent of the striated muscle cells present in both skeletal muscle and the cardiac muscle (myocardium) that comprises the bulk of the heart wall. More specifically, the troponin complex is a structural component of the intracellular contractile assembly (myofibrils) that enables muscle contraction [222]. It comprises three protein subunits, each encoded by separate genes. The three subunits are: troponin C (TnC), troponin I (TnI) and troponin T (TnT). The whole troponin complex is bound via TnT to the actin filament of the myofibril. The interaction between actin and myosin filaments that facilitates coordinated muscle cell contraction is initiated by calcium ions binding to TnC. TnI binding of actin inhibits contraction. By these two opposing effects, one initiating contraction of myofibrils and the other inhibiting the process, the troponin complex plays a major role in regulating contraction of both skeletal and cardiac muscle [222].

Troponin I and troponin T (TnI and TnT) derived from skeletal muscle are structurally distinct from those present in cardiac muscle. It is the tissue specificity of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) that ensures the clinical utility of measuring the concentration of cTnT and cTnI in blood, for the detection of myocardial necrosis and diagnosis of myocardial infarction [223].

Cardiac troponins and Myocardial Infarction

Myocardial infarction (MI) is the death of myocardial cells (myocardial necrosis) due to ischemia (reduced blood supply). In classical (type 1) MI, the precipitating ischemia is due to sudden thrombotic blockage of a coronary artery, consequent on rupture of a long-standing atherosclerotic plaque [224]. There are other mechanisms that can give rise to MI, allowing classification of types 2, 4 and 5 MI (in type 3 patient died before cardiac blood tests could be performed) but by comparison with type 1 MI these are less common etiologies [224]. Early diagnosis of MI and institution of emergency reperfusion therapy can limit cardiac damage and ultimately, preserve life [227]. Since measurement of cardiac troponins (either cTnI or cTnT) in plasma provide the means for early and rapid demonstration of myocardial necrosis following onset of cardiac ischemia, the tests have a well-established role in the diagnosis of MI [224, 225]. Indeed, increased troponin is a defining feature of myocardial infarction.

The following criteria satisfy a diagnosis of acute, evolving or recent MI [224]:

- Detection of a rise or fall of cardiac biomarkers (preferably cTnI or cTnT) with at least one value above the 99th percentile upper reference limit (URL) of a healthy population, and at least one of the following:
  - Symptoms of cardiac ischemia
  - ECG changes consistent with new ischemia
  - Imaging evidence of new loss of viable myocardium
  - Identification of an intracoronary thrombus by angiography or at autopsy

When should cTnI/cTnT be measured

It is recommended that only troponin assays with optimal levels of analytical imprecision at the 99th percentile value be used; this optimal level has been defined as coefficient of variation (CV) preferably less than 10 %, and no higher than 20 % [224, 226].

The dynamic rise in cTnI and cTnT associated with MI is usually first detectable within 4–8 hours after the onset of chest pain; levels peak at around 12–48 hours. Plasma cTnI and cTnT levels remain elevated several days after severe MI: 4–7 days in the case of cTnI, 10–14 days in the case of cTnT [225]. Demonstration of a rise and/or fall in cTnI or cTnT is required to identify MI as the cause of increased troponin, so that serial measurements are necessary. It is recommended that blood be collected for troponin measurement at presentation and then again 3–6 hours later [224, 227]. It may be necessary to test again at 6–12 hours to definitively exclude a diagnosis of MI if clinical doubt remains after the initial testing and clinical assessment. A diagnosis of MI can usually be excluded if troponin remains negative over a period of 6–12 hours after onset of symptoms. MI is also excluded if an abnormal result remains unchanged (<20 % change) during the hours after onset of symptoms.

In practice, patients presenting with typical symptoms of cardiac ischemia and unequivocal electrocardiogram (ECG) evidence of current ischemia (ST-segment elevation) are given a diagnosis of ST-segment elevation myocardial infarction (STEMI) without necessarily demonstrating a rise in cTnI or cTnT [228]. This is because the combination of typical ischemic chest pain and ST-segment elevation is virtually diagnostic of MI; reperfusion treatment of these patients need not and should not be unnecessarily delayed by serial troponin testing [225, 228]. The value of troponin testing in the context of STEMI is less diagnostic (although it can provide confirmatory diagnostic evidence if raised at presentation), and more prognostic: the higher the troponin level at presentation, the poorer the prognosis for patients with STEMI [229].

It is in the absence of unequivocal ECG evidence of MI that serial troponin measurement has the greatest diagnostic value. A diagnosis of non-ST-elevation myocardial infarction (NSTEMI) depends on demonstrating a rise (or fall) in cTnI or cTnT [227].

Clinical indications for cTnI or cTnT request

Since the principal clinical utility for measurement of troponins is indicated as an aid in the diagnosis of MI, these tests should be reserved for those patients presenting with symptoms and medical history suggestive of acute coronary syndrome/MI. Main symptoms [224, 230] include:

- Acute onset of severe chest pain lasting more than 20 minutes that may radiate to neck, shoulder, arms, jaw or back
- Absence of chest wall tenderness on palpation
- Breathlessness
- Sweating
- Nausea/vomiting
- ECG changes suggestive of cardiac ischemia [224]

There are gender differences; the following symptoms are more frequently reported in females than males [231]:

- Pain in left shoulder, throat, jaw and between shoulder blades
- Vomiting
- Nausea
- Dyspnea

Interpreting test results

Unit of measurement

Although the recommended unit of troponin measurement is ng/L [221], it is not necessarily being used in all healthcare institutions. This may lead to confusion in interpreting results. The most widely used alternative units are: ng/mL, µg/L and pg/mL. Example: 20 ng/L = 0.02 ng/mL = 0.02 µg/L = 20 pg/mL.

Defining a positive troponin result

Because of lack of standardization among cTnI assays it is not possible to define a universal reference range for cTnI with which to interpret patient test results (it is easier with cTnT because there is only one licensor). It is therefore imperative that the decision limit determined by the laboratory performing the test be used to interpret patient test results. An increased troponin level (i.e. positive troponin result) is defined as one that is at or above the 99th percentile of a presumed healthy reference population. For 18 currently marketed cTnI assays this 99th percentile value ranges from 13 to 392 ng/L [232]. Depending on the assay used, a positive cTnI result is defined as anything from ≥13 ng/L to ≥392 ng/L.

There is less variation in the 99th percentile value for cTnT because, for license reasons, cTnT manufacturers must have similar analytical and clinical performances. The 99th percentile value for the currently available high-sensitivity troponin T (hs-cTnT) assay is around 14 ng/L [233, 234].

An overview of the multiple situations associated with positive troponin results are is shown in Fig. 20. When working a diagnosis of MI, it is imperative to consider the pretest probability that the patient is having an MI. With that in mind, the example of decision trees shown in Fig. 21 and Fig. 22 provide a reasonable and practical approach. To date, there is no expert consensus guidance on a procedure for establishing or confirming delta values. Until this is available, medical institutions should agree on a delta value based on available data (peer-reviewed journals, manufacturer's documentation), and then modify based on experience and feedback.

In any case, the clinical judgment remains paramount.

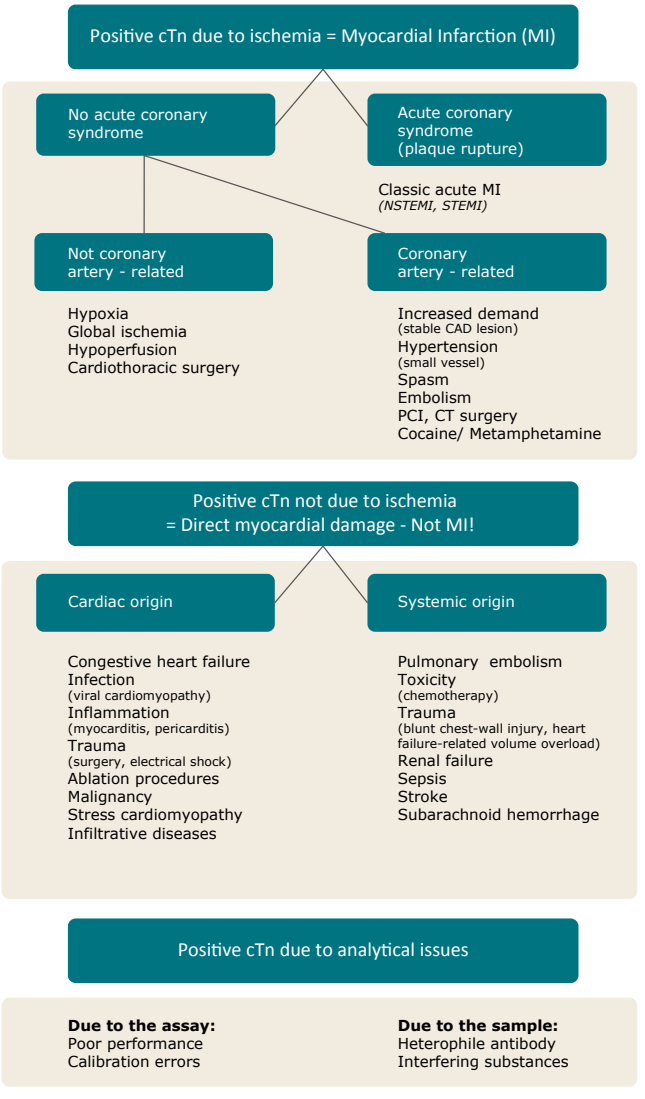


FIG. 20: Defining a positive troponin result. Modified from [221].

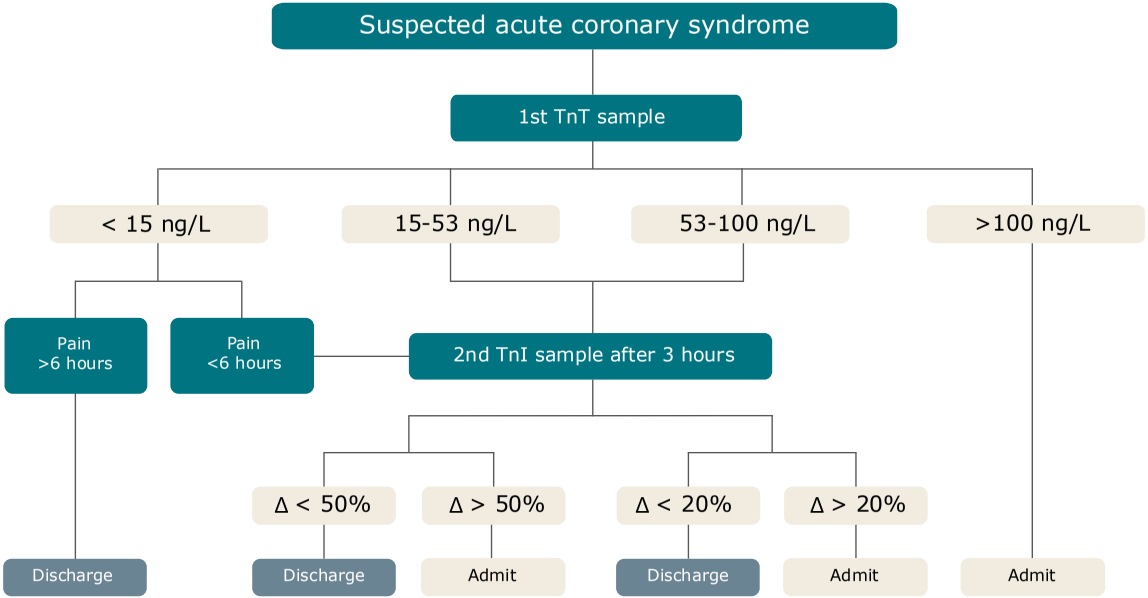


FIG. 21: Example of troponin T result interpretation decision tree.
△: rise or fall between samples 1 and 2. Modified from [235].

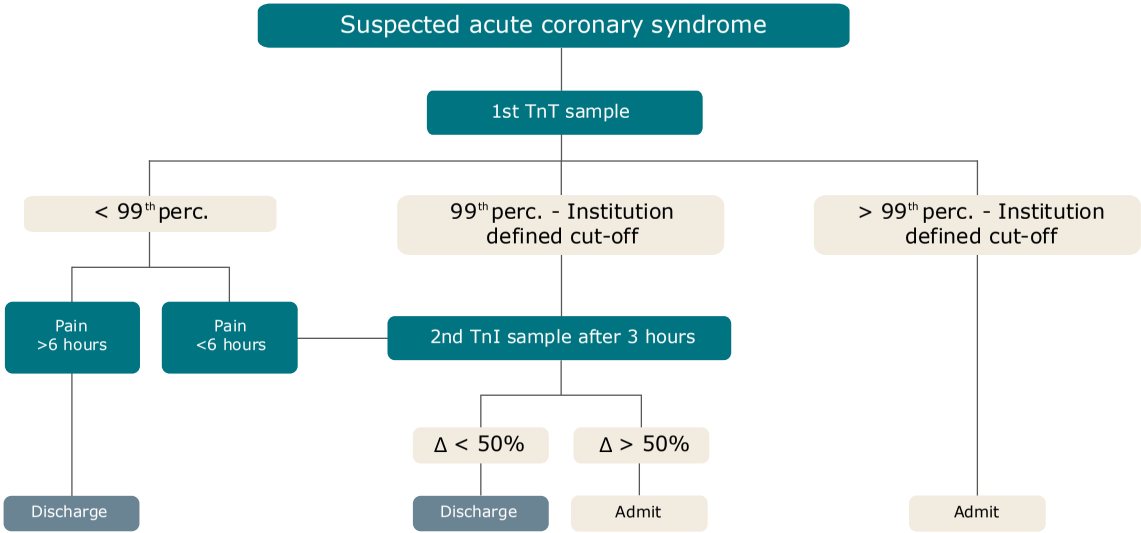


FIG. 22: Example of troponin I result interpretation decision tree.
△: rise or fall between samples 1 and 2. Modified from [235] .

Troponin levels in patients suffering from MI

Troponin levels in patients with MI depend on the amount of myocardial damage sustained. In the case of minimal damage (sometimes referred to as microinfarction) the peak of troponin levels may not exceed 50–100 ng/L, whilst the most severe myocardial infarction may cause troponin levels to rise to 100,000 ng/L [236].

Non-MI causes of increased cTnI and cTnT

Although cTnI and cTnT are both highly specific for myocardial necrosis, neither is specific for MI. There are a number of conditions other than MI that are associated with myocardial necrosis and thereby increased cTnT and cTnI [221, 236, 237]. Therefore, it is not possible to diagnose MI on the sole basis of increased cTnI or cTnT; there must be additional clinical evidence (e.g. symptoms) of cardiac ischemia. Demonstration of the typical acute elevation in troponin values associated with MI helps to distinguish troponin increases due to MI from those due to other causes. A moderately elevated and relatively stable troponin concentration, for example, is often indicative of a chronic cardiac condition but is not consistent with the acute nature of MI.

Causes other than MI that can be associated with increased cTnI and cTnT include:

- Heart failure
- Tachyarrhythmia
- Cardiomyopathy
- Myocarditis, pericarditis
- Renal failure (ESRD)
- Blunt chest trauma
- Pulmonary embolism
- Sepsis/septic shock
- Aortic valve disease
- Cerebrovascular accident (stroke)
- Cardiotoxic drugs
- Extreme exertion (e.g. marathon running)

The clinical value of troponin testing in these causes is not well established. However, for a number of them, increased troponin is a poor prognostic sign [221].

Natriuretic peptides – BNP and NT-proBNP

B-type (or brain) natriuretic peptide (BNP) and N-terminal-pro-B-type natriuretic peptide (NT-proBNP) are derived from heart muscle cells and are present in blood at very low concentrations in healthy individuals. Increased concentrations are associated with many cardiac and some non-cardiac diseases. Clinically, measurement of both BNP and NT-proBNP aids in the diagnosis of heart failure. Both tests have equal diagnostic value [238], although study suggests that NT-proBNP may have a more favorable *in vitro* stability profile than BNP [239].

BNP and NT-proBNP – background physiology

BNP is a cardiac hormone derived from a larger peptide (pro-hormone) called proBNP [240]. This pro-hormone is produced predominantly within the muscle cells (myocytes) of the heart [241]. proBNP, a peptide comprising 108 amino acids, is enzymatically cleaved; the two products of this enzyme action are the active hormone BNP, comprising 32 amino acids, and NT-proBNP, comprising 76 amino acids (Fig. 23). The physiological trigger for synthesis of proBNP within cardiac myocytes and release of BNP and NT-proBNP to the circulation is cardiac-wall stress induced by increased pressure/volume [240].

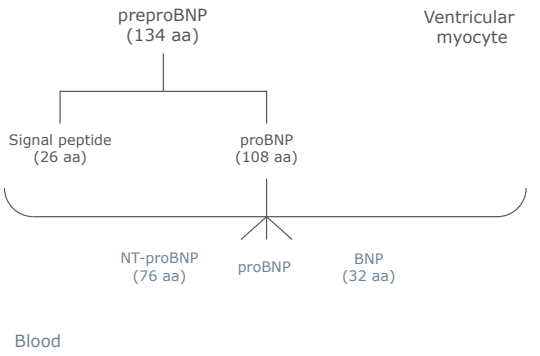


FIG. 23: Origin of BNP and NT-proBNP.
aa: amino acid.

BNP has a multiplicity of hormonal effects that together contribute to cardiovascular homeostasis, including regulation of blood volume and blood pressure [242, 243]. For example, BNP promotes increased sodium and water loss in urine (natriuresis and diuresis) and induces vasodilation. It has an inhibitory effect on the renin-aldosterone axis. In addition to these endocrine effects, BNP has direct paracrine effect on the heart, protecting it from two pathological processes (fibrosis and hypertrophy) [244]. By contrast with BNP, NT-proBNP has no known function; it is an apparently biologically inert peptide [243].

Although BNP and NT-proBNP are secreted in equimolar amounts, circulating concentration of BNP is significantly lower than that of NT-proBNP due to different rates of disposal; the half-life of BNP is 20 minutes compared with 120 minutes for NT-proBNP [245]. The only route for elimination of NT-proBNP is via the kidneys, whereas BNP is eliminated via renal and non-renal routes. Renal dysfunction is thus associated with increase in NT-proBNP and to a lesser extent, BNP [246].

Specimen collection for BNP and NT-proBNP

For BNP measurement, blood must be anticoagulated with EDTA; the analysis may be performed on either plasma recovered from this EDTA sample, or directly on the whole-blood sample [247].

For NT-proBNP measurement, either serum or plasma (anticoagulated with EDTA or heparin) is suitable. EDTA and heparinized whole blood may also be used [247].

Interpreting test results

Units of measurement

BNP and NT-proBNP are expressed either as pg/mL, ng/L or pmol/L.

1 pg/mL (or ng/L) BNP is equal to 0.289 pmol/L BNP.
1 pg/mL (or ng/L) NT-proBNP is equal to 0.118 pmol/L NT-proBNP.

BNP and NT-proBNP in healthy individuals [248, 249, 250]

Both BNP and NT-proBNP increase with age there is also significant gender difference. These effects are demonstrated in the following tables [248, 249].

Median BNP pg/mL (25th–75th percentile range)

	45–54 years	55–64 years	65–74 years	75–83 years
Male	12 (3–34)	21 (5–49)	23 (7–58)	29 (17–44)
Female	23 (10–55)	29.5 (15–68)	37 (19–111)	62.5 (26–172)

Median NT-proBNP pg/mL (upper ref. limit 97.5th percentile)

	45–59 years	≥60 years
Male	20 (100)	40 (172)
Female	49 (164)	78 (225)

NT-proBNP reference range for infants and children

Age interval	Median NT-proBNP pg/mL (5th–97.5th percentile range)
0–2 days	3183 (321–13222)
3–11 days	2210 (263–6502)
> 1month to 1 years	141 (37–1000)
> 1 to 2 years	129 (39–675)
> 2 to 6 years	70 (23–327)
> 6 to 14 years	52 (10–242)
> 14 to ≤ 18 years	34 (6–207)

BNP and NT-proBNP for diagnosis of heart failure

Heart failure is a complex clinical syndrome in which, usually due to ventricular dysfunction, the heart is unable to pump a sufficient blood volume [251]. Patients with reduced cardiac output usually present with shortness of breath (dyspnea) at rest or during exertion, and fatigue. It is a common, chronic, progressively debilitating condition, predominantly affecting the elderly. The general population prevalence of heart failure is 0.8–2.0 %, but 10–20 % among those aged 70–80 years [251]. Pre-existing coronary heart disease (e.g. history of myocardial infarction) is by far the most common cause; other causes include chronic hypertension and diabetes. Consideration of presenting symptoms, results of clinical examination, electrocardiogram (ECG) and chest X-ray may suggest heart failure, but these modes of clinical assessment are individually relatively non-specific for heart failure, or lack sufficient sensitivity, and the "gold-standard" diagnosis depends ultimately on imaging of the heart, usually by echocardiography [251].

Heart failure is associated with increased ventricular wall stretch and consequent increased circulating concentration of BNP and NT-proBNP [245]; the levels correlate with severity of heart failure. So, consistent with this finding, using a suitable diagnostic cut-off value, test can be used to help rule out a diagnosis of heart failure, eventually without recourse to echocardiography [252, 253, 254].

Cut-off values

The European Society of Cardiology (ESC) recommended cut-off values to help rule out a diagnosis of heart failure in an acute setting for patients presenting with acute onset or worsening of symptoms [251]:

- BNP <100 pg/mL
- NT-proBNP <300 pg/mL

For patients presenting with non-acute symptoms, the recommended cut-off values are lower:

- BNP <35 pg/mL
- NT-proBNP <125 pg/mL

UK guidelines [252] make no distinction between acute and non-acute presentation and recommend the use of the following cut-off values to help rule out a diagnosis of heart failure for all patients:

- BNP <100 pg/mL
- NT-proBNP <400 pg/mL

Although a "normal" BNP or NT-proBNP result can be used to rule out heart failure (high negative predictive value), it is less useful as a positive predictor for heart failure since other conditions are associated with increased BNP and NT-proBNP [255–263]:

- Hypertension
- Myocardial infarction
- Angina (stable and unstable)
- Myocarditis
- Cardiac arrhythmias
- Primary pulmonary hypertension
- Pulmonary embolism
- Chronic obstructive pulmonary disease (COPD)
- Renal failure
- Sepsis/septic shock
- Anemia
- Cirrhosis
- Stroke
- Hyperthyroidism

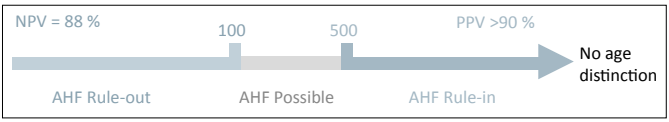
Usually, these conditions are associated with a less pronounced increase than that seen in patients with heart failure. The higher the circulating concentration of BNP or NT-proBNP, the greater is the likelihood that heart failure causes the increase. The following values are proposed as cut-off values for BNP and NT-proBNP, above which heart failure can be considered highly likely [264]:

	years	pg/mL
BNP		>400
NT-proBNP	<50	>450
	50–75	>900
	>75	>1800

The overall average sensitivity and specificity of these values to aid in the rule in of heart failure are 92 % and 84 %, respectively [265].

The algorithm for diagnosis of heart failure incorporating these cut-off values is provided in Fig. 24. For patients whose BNP or NT-proBNP falls within the grey zone (between the rule-in and rule-out values), natriuretic peptide measurement provides limited diagnostic information. However, this only occurs for a minority (~25 %) of patients in whom the clinical assessment suggests heart failure [265]. A major proportion (~70 %) of patients whose clinical assessment suggests heart failure and whose initial BNP or NT-proBNP values lie within the indeterminate grey zone turn out on further investigation (e.g. echocardiogram) to be suffering from heart failure [265].

BNP



NT-proBNP

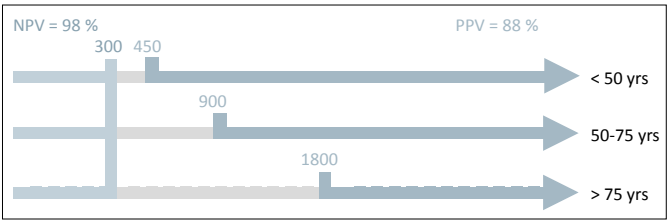


FIG. 24: The BNP and NT-proBNP cut-off values.

NPV: negative predictive value; PPV: positive predictive value; AHF: acute heart failure

The prognostic utilization of BNP and NT-proBNP in heart failure

Both BNP [266] and NT-proBNP [264] levels correlate with severity of heart failure as determined by the New York Heart Association (NYHA) classification. This defines four classes of functional impairment (Class I – symptomless mild heart failure; Class IV – most severely debilitating heart failure).

NYHA Class	Mean BNP pg/mL
Class I (asymptomatic)	244 ± 286
Class II (mild)	389 ± 374
Class III (moderate)	640 ± 447
Class IV (severe)	817 ± 435

NYHA Class	Median NT-proBNP pg/mL	Interquartile range pg/mL
Class II	3512	1395–8588
Class III	5610	2260–11001
Class IV	6196	2757–13295

Prognostic significance of both BNP and NT-proBNP measurements is demonstrated by the Valsartan Heart Failure Trial [267]. The adjusted hazard ratio for death among those with the highest BNP (>442 pg/mL) and NT-proBNP (>3863 pg/mL) was four times than that for those with the lowest BNP (<31 pg/mL) and NT-proBNP (<304 pg/mL). An increment of 500 pg/mL from baseline NT-proBNP and/or an increment of 50 pg/mL from baseline BNP corresponded to an increase of 3.8 % and 5.7 % in risk of death, respectively. Others found positive correlations between BNP/NT-proBNP levels and the risk of morbidity from heart failure, supporting their role as powerful predictors of mortality and morbidity among patients with heart failure.

Serial measurement of BNP or NT-proBNP may be useful in guiding drug therapy of heart failure [268–270] since drug-induced improvement in heart function is associated with reduction in circulating natriuretic peptides. Whether drug dosage should be adjusted to achieve a "target" BNP or NT-proBNP concentration is still a matter of investigation [268].

D-dimer

An increased blood concentration of D-dimer provides evidence of on-going fibrinolysis and therefore fibrin clot formation, a feature of thromboembolic diseases and other conditions associated with a hypercoagulable state. The principal clinical utility of the D-dimer test is in the assessment of patients suspected of suffering from two related thrombotic conditions: deep vein thrombosis (DVT) and pulmonary embolism (PE), together known as venous thromboembolism (VTE).

What are D-dimers?

D-dimers are derived from the fibrin clots that in health are formed to protect against blood loss following blood vessel injury. The fibrin contained within a fibrin clot is the product of a complex process known as the blood coagulation cascade [273]. The final part of this cascade involves formation of fibrin from fibrinogen, a soluble plasma protein that contains within its structure the so-called D-domain. Activation of the coagulation cascade results in production of thrombin, an enzyme that cleaves fibrinogen [273]. The product of this cleavage is fibrin monomers that retain the D-domain. Fibrin monomers join together spontaneously to form long double-stranded protofibrils. Thrombin also activates another enzyme, blood clotting factor XIII, which catalyzes cross-linking between the D-domain of fibrin monomers contained within fibrin protofibrils. The resulting accumulating mass of threadlike cross-linked fibrin polymers forms an insoluble gel; this is a fibrin clot.

Fibrinolysis, the process of fibrin clot degradation, is part of the healing process following blood vessel injury but also serves to limit clot growth within patient blood vessels. A central component of the fibrinolytic system is the enzyme plasmin, which cleaves bonds within fibrinogen and fibrin, including the cross-linked fibrin within a fibrin clot [274]. The product of its action are a heterogeneous collection

of peptides known collectively as fibrin(ogen) degradation products (FDP). D-dimers are a specific group of FDPs that are derived from the cross-linked fibrin polymers present in a fibrin clot; the D-domain cross-linking induced by activated factor XIII is preserved – it is this structural detail, the so-called D-dimer motif that defines D-dimers and distinguishes them from other FDPs. Since D-dimers are derived from the cross-linked fibrin contained within a fibrin clot, they provide a blood marker of on-going fibrinolysis and thereby coagulation activation [274].

D-dimer and venous thromboembolism (VTE)

VTE includes DVT and its life-threatening sequela, PE [275]. The thrombus (blood clot) responsible for DVT is most commonly formed in the deep veins of the leg or pelvis. A distinction is made between distal DVT (thrombus in lower-leg veins passing through the calf muscle) and proximal DVT (thrombus in the deep veins above the knee) [276]. Upward growth (propagation) of a distal DVT can result in proximal DVT. Pulmonary embolism occurs predominantly in those with proximal DVT when a thrombus or thrombus fragment breaks free and is swept away in the venous system to the right side of the heart and then onwards via the pulmonary artery, finally coming to rest in the vasculature of the lungs. As a consequence, blood perfusion of an area of the lung is compromised, with resulting loss of pulmonary function (see oxygen status chapter), and an increase in pulmonary vascular resistance with resulting strain to the right heart. Ultimately, if blood flow is not restored, pulmonary infarction and/or cardiac failure develops.

The embolized thrombus may be sufficiently large to block a large pulmonary vessel causing sudden acute circulatory and respiratory failure (see pO_2 and pCO_2) and death before thrombolytic treatment can be administered; it is estimated that in 10 % of cases, PE is rapidly fatal [277].

Blood concentration of D-dimer is increased in almost all patients with VTE (both DVT and PE). Unfortunately the diagnostic value of the D-dimer test implied by its high sensitivity for VTE (90–98 % depending on the assay) is limited by its low specificity for VTE (<50 %); there are many diseases/conditions other than VTE that can be associated with increased D-dimer [278] including:

Causes of increased D-dimer not associated with VTE

Arterial thrombotic diseases:

- Myocardial infarction (MI)
- Stroke
- Limb ischemia
- Atrial fibrillation (AF)

Venous thrombotic disease:

- Deep vein thrombosis (DVT)
- Pulmonary embolism (PE)

Other:

- Disseminated intravascular coagulation (DIC)
- Aortic dissecting aneurysm
- Severe infection/sepsis
- Severe inflammation
- Systemic inflammatory response syndrome (SIRS)
- Surgery/trauma
- Sickle cell crisis
- Cancer
- Acute kidney injury (AKI)
- End-stage renal disease (ESRD)
- Heart failure (HF)
- Severe liver disease (cirrhosis)
- Preeclampsia and eclampsia
- Normal pregnancy
- Use of thrombolytic drugs

Why measure D-dimer?

It is thus not possible to make a diagnosis of DVT or PE on the basis of increased D-dimer, but a normal D-dimer result is useful in helping to exclude the diagnoses.

Diagnosis of DVT and PE depends on imaging study of veins – usually compression ultrasound scan of the legs, in the case of suspected DVT [275, 279]; and computed tomography angiography scan of the chest, in the case of suspected PE [275, 277, 279]. The primary value of the D-dimer test is in patients with low pretest probability of VTE (either DVT or PE). In such cases, the diagnosis of VTE can be reliably ruled out if the D-dimer concentration is below a preset diagnostic cut-off value [275, 277, 279].

This means that a significant number of patients do not need to be subjected to expensive and time-consuming imaging investigations. The detail of assessing pretest probability of DVT and PE using Wells clinical criteria [280, 281] along with a diagnostic algorithm for the two conditions is contained in Fig. 25.

In addition to its well-established role in excluding a diagnosis of VTE, the D-dimer test may also be helpful in identifying those patients who are at high risk of recurrent VTE and therefore require long-term anticoagulation therapy. A negative D-dimer test after cessation of anticoagulation therapy indicates a low risk of recurrence [282].

WELLS SCORE FOR DVT	Points
Active cancer	+1
Paralysis or recent cast immobilisation	+1
Bed rest > 3 days or surgery < 4 weeks ago	+1
Pain on palpation of deep veins	+1
Swelling of entire leg	+1
Calf swelling > 3 cm difference in affected limb	+1
Pitting oedema in affected limb	+1
Dilated superficial veins in affected limb	+1
Previously documented DVT	+1
Alternative diagnosis at least as likely as DVT	-2

Score 2 points or more - high probability of DVT
Score 1 point or less - low probability of DVT

WELLS SCORE FOR PE	Points
Previous PE or DVT	+1.5
Heart rate > 100 beats per minute	+1.5
Recent surgery or immobilisation	+1.5
Clinical signs of DVT (leg swelling pain)	+3
Alternative diagnosis less likely than PE	+3
Haemoptysis (coughing up blood)	+1
Cancer	+1

Score more than 4 points - high probability of PE
Score 4 points or less - low probability of PE

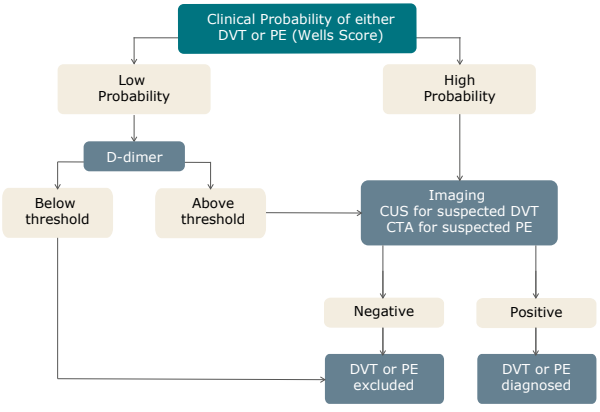


FIG. 25: Wells clinical scoring to determine probability of DVT/PE and (below) how that score is used in diagnosis/exclusion of DVT/PE based on D-dimer testing and imaging studies. Modified from [275, 279].
DVT: Deep venous thrombosis; PE: Pulmonary embolism
CUS: compression ultrasonography; CTA: Computed tomography angiography

Clinical utility of D-dimer test not confined to VTE

Disseminated intravascular coagulation (DIC) is a potentially life-threatening complication of many critical illnesses that are associated with inappropriate fibrin clot formation within blood vessels, increased fibrinolysis and thereby, marked increase in blood D-dimer concentration. Diagnosis of DIC depends on a scoring system devised by the International Society on Thrombosis and Haemostasis (ISTH) that takes account of the results of a number of laboratory tests, including the D-dimer test [283].

There is some evidence to suggest that the D-dimer test may have a role in the investigation of patients suspected of suffering from acute aortic dissection; close to 100 % of patients with this condition have increased D-dimer, and it has been proposed that a negative D-dimer test result may be sufficient evidence to exclude the diagnosis, although this remains controversial [284].

When should the D-dimer test be considered?

Symptoms consistent with possible DVT [285] that might provoke D-dimer measurement include:

- Localized leg pain/tenderness
- Leg swelling – pitting edema
- Reddening/blue-red discoloring of skin of affected leg
- Low-grade pyrexia

Symptoms consistent with possible PE [285] that might provoke D-dimer measurement include:

- Sudden-onset dyspnea
- Chest pain
- Cough (with or without hemoptysis)
- Hypoxia (pO_2(a) <80 mmHg, SpO_2 <95 %)
- Increased heart rate >100mmHg
- Symptoms of DVT

None of these symptoms are specific for DVT/PE, and both DVT and PE can be asymptomatic. Suspicion of DVT/PE and consequent need for D-dimer measurement is increased for those with a history of any of the following [285]:

- Active cancer
- Recent major surgery/trauma
- Extended period of recent immobilization (e.g. bed rest/long-haul air travel)
- Hormonal therapy
- Pregnancy/recent pregnancy
- Previous history of VTE

The take home message is that D-dimer should only be ordered in cases of suspected DVT or PE if, on application of Wells criteria, there is low probability of either condition (Fig. 25).

D-dimer measurement may also be indicated for critically ill patients with symptoms of excessive bleeding and thrombocytopenia, a signal of possible DIC.

Interpretation of D-dimer test results

The assays used to measure D-dimer vary greatly and there is no single standard against which all assays are calibrated [286]. This means that D-dimer reference intervals and diagnostic cut-off values used to exclude VTE are assay-specific; there is currently no universal D-dimer reference interval or diagnostic cut-off value for exclusion of VTE. There is also variation both in the literature and between manufacturers of D-dimer assays, with regards to units of D-dimer measurement. The results are expressed as either fibrinogen equivalent units (FEU) or D-dimer units (DDU), using various concentration modalities: ng/mL; µg/mL; µg/L and mg/L. Note that D-dimer expressed in FEU is twice as high as D-dimer expressed in DDU (i.e. D-dimer 100 ng/mL FEU is the same as D-dimer 50 ng/mL DDU). However, conversion of results from FEU to DDU or vice versa is not recommended.

Example: 500 ng/mL = 0.5 µg/mL = 500 µg/L = 0.5 mg/L.

Given these assay, standardization and unit differences, it is imperative that only the diagnostic cut-off/reference interval published by the laboratory performing the test is used to interpret patient D-dimer test results.

The issues discussed in this section are more fully addressed in a presentation available on the internet [287].

C-reactive protein – CRP

C-reactive protein (CRP) is a highly conserved, relatively large protein (MW ~120,000) consisting of five identical polypeptide chains that are synthesized in the parenchymal cells of the liver (hepatocytes). In healthy individuals, plasma concentrations of CRP are normally below 5.0 mg/L, but during the innate immune response to infection or any major tissue injury/insult, hepatic synthesis increases, and plasma concentration rises. In this sense CRP, in common with the erythrocyte sedimentation rate (ESR) test, is a non-specific blood marker of organic disease. Measurement of plasma CRP concentration has proven clinically useful in the diagnosis and management of infectious disease and the monitoring of a range of non-infectious inflammatory conditions. In recent years, it has emerged that CRP measurement can be useful in assessing individual risk of cardiovascular disease but this requires the use of high-sensitivity CRP assays [288].

Background pathophysiology

CRP's first property was identified in 1930, at the time of its discovery in the blood of patients suffering from streptococcal pneumonia. CRP was shown to have the ability to bind to the C-polysaccharide constituent of the streptococcal bacteria wall [289]. CRP is the archetypal acute-phase response protein, the acute-phase response being one element of the complex physiological response to infection, inflammation, tissue injury or malignant neoplasia [290]. The stimulus to hepatic synthesis of CRP is the cytokine, interleukin-6 (IL-6) released from activated macrophages at the site of infection, injury or inflammation. CRP is sometimes called a "surrogate marker" of IL-6. Within hours of the initial insult that induces an acute-phase response, plasma CRP concentration begins to rise rapidly, reaching a plateau at 24–48 hours. Peak CRP concentration varies greatly depending on the nature and severity of the stimulus that provokes the acute-phase response, but following a severe stimulus, e.g. sepsis or acute myocardial infarction, the rise from normal baseline

concentration can be more than 1000-fold [291]. When the stimulus is removed or resolves, plasma CRP concentration rapidly falls, diminishing by half every 19 hours [289].

The precise function of CRP remains undetermined, but it is assumed that it helps defending against microbial invasion or mitigates the effects of microbial invasion. Its ligand-binding properties suggest a role in the safe disposal of damaged cellular material [291].

CRP reference values – what is normal?

The shape of the distribution of CRP concentration in apparently healthy populations is heavily skewed to the right, with median concentration close to 0.8 mg/L, and an interquartile range of 0.3–1.7 mg/L [292]. Only around 1 % of apparently healthy adults have CRP >10 mg/L, and for most (90–95 %), CRP is less than 5.0 mg/L. It is assumed that subclinical disease is the reason for the right-skewed distribution [292]. CRP in the range of 5.0–10.0 mg/L probably indicates mild (subclinical) inflammation/infection [293], and CRP >10.0 mg/L is consistent with a clinically evident acute-phase response. The cut-off value used to distinguish normal from abnormal CRP varies between laboratories but is in the range of 5–10 mg/L [289].

Measurement of CRP – the distinction between CRP and hsCRP assays

The analytical sensitivity of CRP assays varies. The term "high-sensitivity CRP" (hsCRP) refers specifically to those assays that have sufficient sensitivity to reliably detect CRP concentration throughout the whole reference range ("normal" from 0.1 to >10 mg/L) [291]. Less sensitive assays (called simply CRP assays) have a detection limit in the range of 2–10 mg/L. Whilst these less sensitive assays are capable of detecting the CRP increase associated with an

acute-phase response, they are not able to reliably measure very low concentrations, and are not suitable for assessing the risk of cardiovascular disease. It is important to note that hsCRP assays and CRP assays measure the same substance, CRP.

Causes associated with increased CRP [299, 294 – 296]

- Bacterial infection
- Viral infection
- Fungal infection
- Sepsis

- Rheumatoid arthritis
- Juvenile chronic arthritis
- Ankylosing spondylitis
- Reiter's disease
- Systemic vasculitis (e.g. Behçet's disease)
- Polymyalgia rheumatica
- Crohn's disease

- Systemic lupus erythematosus (SLE)
- Systemic sclerosis
- Dermatomyositis
- Ulcerative colitis
- Sjögren's syndrome

- Myocardial infarction
- Acute pancreatitis
- Severe trauma
- Burns
- Fractures
- Surgery
- Malignant disease

Clinical utility of CRP

The non-specific nature of the acute-phase response and resulting rise in CRP determines that CRP cannot be used alone to diagnose any condition. However, it can provide supportive evidence for diagnosis of the causes listed above, and for many of these causes, the CRP level accurately reflects disease activity or extent of tissue damage [296].

Infectious disease is often caused by bacteria and are usually associated with a higher rise in CRP than those caused by viruses [292]. This difference is diagnostically useful. Specific applications of CRP measurement in infectious disease include [297–310]:

- Bacteremia and septicemia in adults, children and neonates
- Detection of bacterial and other infections in immunosuppressed patients
- Detection of postoperative infection
- Detection of infection in children with unexplained fever
- Distinguishing bacterial and viral meningitis/pneumonia
- Identification of perforated acute appendicitis
- Predicting severity of infection among the critically ill
- Predicting bacterial infection among patients with symptoms of influenza
- Guiding the rational use of antibiotics for respiratory infection in primary care
- Serial measurement to monitor efficacy of antibiotic therapy in a wide range of serious infectious diseases

As shown above, CRP concentration accurately reflects disease activity/severity in inflammatory disease; the clinical utility of this general observation is most widely applied in the diagnosis and monitoring of rheumatoid arthritis [296]. Whilst CRP levels vary among patients with an apparently similar level of arthritis severity, the changes seen in individual patients accurately reflect changes in severity for that patient, so CRP is useful in confirming the efficacy of treatment (decrease in CRP) or disease progression (increase in CRP) [311].

Other specific applications of CRP measurement in inflammatory disease include:

- Assessing severity at diagnosis and response to therapy in Crohn's disease [312]
- Might be helpful in distinguishing Crohn's disease (marked increase in CRP) from ulcerative colitis (normal or only slightly raised CRP) among patients presenting with symptoms of inflammatory bowel disease [313]
- Monitoring disease activity/response to treatment in polymyalgia rheumatica [314]

Human chorionic gonadotropin – hCG

Human chorionic gonadotropin (hCG; also known as βhCG or total hCG) is a hormone that is normally only produced in significant measurable quantity during pregnancy. The principal clinical utility of measuring hCG is for the early detection of pregnancy, and for the diagnosis and management of some common early pregnancy-related disorders such as spontaneous abortion and ectopic pregnancy [316]. hCG is elaborated by the cells of some rare placenta-derived tumors and by some germ-cell tumors of the testes. Measurement of hCG has an established "tumor marker" role in the diagnosis and monitoring of these rare malignant or potentially malignant conditions. Finally, the observation that pregnant females carrying a child with trisomy 21 (Down's syndrome) have increased concentrations of hCG, has ensured a specialized role for hCG measurement in the process of antenatal screening for Down's syndrome that is offered to pregnant females [315].

hCG and its variants

hCG is a 237-amino-acid glycoprotein hormone composed of two highly glycosylated dissimilar subunits (α and β) [316]. The α-subunit is common to three other related glycoprotein hormones produced in the pituitary: luteinizing hormone (LH); follicular-stimulating hormone (FSH); and thyroid-stimulating hormone (TSH). Although both subunits are required for full biological activity, it is the βhCG subunit that is unique to the molecule and defines hCG biological and immunological specificity.

A number of variants (isoforms) of hCG circulate in plasma and urine [317, 318]. They include:

- Intact active hCG, the regular hCG isoform – produced by syncytiotrophoblast cells of the placenta villi throughout pregnancy – the "pregnancy hormone"

- Hyperglycosylated hCG (h-hCG) – intact active hCG with longer sugar side chains. This isoform is produced physiologically by cytotrophoblast cells of the placental villi only during very early pregnancy (3–5 weeks of gestation). For this short period of pregnancy it is the principal form of hCG in plasma. It is detectable in the blood of those suffering from some gestational malignant diseases (e.g. choriocarcinoma) where it may account for up to 60 % of total hCG [319]
- Sulfated hCG, unrelated to pregnancy (produced by the pituitary). This form accounts for the very low level (often undetectable) of total hCG present in the blood of healthy non-pregnant females and healthy males
- Nicked hCG is partially degraded hCG, the result of enzyme cleavage at a specific site on the β-subunit (amino acid 47–48); it is biologically inactive
- Free α-and β-subunits (αhCG and βhCG); both physiologically inactive
- βhCG promotes tumor growth and is a form of hCG detected in the blood of those suffering from gestational and non-gestational malignant disease. It accounts for less than 1 % of total hCG during normal pregnancy [320]
- Other inactive hCG degradation products include: nicked βhCG subunit; nicked h-hCG; nicked hCG missing C-terminal peptide; and β-core fragment (the principal urinary hCG degradation product – it accounts for 80 % of hCG in urine during pregnancy [320])
- The variable ability of hCG assays to fully detect isoforms other than intact active hCG and the clinical significance of this variability is recently reviewed [318]. Most importantly, it is vital to appreciate that hCG assays intended to detect pregnancy and/or pregnancy-related disorders may not be appropriate for the detection and monitoring of tumors associated with increased hCG, as outlined in section "Causes of increased hCG outside of pregnancy"

Background physiology – pregnancy and hCG

Following conception, the single-cell fertilized egg (zygote) undergoes a series of mitotic cell divisions every 20 hours as it passes down the fallopian tube to the uterus. The resulting mass of around 100 identical cells – called blastocysts – differentiates at around day 4–5 following conception to either trophoblast cells on the outer surface of the blastocyst or to embryoblast cells. Embryoblast cells are the pluripotential cells that differentiate to form the developing embryo, whilst trophoblasts are the precursor cells of the placenta. Trophoblast cells mediate implantation of the blastocyst in the endometrial lining of the uterus, around 8–10 days after conception [321]. Two types of trophoblast cells are apparent at this stage: cytotrophoblasts; and a differentiated hormone-producing cell type formed from fusion of cytotrophoblasts, called syncytiotrophoblasts [322].

At the time of implantation both of these cell types begin producing hCG. In the case of cytotrophoblasts, this is the hyperglycosylated hCG isoform (h-hCG); syncytiotrophoblasts produce the regular intact active hormone, hCG. For the first week of the hCG production, cytotrophoblastic production of hyperglycosylated hCG predominates, but with the rapid growth of the syncytiotrophoblast from fusion of cytotrophoblast cells below, regular hCG soon becomes the principal form of hCG produced, and eventually syncytiotrophoblasts become the sole source of hCG throughout pregnancy [322].

Prior to implantation, plasma hCG concentration is <5 IU/L, but after implantation the concentration roughly doubles every 2 days, reaching a peak of around 120,000 IU/L at 8–10 weeks of gestation (i.e. 8–10 weeks after the beginning of the last menstrual period). Between 10 and 20 weeks of gestation, concentration gradually declines to around 20,000 IU/L and remains at that level for the rest of the pregnancy [322]. As the reference range values below demonstrate, there can be considerable deviation from these median plasma hCG concentrations.

The principal, and first described function of hCG during pregnancy is to stimulate the corpus luteum in the ovary to secrete progesterone until there are sufficient syncytiotrophoblast cells in the placenta to take over this progesterone production from the fourth week of pregnancy [323].

Other more recently identified functions, which account for hCG secretion throughout pregnancy – not just the first 4 weeks – include [323]:

- Promotion of uterine angiogenesis/vasculogenesis required for ensuring nutrition via the placenta to the developing fetus
- Involvement in the immune process that protects against rejection of the "foreign" fetoplacental unit
- Umbilical-cord growth and development
- Promotion of the differentiation of cytotrophoblasts to syncytiotrophoblast cells and thereby growth of the placental villi into the uterine wall during placentation

Hyperglycosylated hCG has a function in the process of blastocyst implantation, but the function of pituitary hCG (sulphated hCG) remains unclear [323].

Reference plasma hCG values

- Premenopausal (non-pregnant) women and men 0–5 IU/L [320]
- Postmenopausal women 0–14 IU/L [320]

Pregnancy median values according to gestational age (weeks since the beginning of the last menstrual period) [322, 324]:

Weeks	IU/L, mIU/ml
3	22
4	239
5	3,683
6	16,850
7	32,095
8	95,282
9	128,300
10	102,750
11–13	95,600
14–17	32,275
18–26	21,250
27–40	21,025

Weeks	IU/L, mIU/ml
4	5–100
5	200–3000
6	10,000–80,000
7–14	90,000–500,000
15–26	5,000–80,000
27–40	3,000–15,000

Use of hCG in the early diagnosis of pregnancy and early pregnancy loss

The finding of plasma hCG <5 IU/L excludes pregnancy. The rapid (exponential) rise in hCG which begins 6–8 days after conception (i.e. before the expected date of the next menstrual period and before a pregnancy can be visualized by ultrasound) ensures that hCG measurement provides the earliest objective evidence of pregnancy. Early pregnancy loss during the first trimester is, however, common, occurring in 20–30 % of pregnancies [325], so that the finding of hCG >5 IU/L is no guarantee of a viable pregnancy. It is the reason why an hCG level ≥25 IU/L is a good indicator of pregnancy [324]. When a borderline value is reported, a new patient sample should be drawn 48 hours later.

The term "biochemical pregnancy" is used to describe a pregnancy that does not progress to the point at which it can be visualized by ultrasonography, despite an initial rise in hCG consistent with implantation of a fertilized ovum.

Plasma hCG concentration is used to predict a viable pregnancy after assisted reproduction treatment. Median hCG concentration on day 12 following embryo transfer was found to be 126 IU/L (range 5–683 IU/L) among females whose pregnancy progressed, but only 31 IU/L (range 5–268 IU/L) among females who suffered pregnancy loss during the first trimester. An hCG value of 76 IU/L on day 12 proved the most suitable cut-off value to predict a viable pregnancy [327].

Serial hCG measurement is helpful in the assessment of pregnant females who present with vaginal bleeding or spotting during the first trimester, a common feature occurring in an estimated 25 % of all pregnancies [328]. Normal pregnancy is associated with doubling of plasma hCG every 48 hours but there is considerable variability; the minimum hCG rise over a 48-hour period necessary for a viable pregnancy is 53 % [326]. The finding of a normal rate of hCG rise is

reassuring that the bleeding reflects no threat to the pregnancy, but a rate of hCG rise <53 % is indicative of either a failing intrauterine pregnancy (miscarriage) or ectopic pregnancy [328, 329].

Use of hCG in diagnosing ectopic pregnancy

Ectopic pregnancy (i.e. implantation of a fertilized ovum outside the uterus, usually in the fallopian tubes) occurs in approximately 1–2 % of pregnancies [330]. Rupture of ectopic pregnancy is a potentially life-threatening clinical emergency. Diagnosis of ectopic pregnancy depends on study that has defined the hCG level above which a normal uterine pregnancy is detectable by transvaginal ultrasonography. This so-called discriminatory value is in the range of 1500–3000 IU/L. Absence of ultrasound evidence of uterine pregnancy in association with hCG greater than 1500 IU/L is highly suggestive of ectopic pregnancy; the higher the hCG above 1500 IU/L, the greater is the likelihood of ectopic pregnancy [330]. For patients whose hCG is less than 1500 IU/L, it remains unclear where the pregnancy is located; such a level could reflect a normally progressing uterine pregnancy or an ectopic pregnancy. To distinguish between the two, serial hCG measurements can be useful because hCG rises at a slower rate in ectopic pregnancy compared with normal uterine pregnancy, and may be plateauing or even decreasing [329].

Monitoring role of hCG following pregnancy loss and ectopic pregnancy

Following miscarriage, surgical abortion and treatment of ectopic pregnancy, hCG concentration declines to non-pregnancy concentration (<5 IU/L) at predictable rates [329], so that serial hCG measurement is useful for monitoring both conditions. Absence of hCG decline following miscarriage or surgical abortion, for example, is indicative of retained trophoblastic tissue requiring further treatment.

Causes of increased hCG outside of pregnancy

Although pregnancy is the most common cause of increased hCG, there are other causes. These fall into three categories:

- Gestational trophoblastic neoplasia (GTN)
- Other malignant conditions
- Falsely increased hCG

GTN

The most common GTN is hydatidiform mole, a usually non-malignant uterine tumor that originates from either fusion of a sperm with an ovum lacking chromosomal material (complete mole) or fusion of two sperms with a normal (haploid) ovum (partial mole) [331]. Complete hydatidiform mole is associated with massive increase in plasma hCG (median value ~200,000 IU/L; range 25,000–>3,000,000 IU/L). Partial mole is associated with a lesser degree of increase (median hCG ~50,000 IU/L; range 11,000–220,000) [322].

Evacuation or surgical removal of hydatidiform mole is followed by a predictable decline in hCG to normal (non-pregnant) values, so serial hCG measurement provides the means for confirming the efficacy of such treatments.

Malignant conditions

Choriocarcinoma is an aggressive malignancy of transformed placental (cytotrophoblast) cells that can arise following hydatidiform mole or, more rarely, a normal pregnancy (1 in 20,000 live births are followed by choriocarcinoma). hCG is a useful tumor marker for choriocarcinoma with levels exactly reflecting tumor mass [322]. In advanced disease, prior to treatment plasma hCG may be as high as 5,000,000 IU/L. Over half of this hCG (60 %) is the hyperglycosylated isoform. The efficacy of chemotherapy is monitored by a decline in plasma hCG, preferably using an assay that detects 100 % of any hyperglycosylated isoform present [322].

A falsely low level of hCG can occur in patients with choriocarcinoma and complete hydatidiform mole due to the "high-dose hook effect". This occurs if the patient's hCG concentration exceeds the measuring range of the hCG assay (usually >1,000,000 IU/L) [331].

Some forms of testicular cancer secrete hCG and for these tumors, serial hCG monitoring provides the means for monitoring the efficacy of chemotherapy [320].

Other common (non-placental) malignant conditions that may be associated with moderately increased plasma hCG (predominantly the free β-subunit) include: uterine cancer, ovarian cancer, cervical cancer and lung cancer [320, 322].

Falsely increased hCG

A false increase in hCG is a relatively common cause of moderately increased hCG outside of pregnancy, accounting for 42 % of such occurrences in one survey [333]. These "false positives" are due to the presence of heterophilic antibodies in patient plasmas that interfere with immunological hCG assays. In such cases urine hCG concentration is not increased because the interfering antibodies do not appear in urine [333].

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