Review Article

Investigative algorithms for disorders affecting plasma proteins with a focus on albumin and the calculated globulin fraction: a narrative review

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Background and Objective: The following article is part of a special series to aid the reader in diagnosing the cause of various protein derangements. By the end of the article, the reader will be able to order and interpret appropriate investigations when faced with a patient with hypoproteinaemia or hyperproteinaemia.

Methods: A narrative, focused literature review was performed using Medline, OMIM and Google during December 2022 to January 2023 to identify references published from database inception to January 2023; reference lists from these articles were also used. Language was restricted to English.

Key Content and Findings: There is huge abundance of protein within the body with a spectrum of over 12,000 different proteins. The main contributors to serum total protein measurement are the albumin and globulin fraction. Excesses and deficiencies can occur in these two fractions due to many different disease states that can lead to significant morbidity and mortality. A laboratory approach to the investigation of hypoalbuminaemia, hypoglobulinaemia and hyperglobulinaemia is present. There are many conditions that can cause protein imbalances and the clinical status and condition of the patient should be considered to carry out a targeted investigation.

Conclusions: Diagnostic flow charts have been created to help aid healthcare professionals rapidly diagnose and elicit the cause of hypoproteinaemia or hyperproteinaemia in their patients. These algorithms have been present and created within the limitations of the laboratory tests discussed within the paper.

Keywords: Protein; albumin; calculated globulin fraction; investigation; diagnosis

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Introduction

The measurement of total protein is one of the most requested tests within the biochemistry laboratory and is routinely carried out on an array of different biological fluids. Serum total protein refers to a quantitative determination of all proteins present excluding the clotting factors. Serum samples principally contain about 60–80 g/L of protein in healthy basal states and an approach to the diagnosis of high or low concentrations

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will be presented to aid test selection for diagnosis (1). We present this article in accordance with the Narrative Review reporting checklist (available at https://jlpm.amegroups.com/article/view/10.21037/jlpm-23-15/rc).

Methods

The narrative literature review was created by searching Medline, Google Scholar, OMIM and seminal texts over the period December 2022 to January 2023. The diagnostic algorithms were then created based on the literature review. The language was restricted to English. For further information please see Table S1.

Results

Plasma proteins

Plasma contains many proteins, which have a variety of roles and reflect the state of the tissues. While over 12,000 individual proteins have been characterised within the serum, the two major protein components are albumin and immunoglobulins (*Figure 1*) (2). Albumin is a protein exclusively synthesised by the liver whereas immunoglobulins are glycoproteins synthesised by B lymphocytes of the humoral immune system. Many other proteins are included in the determination of total serum protein, such as transferrin, α2-macroglobulin, and complement proteins. None of these individually contribute more than 5% of the total and so do not significantly affect total protein concentrations (3). Therefore serum total protein measurements are not diagnostic for a particular condition (4). Albumin is commonly measured in tandem with serum total protein. Generally, the laboratory will also calculate the globulin fraction:

$$Globulin = (total\ protein) - (albumin) \quad [1]$$

Eq. [1] used to calculate the globulin fraction using the total protein and albumin concentration (all values should be in the same units e.g., g/L) (3).

Albumin

Albumin is synthesised in the liver (*Figure 2*) accounting for 55–60% of the serum protein mass (5). Dietary protein intake varies widely but should not exceed about 2 g/kg of body weight/day (6). Albumin is a water-soluble 585 amino acid peptide chain, with a turnover time of 25 days and a weight of 65 kDa (7). It distributes between the intravascular (30–40%) and extravascular spaces (60%) predominately with significant pools in the lymph and skin (8). Healthy individuals lose 6% of their albumin via the kidneys and 10% via the gut, per day (7). Although albumin is freely filtered in the kidneys (9), its anionic charge limits this and it is actively reabsorbed in the proximal tubules. In health, a urinary albumin creatinine ratio is less than 3 mg/mmol (8). Albumin has many functions including the regulation of osmotic pressure, buffering of plasma pH, transport of hormones and more (*Table 1*) (10-17).

Albumin synthesis is primarily thought to be regulated by alterations in the colloidal oncotic pressure rather than the albumin concentration (7). For example, an acute loss of albumin (7%) during plasmapheresis is slowly replaced by the liver increasing albumin synthesis by 25% over 30 days (18). Both insulin and growth hormone stimulate albumin synthesis (19-21). A protein meal stimulates synthesis (21) but is not related to the concentration of amino acids in the circulation (22). Mild acidosis reduces albumin synthesis (23) and catecholamines have been documented to increase synthesis (24).

Immunoglobulins

There are five classes of immunoglobulins, although only three are routinely measured i.e., immunoglobulin G (IgG), immunoglobulin A (IgA) and immunoglobulin M (IgM). The five classes are as follows, in descending concentration order: IgG (7.0–16.0 g/L), IgA (0.7–4.0 g/L), IgM (0.4–2.3 g/L), immunoglobulin D (IgD) (<152.7 mg/L), immunoglobulin E (IgE) (5–120 KU/L) (25-27). The ranges provided in brackets are a guide and will vary with certain factors i.e., age, sex, and methodology. Immunoglobulins can either be membrane-bound antigen receptors or a secreted product of the adaptive immune system.

The basic monomer structure consists of a pair of identical heavy and light-chains arranged into a Y shape via disulphide bonds (4). The heavy chain defines the isotype, light-chains are either lambda or kappa providing additional subgrouping as each isotype exists in a lambda and kappa form, i.e., IgG kappa and IgG lambda (28). In addition to this there are four subclasses for IgG and two subclasses of IgA (29). An in-depth review of immunoglobulin synthesis and metabolism is outside the scope of this algorithm, but is summarised in *Figure 3* (30-34).

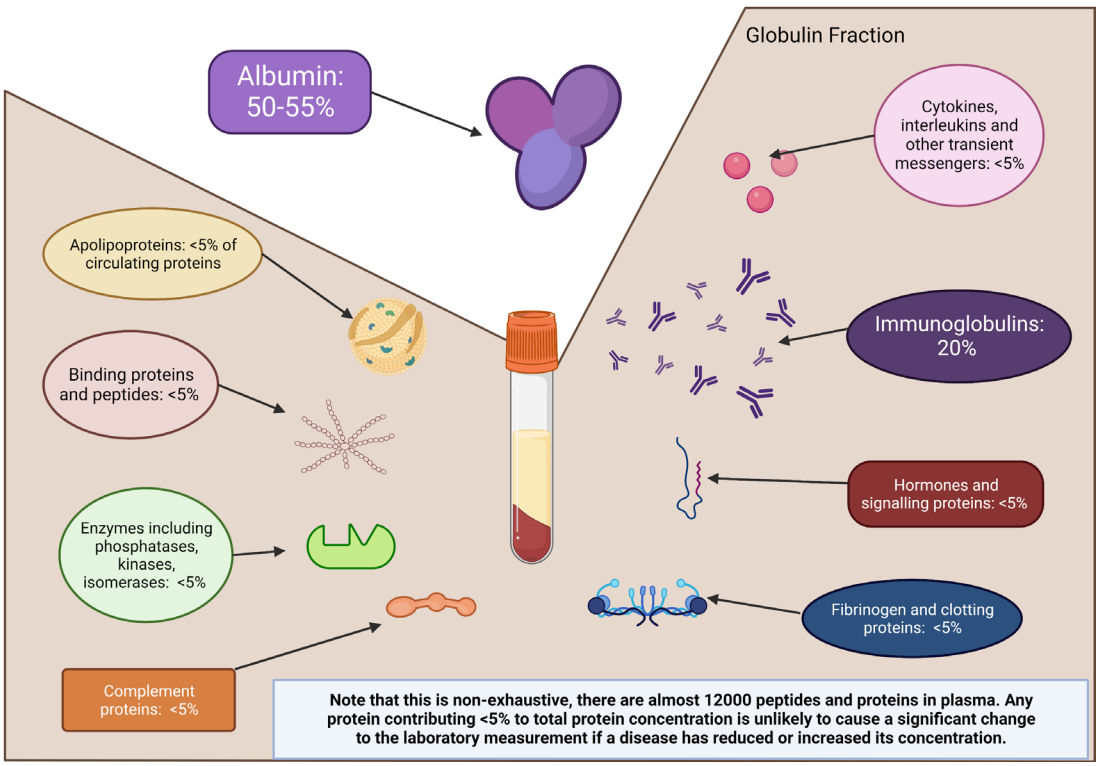


Figure 1 The range and approximate quantity of proteins found in human plasma. Human plasma proteins, note that this is non-exhaustive, there are almost 12,000 peptides and proteins in plasma. Any protein contributing <5% to total protein concentration is unlikely to cause a significant change to the laboratory measurement if a disease has reduced or increased its concentration.

Laboratory techniques

In the following section some of the commonly used analytical methods will be discussed.

Total protein method

Total protein methods include chemical methods (such as the biuret reaction), turbidimetry, direct optical methods, dye-binding methods, refractometry, capillary electrophoresis, western blotting, mass-spectrometry, immunofixation electrophoresis, Kjeldahl method, Lowry method, and nephelometry (3). The biuret reaction is one of the most commonly available techniques with good linearity between 15 to 120 g/L (3,35). Interferences include ethylenediaminetetraacetic acid, sucrose, phospholipids, glycerol, triton X-100, and dithiothreitol (36). Hyperbilirubinaemia induces a negative interference causing pseudohypoprotinaemia and pseudohypoglobulinaemia (37). Spuriously raised protein concentrations can be produced by turbid specimens or those with particulates within the serum as well as moderate to marked haemolysis and lipaemia (38).

Albumin method

The most commonly available albumin assays are dye based (bromocresol green or purple) spectrophotometry methods (39). Other methods include immunonephelometric, immunoturbidometric techniques, and conventional, capillary serum protein electrophoresis (40,41). Reasons for possible spurious results related to method selection are summarized in *Table 2*. Spurious results can also be caused by taking blood samples from an arm attached to an intravenous drip (dilution artefact), or contamination with blood tube additives e.g., sodium citrate contamination (dilutional drop in total protein, as well as other characteristic changes such as hypocalcemia) (42).

Calculated globulin fraction {Eq. [1]}

The globulin fraction is a calculated metric produced from the subtraction of albumin from the total protein. This

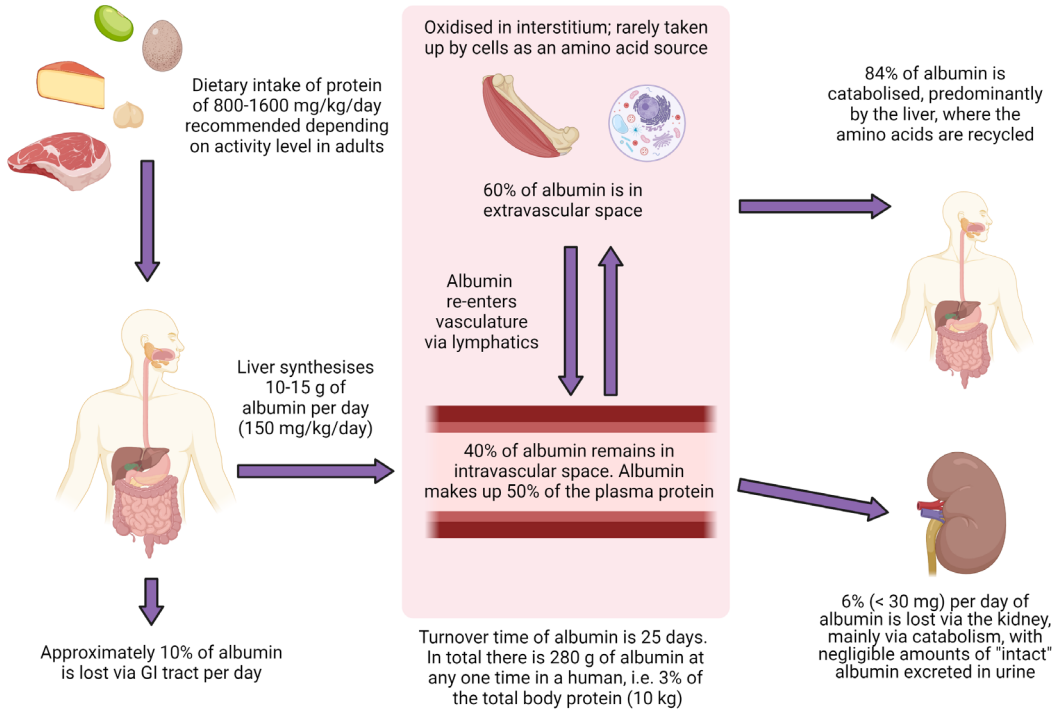


Figure 2 A basic overview of the distribution and homeostasis of albumin in humans. GI, gastrointestinal.

Table 1 Functions of albumin

Role	Details of function
Regulation of osmotic pressure	Major component (80%) of plasma oncotic pressure
Transport of metabolites and ions	Transports molecules (e.g., free fatty acids, unconjugated bile acids, bilirubin) to the liver for metabolism and storage. Binding may regulate activity, clearance, or act as a reservoir e.g., calcium ions
Binding and transport of hormones	Binds hormones including those with specific transport proteins e.g., thyroxine, cortisol, vitamin D and testosterone
Binding and transport of drugs	Some drugs are protein bound therefore albumin concentration affects their pharmaco-kinetics and -dynamics e.g., phenytoin
Buffering plasma pH	Reservoir donating and binding H^+ ions
Scavenging reactive oxygen species	Albumin is thought to be responsible for approximately 50% of the antioxidant properties of plasma
Reservoir of nitric oxide	Blood pressure regulation
Anticoagulant effect	Prevents coagulation and platelet aggregation
Reduces inflammation	Inhibits the actions of cytokines e.g., tumour necrosis factor alpha (TNF-α) and complement proteins

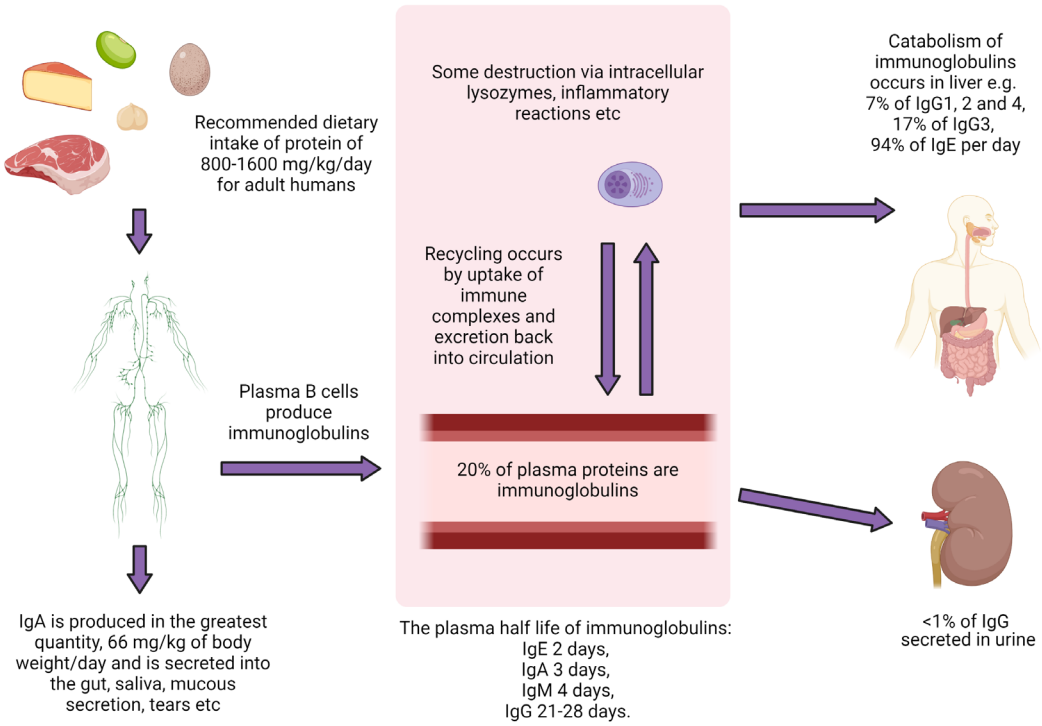


Figure 3 A basic overview of the distribution and homeostasis of immunoglobulin in humans.

Table 2 Laboratory techniques for albumin measurement and factors to consider

Test name	Factors that can affect the reliability of the results
Bromocresol green dye	Can give a falsely high reading in hypoalbuminemia and a falsely low reading in hyperalbuminemia; poor performance if albumin <20 g/L; low specificity, other proteins interfere
Bromocresol purple dye	Falsely low when the albumin, and albumin: globulin ratio, is low (nephrotic syndrome); more specific with less protein interference than bromocresol green, but more difficult to calibrate; heparin negatively interferes
Immunoassay	Imprecise and weak reactions from albumin fragments causing falsely high readings; not commonly used
Serum electrophoresis	Falsely high albumin if other proteins cluster around the densitometry peak (e.g., pre-albumin and α1 acid glycoprotein); protein can be absorbed by the matrix (e.g., the paper) and give falsely low readings; high interlaboratory variation

fraction consists of immunoglobulins (30%) with enzymes, carrier and complement proteins, among others, contributing to the remaining 70% (*Figure 1*) (4). Globulin fraction cut-offs are often locally decided by laboratories i.e., (20–39 g/L), poor consensus on this cut-off is thought to arise due to variation in methodology performance and local population differences (43). A clear lower limit for globulin is required to reduce the current 5- to 6-year delay there is on detecting primary hypogammaglobulinaemia (43-47). A few studies have suggested that a globulin less than 18 g/L (children ≥10 years and not under haematology or oncology review) should indicate screening for hypogammaglobulinaemia, but there is less certainty in regard to adult thresholds (43,44). The globulin fraction in hypogammaglobinaemia, may also be spuriously normal in the acute phase response, due to the rise in concentration of other proteins (43).

Measurement methods for serum immunoglobulin

Immunoglobulins are measured as part of total protein

quantification, and during serum electrophoresis and immunofixation. Direct individual measurement of the separate subclasses can be carried out by automated nephelometry or sandwich enzyme-linked immunosorbent assays (ELISA). Specific immunoglobulins can also be measured, but are outside the scope of this paper (4,48,49). Immunoglobulin isotype assays can be subject to prozone/antigen excess errors. Monoclonal proteins, in particular, can result in non-linearity and misreporting. Nephelometry methods are affected by turbid samples or particulates (48,50). Mass spectrometry, a highly specific and sensitive method, is currently not yet widely used (51).

Serum electrophoresis

Serum electrophoresis segregates proteins into six fractions based on size, shape, and electrical charge (48). Traditionally this was carried out on agarose gel, which has been mostly replaced by capillary zone electrophoresis (CZE) (3). Interpretation of electrophoretograms is complex and requires specialist training (48). Traditional gel electrophoresis is subject to point of application artifacts with some precipitants unable to migrate into the gel, such as euglobulin and cryoprecipites, which may contain monoclonal proteins leading to spuriously negative results. Discrepancies in interpretation and reporting may also occur and rarely low concentrations of monoclonal proteins are missed (3).

Serum free light-chains

Free circulating kappa and lambda light-chain components within the serum can be used as a diagnostic marker for monoclonal proteins (52). Lambda concentrations in health are twice that of kappa due to kappa's smaller molecular weight and thus increased renal filtration (48). Serum free light-chains analysis is more sensitive (sensitivity: 0.76, specificity: 0.96) than urine Bence Jones protein quantification (monoclonal globulin protein or immunoglobulin light chain in the urine), as the rate of paraprotein synthesis needs to exceed the renal metabolism rate before being detectable in urine (53,54). However, it is argued that urine light-chain assessment is more specific than serum free light-chain analysis (as it quantifies all/polyclonal free light-chains, as well as monoclonal, which may result in a spuriously normal kappa: lambda ratio) (55).

The Freelight® immunoturbidimetric assay is a latex-enhanced conjugated polyclonal antibody nephelometric assay (56). Other methods include immunonephelometric assays or sandwich ELISA kits (3). Serum free light-chain assays are very prone to prozone antigen excess errors and cross-reactions. The prozone error/antigen excess is a reaction kinetic error where there is such a high concentration of the target analyte this causes incomplete reagent antibody binding, a falsely low concentration (due to a diminished signal), and is overcome by diluting the sample (48,57). This assay type is also prone to non-linear results, discrepancies in dilution, and polymerisation effects. Some patients may also have a unique light-chain epitope that is not recognised (52,57).

Urine electrophoresis

Urine electrophoresis can characterise urinary proteins and detect Bence Jones protein, which can be typed by immunofixation (48). Urine electrophoresis is generally carried out via a 2D gel electrophoresis method, applying a high electric current to a high-resolution agarose gel from a commercial kit or via CZE (58,59) and is subject to the same analytical errors as serum (3). It is time-consuming and, if the urine is too dilute, it may require a concentration step or a repeat sample. The quantification of urine protein is normally carried out on densitometry, which can be inaccurate and is dependent on the measurement of urine total protein. This is often measured by a dye binding method, which has its own inaccuracies as discussed above. In urine electrophoresis monoclonal bands however can be defined from the polyclonal background (55).

Other tests

Most liver function and bone profiles consist of albumin and total protein. The other parts of these profiles will be discussed in the companion articles in these series.

Urine protein creatinine ratio (PCR) uses creatinine to correct for diurnal variation in urine concentration. Urine PCR is a fast, simple way of detecting if proteinuria is present, and if so, to what degree (60-62). The most commonly reported thresholds for a random PCR sample are >20 mg/mmol (>0.2 mg/mg) for detecting proteinuria and >350 mg/mmol (>3.5 mg/mg) for nephrotic range proteinuria (62).

Urine albumin creatinine ratios (ACR) are often recommended instead of PCR as this test has greater sensitivity for low levels of proteinuria but can miss cases of proteinuria without albuminuria. For example, urine PCR

testing has greater utility over ACR when screening for pre-eclampsia in pregnancy (63). Urine albumin and creatinine are generally measured using immunonephelometry, immunoturbidimetry, and occasionally high-performance liquid chromatography. Urinary albumin can be carried out at the bedside via dipstick testing, although this method is considered to have a false negative rate (around 40%) and is not quantitative (64).

Albumin present in trace amounts, microalbumin, can point to nephropathy (65) but false positives can occur e.g., urinary tract infections, intercurrent illnesses and exercise (65,66). Initial positive results should be repeated within 2–12 months and a mid-stream urine sent for microbiology cultures (66). The most commonly quoted reference ranges for urine albumin: creatinine ratio is from Kidney Disease: Improving Global Outcomes (KDIGO) program and are as follows: <3 mg/mmol normal, 3–30 mg/mmol moderate increase, >30 mg/mmol severely increased urine protein excretion, and >220 mg/mmol nephrotic syndrome (67).

Detection of α1 antitrypsin in stool, or albumin scintigraphy, may be used to screen for excess gastrointestinal loss of protein (68-70). Enzyme linked immunoassay quantification of faecal α1 antitrypsin is the most used and reliable method of diagnosis of protein losing enteropathy (PLE) (71-75). Additional tests, such as serum anti transglutaminase IgA, faecal cultures/microscopy, faecal calprotectin, imaging studies, and intestinal biopsies, should be considered to elucidate the cause and hence treatment strategy of PLE (75).

Natriuretic peptides are produced by a failing heart (causing a dilutional state and low protein) (4,76). The measurement of a pre-cursor prohormone N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) is more commonly measured than BNP as it has a longer half-life and better stability profile (77). The quoted NT-proBNP cut-offs for referral are 400–2,000 ng/L indicating echocardiography within 6 weeks for a new case, and >2,000 ng/L requiring urgent referral (78). Others suggest a lower threshold of >125 ng/L (79). Note pg/mL is equivalent to ng/L.

BNP or NT-proBNP are often measured by immunoassays and may be subject to interference with biotin, human anti-animal or heterophilic antibodies, prozone/antigen excess, and rarely macro complexes (80). BNP and NT-proBNP are non-specific e.g., raised in sepsis, older age (>70 years), renal failure (eGFR <60), exercise, cirrhosis, ventricular overload, acute coronary syndrome, hypoxaemia, etc. Low values can be seen with obesity, diuretics, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers and aldosterone antagonists (81,82).

Albumin can be measured on ascitic fluid samples to calculate the serum-ascitic albumin gradient. This is used in the differential diagnosis of ascites formation and a gradient of >11 g/L is suggestive of portal hypertension (83).

The serum-ascites albumin gradient formula is as follows (83):

$$\begin{aligned} &\textit{Serum-ascites albumin gradient} \\ &= \textit{serum albumin} - \textit{ascites fluid albumin} \end{aligned} \quad [2]$$

Blood and ascitic samples should be collected simultaneously and measured in the same units. The original paper that defined this cut-off was not explicit in analytical method simply stating a commercial Abbott kit was used (84). Therefore, it would be prudent to verify and modify this cut-off depending on the local methods used.

Fluids (other than serum and urine) tend not to have a consistent albumin composition and results may have high variability. The main methods used for fluid albumin measurement include dye binding methods (bromocresol purple and green) or immunoturbidimetry. The bromocresol green method has been found to have a positive bias of 1–3 g/L when compared to immunoturbidimetry. Dye binding methods in fluid can have a positive bias compared with serum which may lead to a significantly lower serum-ascitic albumin gradient threshold (85).

Hypoproteinaemia

Normal serum total protein measurement is reported to range from 60 to 80 g/L (1,86). Therefore 59 g/L and below defines hypoproteinaemia (87,88). Hypoproteinaemia can present with a spectrum of symptoms including muscle wasting, hair loss, oedematous limbs, failure to thrive, infections, fatigue (4), and causes are multiple (*Table 3*) (88). Hypoproteinaemia occurs principally due to decreases in albumin and immunoglobulin (particularly if affecting IgG) (4). Most causes can be identified by history and examination e.g., pregnancy and sepsis, however diagnostic algorithms are presented below for hypoalbuminaemia and hypogammaglobinaemia.

Hypoalbuminaemia

Hypoalbuminaemia causes fluid shifts therefore peripheral or pulmonary oedema, and ascites; an algorithm for

Table 3 Causes of serum hypoproteinaemia

General cause	Subtype	Example
Dilutional states	Fluid overload/ oedema	Cardiac failure
		Cirrhosis
		Nephrotic syndrome
		Third trimester pregnancy
	Iatrogenic	Intravenous fluid
Physiological stress	Starvation	Kwashiorkor
		Marasmus
	Genetic disease	Cystic fibrosis
	Chronic intestinal disease	Coeliac disease
		Pernicious anaemia
	Age	Frailty (elderly)
Protein loss	Fluid imbalance and loss	Haemorrhage
		Peritoneal effusion
		Pleural effusion
	Intestinal disease	Protein losing enteropathy
		Ulcerative colitis
	Exudative skin disease	Severe burns, erythroderma
	Renal disease	Chronic kidney disease
		Diabetes nephropathy
		Nephrotic syndrome
		Renal cell carcinoma
Decreased synthesis	Humoral immunodeficiency	Bone marrow failure
		Common variable immunodeficiency
		Hypogammaglobulinaemia
	Liver disease	Alcoholic liver disease
		Chronic hepatitis
		Cirrhosis
	Cachexia	Malignancies
	Inflammation	Bacterial infection
		Sepsis
		Severe trauma

Some of the causes of hypoproteinaemia in the table have complex pathologies that have many contributing factors, i.e., a state of inflammation leading to decreased synthesis as well as an increased loss of protein (3,45,87-96).

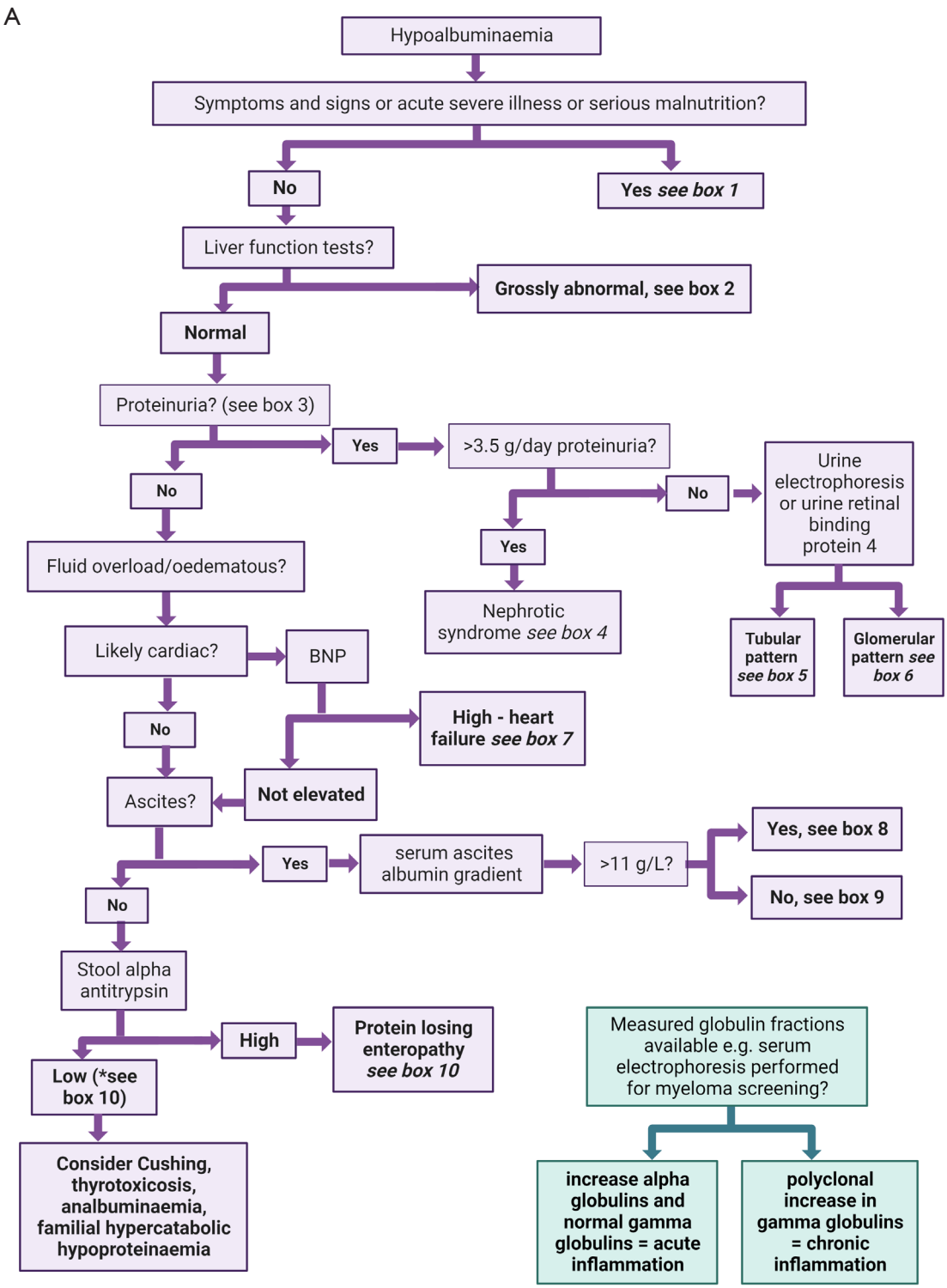
a laboratory approach to its investigation is shown in *Figure 4*. Low serum albumin concentration is associated with poorer prognosis during illness (97-99). However, albumin supplements do not significantly affect outcomes (100,101). Albumin decreases in states of inflammation and is thus a negative acute phase protein (91), partly as increased capillary permeability leads to albumin extravasation that outstrips the rate of albumin synthesis (3).

Only severe malnutrition states can lead to protein deficiencies, such as kwashiorkor and marasmus (102,103). Kwashiorkor primarily affects children and arises from a predominant protein deficiency and marasmus is a global deficiency of macronutrients including protein, lipids, and carbohydrates (102-105).

Albumin may be used, with extreme caution, as one of the indicators of nutritional status in patients with severe gastrointestinal disease on supported nutrition, as well as those at risk of refeeding and cancer patients, but many nutritional assessment and screening tools do not include protein nor albumin concentrations (91,106). In patients with simple starvation the rate of albumin catabolism and synthesis falls so albumin remains normal despite a prolonged state of malnutrition e.g., concentrations are normal in anorexia nervosa (106,107).

Albumin may reduce with age, likely a reflection of the extent of physiological stress from underlying age-related inflammatory conditions rather than a long-term indicator of nutritional status (108,109). During sepsis and general physiological stress, albumin extravasation increases, and the liver shifts synthetic function to inflammatory, and other, proteins thereby reducing albumin synthesis. Depending on the severity, the source, and progression of healing, it may take 3–4 months for albumin concentrations to normalise despite only taking 3–7 days to decrease post the initial inflammatory insult (109). C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and procalcitonin may help elucidate the cause of the active phase response depending on local availability and condition specific pathways (110).

Fluid imbalances in the extracellular plasma compartment can cause dilution of the plasma proteins leading to low serum total protein e.g., cardiac failure, liver failure and nephrotic syndrome. However, these conditions are also associated with cachexia, malnutrition, inflammation, liver dysfunction, PLE, increased extravascular loss and haemodilution (10). Spurious results can be due to intravenous drip fluid contamination, as discussed earlier, (111) and so if the results unexpected



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Box 1 - Unwell and severe malnutrition
Albumin is a negative phase reactant therefore sepsis, trauma, cachexia, erythroderma, burns etc can all cause low albumin. The drop is rapid, within days of onset of illness, and fluid replacement and other losses may exacerbate. Recovery is very slow. Note malnutrition is not usually a cause except in Kwashiorkor. If degree of hypoalbuminaemia is unexpected then follow the rest of the algorithm assuming you selected 'no'.

Box 2 - Liver Disease
In cases of liver failure, e.g.cirrhosis, then albumin synthesis reduces and fluid shifts result in hypoalbuminaemia. It is worth noting that people with end stage liver disease can have remarkably normal liver function tests.

Box 3 - Identifying Proteinuria
Consider urine dip to screen or to quantify: albumin or protein creatinine ratio, or 24 hour albumin excretion

Box 4 - Nephrotic syndrome
Causes of nephrotic syndrome included genetic, glomerulonephritis, diabetes mellitus, malaria, drugs, toxins, renal vein thrombosis

Box 5 - Tubular Pattern
If these tests are available, the presence of retinol binding protein, α1 microglobulin and α2 microglobulin indicates renal tubular damage e.g. Fanconi syndrome, heavy metal poisoning, drugs (e.g. cisplatin)

Box 6 - Glomerular Pattern
If tests are available then albumin and larger proteins are seen in urine. This can be due to reflux obstruction, hypertension, diabetes mellitus, glomerulo- and interstitial nephritis

Box 7 - BNP and Heart Failure
BNP is not specific to heart failure and is also raised in left ventricular hypertrophy, myocardial ischaemia, arrhythmia, heart valve disease, pulmonary embolism, pulmonary hypertension/cor pulmonale, hyperthyroidism, sepsis, renal failure, and fluid overload of any other cause

Box 8 - Serum albumin ratio > 11
Seen in portal hypertension e.g. heart or liver failure (consider echo or liver imaging based on clinical picture).

Box 9 - Low serum albumin ratio
Seen in peritoneal carcinomatosis, TB, nephrotic syndrome, pancreatitis and serositis. Other measurands in ascitic tap may aid in the differential.

Box 10 - Protein Losing Enteropathy
Alpha 1 antitrypsin (A1AT) is resistant to gut enzymes so a good marker of increased protein loss when measured in stool. Technetium 99 labelled albumin is an alternative test. Causes include inflammatory bowel disease, lymphangiectasis, coeliac, eosinophilic gastritis, cardiac disease, lupus. *Note a low faecal A1AT does not exclude protein losing enteropathy

Figure 4 An investigative algorithm for hypoalbuminaemia. Diagnostic laboratory algorithm for hypoalbuminaemia in humans (A) with additional supportive information (B). (A) Diagnostic laboratory algorithm for hypoalbuminaemia in humans. (B) Boxes providing supportive information for diagnostic algorithm *Figure 4A*. BNP, brain natriuretic peptide; TB, tuberculosis.

consider repeating the venesection (3,111).

Liver disease, when severe and chronic, causes hypoalbuminaemia. Cirrhosis, for example, can lead to a reduced albumin production by 60–80%, a feature further exacerbated by dilution, shift of albumin to a different compartment, and post transcriptional changes to albumin (112). Acute liver disease does not present with hypoalbuminaemia typically; for an algorithm on the interpretation of abnormal liver function tests please see other useful papers and the companion articles in this series (113).

Physiological stress suppresses albumin synthesis (114). Negative feedback from a high osmotic pressure will also decrease albumin synthesis, as can diabetes mellitus (insulin stimulates albumin gene expression) (115).

The autosomal recessively inherited condition analbuminaemia causes a complete lack of albumin (OMIM) (116). Heterozygotes are disease free and distinct bands are seen on serum electrophoresis (bisalbuminaemia

Table 4 Causes of nephrotic syndrome

Commonest causes in children	Commonest causes in adults
Idiopathic, primary, and genetic Biopsies normally show minimal change disease, membranoproliferative glomerulonephritis or focal segmental glomerulosclerosis	Usually secondary: toxins, drugs, heavy metals, autoantibodies, post-infections antibody complexes (e.g., group B streptococcus) or, myeloma and neoplasm related immune complexes

or alloalbuminaemia) (48,116). Analbuminaemia is often asymptomatic but there can be perinatal problems due to shifts of fluid across the placenta (117). Depending on where the mutation is it may affect albumin's ability to carry thyroid hormone, divalent copper or nickel ions, fatty acids and drugs, therefore may cause abnormal blood tests, if not morbidity, due to reduced function, especially in the lipid pathway (16,116,118). Analbuminaemia may also lead to a degree of pulmonary oedema and increased risk of respiratory infections leading to infant mortality (119). Symptoms of analbuminaemia are minimised by a compensatory increase in other circulating proteins (118).

Protein loss can occur through the gastrointestinal tract i.e., in PLE (any state in which oedema and increased lymphatic pressure adversely affects gastrointestinal function) e.g., inflammatory bowel disease, lymphangiectasis, coeliac, eosinophilic gastritis, cardiac disease and lupus (120-122). In PLE 60% of total albumin is lost via the gut along with indiscriminate loss of immunoglobulin, fibrinogen, lipoprotein, transferrin, and other proteins (68,69,75,123,124).

Proteinuria generally arises due to abnormal transglomerular passage of proteins due to the increased permeability of the glomerulus capillary walls or impaired absorptive capacity of the proximal tubules (125). Proteinuria is used as a biomarker of renal risk in the general population, non-diabetic chronic kidney disease (CKD) and diabetic CKD patients and in CKD staging criteria (63).

Significant loss of albumin from the kidneys occurs in nephrotic syndrome the causes are listed in *Table 4* (8,126). End stage renal failure can result in the loss of 30–300 mg of albumin a day, but interpretation of laboratory results can be affected by fluid status and shifts (127). Dietary protein supplementation only increases the renal protein loss and does not increase the plasma albumin concentration (128). Usually the renal loss of albumin in diabetic nephropathy is not sufficient to cause hypoalbuminaemia unless the loss becomes nephrotic (7). However, microalbuminuria, seen in diabetes and hypertension, probably has a deleterious effects on the kidneys and accelerates the decline in renal function (8).

During illness it is proposed tissue utilisation and catabolism of albumin increases thus providing amino acids for tissue repair (129). Biochemically altered albumin is taken into cells and catabolised, predominantly in muscle and skin, whereas unaltered albumin is released back into the extracellular fluid (9,130).

Various medication have been linked to hypoalbuminaemia including (131):

- Dapsone (only reported in long term use in dermatology (autoimmune bullous disorders); may reflect the extent of the skin or gut involvement but all cases recovered rapidly on cessation of dapsone) (132);
- Dexmedetomidine (used as a sedative in ITU so could be spurious);
- Micafungin (no case reports found, used to treat invasive fungal infections so may be spurious, i.e., caused by the infection);
- Glycerol phenylbutyrate (no case reports found, used to treat people with urea cycle defects on very low protein diets);
- Isavuconazole (no case reports found, used to treat invasive fungal infections so may be related to tissue inflammation).

Also, caution is required when prescribing certain drugs that bind protein in the case of hypoalbuminaemia e.g., benzodiazepines (131). Anti-tumour medication (e.g., cytotoxic, antimetabolites, tyrosine kinase inhibitors, immunotherapy, histone deacetylase inhibitors, proteasome inhibitors, topoisomerase inhibitors, VEGF and EGFR antibodies) is often associated with hypoalbuminaemia, but it is likely that the cancer, and tumour lysis, leads to fluid shifts and is the cause rather than the drugs themselves (133).

Hypogammaglobulinaemia

Humoral immunodeficiency refers to a spectrum of conditions that result in a deficiency of one or some of the classes of immunoglobulins (134). These conditions may be acquired or congenital and arise due to a malfunction of maturation of B-cell lymphocytes (47). Other reported

causes of low calculated globulin fraction include, cirrhosis, kidney conditions such as nephrotic syndrome, malnutrition, acromegaly, and malignancies (135-137). For a diagnostic algorithm please see *Figure 5*.

Primary immunodeficiencies arise from chromosomal or genetic abnormalities e.g., X-linked agammaglobulinemia, common variable immunodeficiency (CVID), transient hypogammaglobulinaemia, selective IgA deficiency and hyper IgM syndrome (43-45,47,48). The diagnostic work up for primary immunodeficiencies includes the measurement of serum immunoglobulins (IgG, IgM, and IgA), full blood count with white blood cell differential, flow cytometry for B-cell and T-cell evaluation as well as genetic testing, e.g., BTK gene mutations (43,44,48).

Common variable immunodeficiency (CVID) presents with hypogammaglobulinaemia and an increased susceptibility to bacterial infections and is one of the most common antibody deficiencies accounting for around 40% of primary immunodeficiencies in the western world (45,138). In CVID serum IgG is markedly reduced and, to a lesser and variable extent, so are IgA and/or IgM (138). Around 12% of those with CVID go on to develop chronic liver disease, therefore in liver disease cases with hypogammaglobinaemia some may be undiagnosed CVID (139). CVID mimics the symptoms of gastrointestinal diseases, and studies have confirmed there is a high prevalence of inflammatory, infectious and malignant gastrointestinal disorders (140). CVID patients may also develop haemolytic anaemia and malignancies due to reduced immune function (141,142).

Acquired causes of reduced immunoglobulin synthesis include malignancies, drugs, (discussed below), malnutrition, and environmental conditions such as ionising radiation exposure. Sometimes metabolic disorders such as propionic acidaemia can produce hypogammaglobinaemia, but it is unlikely that hypoproteinaemia would be the presenting feature (44,47,143-145). Patients with liver disease can present with both hypoglobulinaemia and hyperglobulinaemia depending on the aetiology of the organ damage, however the latter is much more common (4,112,146).

Severe malnutrition has diverse effects on the humoral immune system but decreased immunoglobulin synthesis can occur (95). Hypogammaglobinaemia can be observed with gastrointestinal conditions such as coeliac, pernicious anaemia, and irritable bowel disease (i.e., Crohn, ulcerative colitis) (147,148).

Infectious agents such as viruses, bacteria, or recovery from severe sepsis can cause immunoparesis (suppression of polyclonal immunoglobulins). Viral causes of hypogammaglobulinaemia include Epstein-Barr virus, cytomegalovirus, rubella, and human immunodeficiency virus (143). This phenomenon is also well documented in multiple myeloma patients; however, these patients do not tend to present with hypoproteinaemia due to the increased production of a monoclonal protein. Other haematological malignancies can also cause hypogammaglobinaemia, as can their treatment with stem cell transplantation (143,149).

Immunoglobulins will also be lost in nephrotic syndrome, in PLE, and in severe burns (143). There are a few reports in the literature of acromegaly or excessive growth hormone being associated with apparent hypoglobulinaemia or hypoproteinaemia (135,150,151). The pathophysiology behind this is unknown as growth hormone stimulates protein synthesis so may be altered protein metabolism or increased loss (151). Reports of hypoproteinaemia associated with acromegaly are sparse however and therefore it is unlikely that a low total protein would be a presenting symptom.

The British National Formulary (BNF) lists hypogammaglobulinaemia as a side effect of the following drugs: mycophenolate, tacrolimus, cyclosporin A, tafasitamab, diazoxide, daratumab, blinatumomab, carbamazepine, belatacept, rituximab and pencilliamine (131). Other drugs that have been associated with hypogammaglobulinaemia include corticosteroids, chemotherapy, valproic acid, phenytoin, sulfasalazine, chloroquine, fenclofenac, hydantoin, and lamotrigine (152,153).

Hyperproteinaemia

A total protein concentration of 81 g/L and above is considered to be hyperproteinaemic (1). Causes include dehydration, inflammation, autoimmune disease and bone marrow dysfunction (*Table 5*). Clinically it is difficult to distinguish the effects and symptoms of the primary disease from hyperproteinaemia (105). Symptoms and signs associated with hyperproteinaemia may include anaemia, renal dysfunction, liver dysfunction, or the laboratory phenomena of apparent hypercalcaemia, pseudohyponatremia, etc. (54,155). Renal involvement with monoclonal gammopathy of unknown significance (MGUS), for example, is a clinically important entity (156). MGUS of clinical significance, is a term used for when a disease pathology is caused by end organ build-up of the protein (157). These diseases can present to the dermatologist (e.g., finger vasculitic

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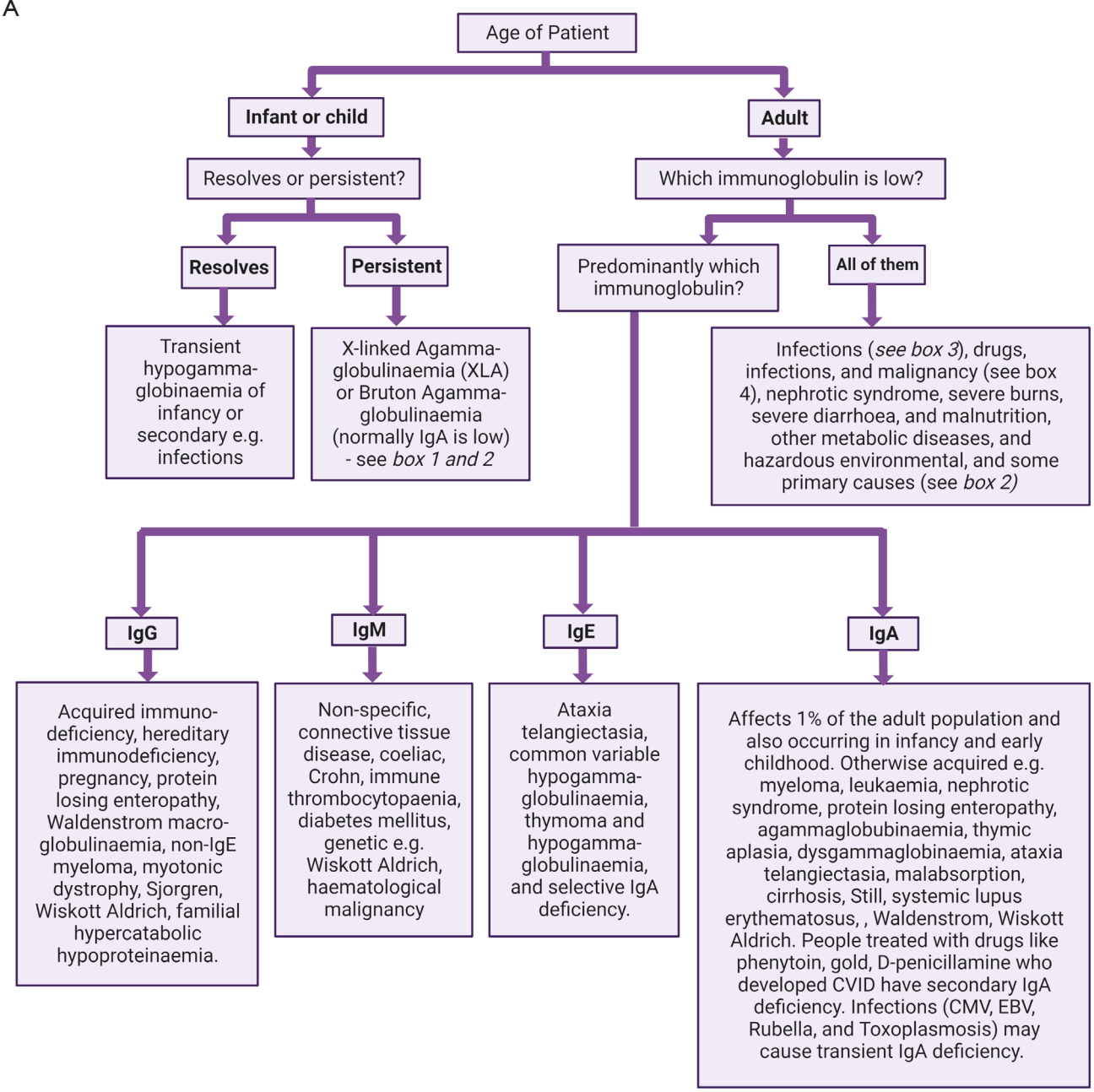

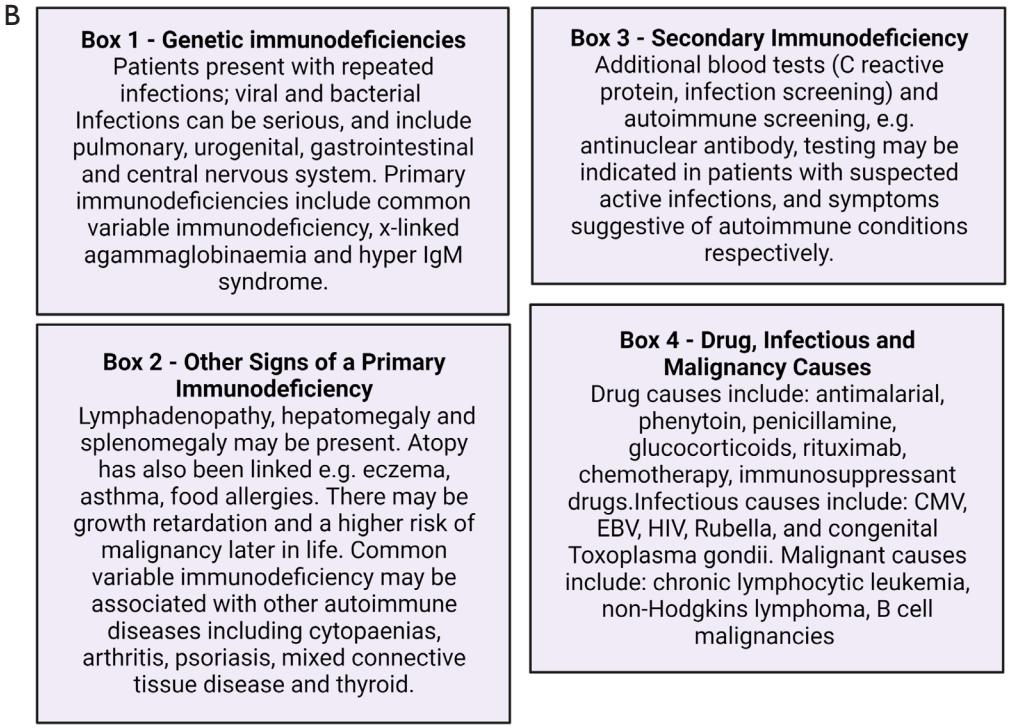

Figure 5 An investigative algorithm for hypogammaglobinaemia. Diagnostic laboratory algorithm for hypogammaglobinaemia in humans (A) with additional supportive information (B). (A) Diagnostic laboratory algorithm for hypogammaglobinaemia in humans. (B) Boxes providing supportive information for diagnostic algorithm *Figure 5A*. CVID, common variable immunodeficiency; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus.

lesions caused by a cryogloblinaemia, ecchymoses of AL amyloidosis, urticaria caused by Schnitzler syndrome, xanthelasma like periorbital deposits of necrobiotic xanthogranulomas, tissue thickening of scleromyxoedema or the telangiectasis of the TEMPI syndrome); the neurologist (peripheral neuropathy caused by distal acquired demyelination or cerebellar ataxia of CANOMAD); the respiratory physician (in Clarkson disease or crystal storing histiocytosis); or the renal physician (glomerulonephritis or tubulopathy) to name a few (158). Rarely oncology patients, particularly those with Waldenstrom macroglobulinaemia, may develop hyperviscosity syndrome. This emergency can present with neurological deficits, mucosal bleeding, and visual disturbances (159).

Hyperalbuminaemia

If the tourniquet or cuff is on a limb for a long period during venesection, then one can get a falsely raised albumin concentration in the serum (3). If there is any doubt repeat the sampling. The most common reason, and many argue the only reason, for hyperalbuminaemia is dehydration. Drugs that shift body fluid balance may alter albumin concentration. Cisplatin has also been linked to hyperalbuminaemia due to nephrotoxicity (160). Hyperalbuminaemia has been reported with the use of co-magaldrox (aluminium hydroxide and magnesium hydroxide) and simeticone with aluminium hydroxide and magnesium hydroxide (131), however there are no case reports in the literature and perhaps the metal salts are interfering with the laboratory techniques used to measure albumin in the laboratory (161).

Retinol drugs, commonly used in dermatology for acne, psoriasis, hyperkeratotic disorders and cutaneous lymphomas, up-regulate albumin production and have been linked to hyperalbuminaemia (162). Diet may have a small effect and extreme, protein-heavy diets may lead to hyperalbuminaemia (163).

Table 5 Causes of hyperproteinaemia (147,151-156)

General cause	Subtype	Example
Plasma cell neoplasms and B-cell lymphoma	Plasma cell dyscrasia	Amyloidosis
		Monoclonal gammopathy of known significance
		Monoclonal gammopathy of clinical significance
		Monoclonal gammopathy of renal significance
		Multiple myeloma
		Plasma cell leukaemia
		Waldenstrom macroglobulinemia
Fluid imbalance	Dehydration	Addisonian crisis
		Cholera
		Diabetic ketoacidosis
		Severe vomiting and diarrhoea
		Very poor intake
Inflammatory	Autoimmune disease	Autoimmune hepatitis
		Systemic lupus erythematosus
		Rheumatoid arthritis
		Sjogren
	Infective	Hepatitis
		HIV infection
		Tuberculosis
		Sepsis

HIV, human immunodeficiency virus.

Hypergammaglobulinaemia

An algorithm for a laboratory approach to its investigation is shown in *Figure 6*. A cut-off of 45 g/L is commonly used (164-166). This threshold has a sensitivity of 73.15%, specificity 32.14%, positive predictive value of 44.12% and negative predictive value of 62.1% for detecting the presence of paraproteins in hospital patients (164). Ranges should be method and population dependent. The most important point for clarification, with hypergammaglobinumaemia, is whether the rise is polyclonal or monoclonal.

Polyclonal hypergammaglobulinaemia

Inflammation causes a polyclonal hypergammaglobulinaemia. Inflammatory symptoms can be broad and nonspecific and include headaches, chills, fever, loss of appetite, and muscle stiffness etc. (4). A raised globulin fraction can be seen in many autoimmune and vascular diseases (167,168). Raised polyclonal IgG responses are associated with vasculitis, autoimmune diseases, infections, IgG4-related diseases, and iatrogenic sources such as intravenous immunoglobulins. Elevations in IgA are associated with vasculitis, HIV infections, autoimmune diseases such as SLE, rheumatoid arthritis, coeliac disease, and IgA nephropathy. Polyclonal elevation of IgM can be associated with immunoglobulin class-switching disorders, acute infection, and hyper-IgM syndrome. IgD and IgE polyclonal elevations are rare, but IgD has been associated with hyper-IgD syndrome, and IgE is associated with asthma, allergies, atopy, lymphoma, parasite infections and hyper-IgE syndromes (144,169).

Further testing depends on the suspected condition, autoimmune serology testing should not be requested indiscriminately but based on the clinical presentation and history. For example, suspected inflammatory bowel disease may indicate tests including CRP, full blood count, anti-tissue transglutaminase antibodies, thyroid function, stool cultures and faecal calprotectin or immunochemical testing (168,170,171). Suspected SLE may indicate more general tests such as CRP, ESR, full blood count and a staged approach to specific immunology tests including anti-nuclear antibodies, complement (C3 and C4), anti-phospholipid antibodies and the follow-on tests of anti-double stranded DNA or extractible nuclear antigens (172).

Infective agents such as bacteria and viruses will produce an immune response resulting in a polyclonal antibody response, which may result in hyperproteinaemia and hypogammaglobinaemia. Specific samples may include HIV, infective exacerbation COPD, infective exacerbation of bronchiectasis, syphilis, bacterial endocarditis, mycetoma, acute rheumatic fever, cholecystitis, malaria, etc. (164).

Immunoglobulins are generally raised as part of the picture of liver damage. A raised IgM has been associated with primary biliary cirrhosis, raised IgA with alcoholic injury and portal cirrhosis and IgG was generally raised in chronic active hepatitis. As discussed, liver patients can also have a low albumin due to reduced synthetic function of the liver.

Non-haematological malignancies that may have polyclonal hypergammaglobulinaemia include liver, kidney, lung and ovarian (173). Non-haematological malignancies

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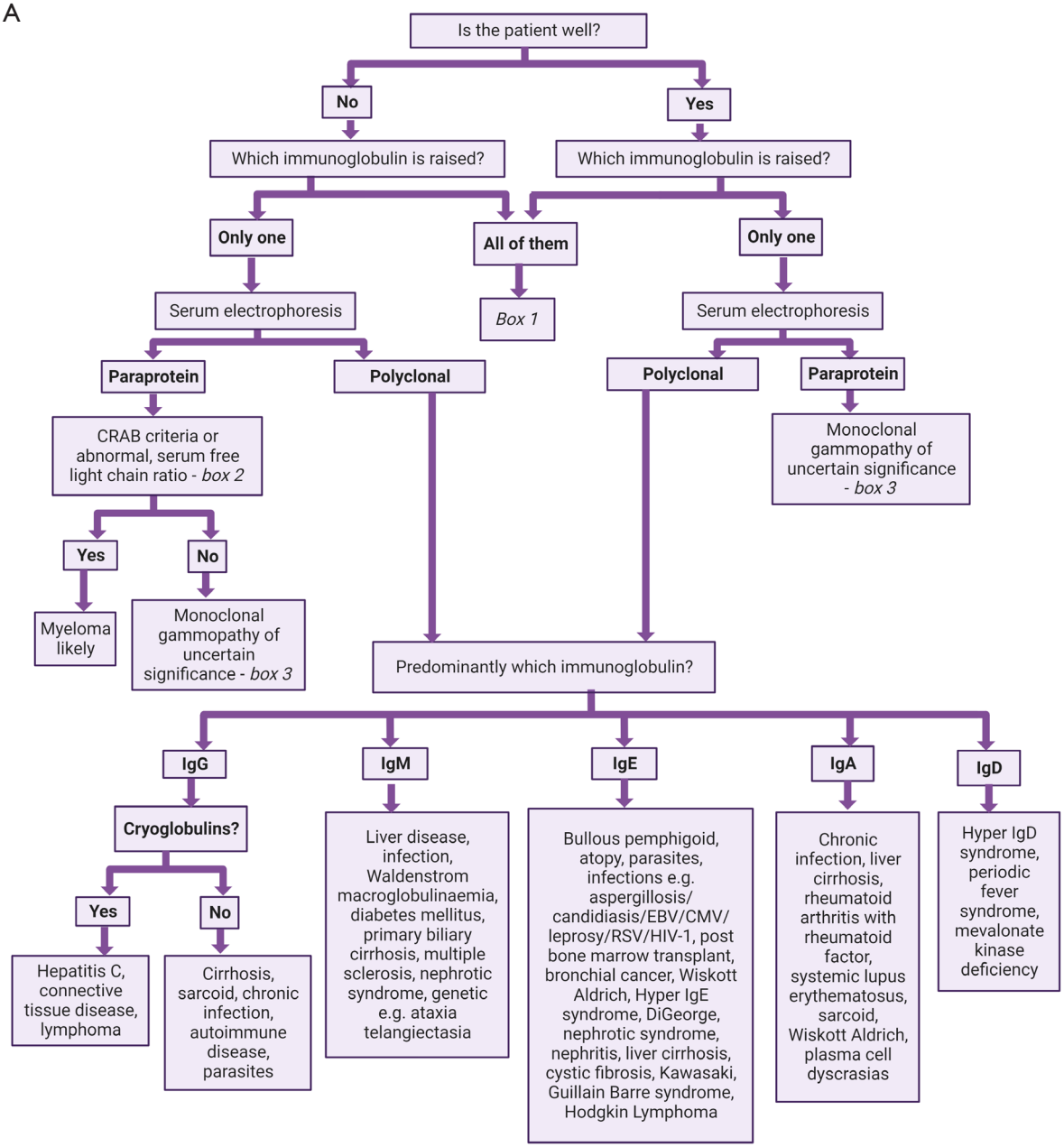

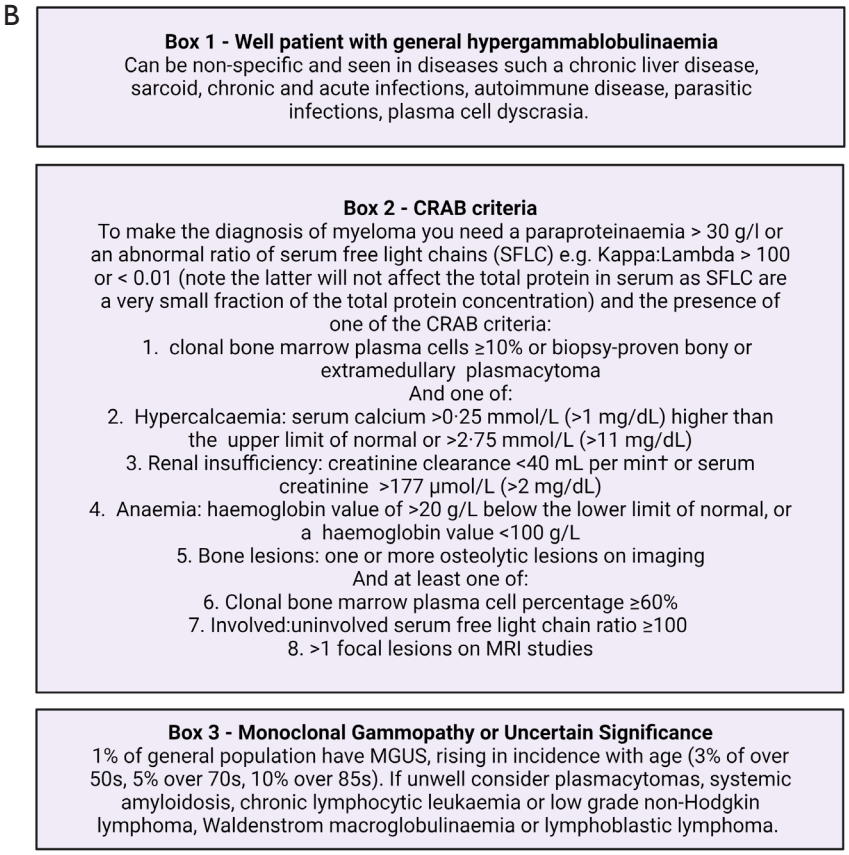

Figure 6 An investigative algorithm for hypergammaglobulinaemia. Diagnostic laboratory algorithm for hypergammaglobulinaemia in humans (A) with additional supportive information (B). (A) Diagnostic laboratory algorithm for hypergammaglobulinaemia in humans. (B) Boxes providing supportive information for diagnostic algorithm *Figure 6A*. EBV, Epstein-Barr virus; CMV, cytomegalovirus; RSV, respiratory syncytial virus; HIV-1, strain of human immunodeficiency virus; SFLC, serum free light chains; MGUS, monoclonal gammopathy of unknown significance.

tend to have a raised IgG concentration; however, the neoplasm needs to induce inflammation to see a polyclonal response (169).

IgG subtypes can be individually raised e.g., IgG4 in a fibroinflammatory disorder, which may present with a raised total protein, calculated globulin fraction, polyclonal IgG and signs such as lymphadenopathy, and laboratory findings such as eosinophilia, etc. (169). It is difficult to diagnose due to non-specific symptoms, ethnic and sex variation in IgG4 and it remains a relatively rarely diagnosed disease (174,175).

Monoclonal gammopathy

A broad spectrum of haematological malignancies may have a monoclonal gammopathy, which may present with a raised globulin fraction. The CRAB criteria are used to help distinguish diagnoses (53). Haematological malignancies may include multiple myeloma, Waldenström macroglobulinaemia, acute myeloid leukaemia, chronic lymphoid leukaemia, chronic myeloid leukaemia (164). MGUS is benign but can progress and develop into multiple myeloma. MGUS of renal or clinical significance refers to diseases where the protein deposition in organs causes a variety of clinical presentations (see above). A paraprotein should stimulate further investigation (*Table 6*).

Bence Jones proteins can be present in the urine of MGUS patients, but it is less frequent, and they generally present in very small quantities (3,4,53,56,164,173).

High concentration of protein, particularly those of an IgM paraprotein source, or of rheumatoid factor, are a common listed interferent on many different commercial assay's kit inserts. It is also well documented

Table 6 The differential diagnostic criteria of monoclonal protein band and myeloma without a typical paraprotein band (53)

Disease	Monoclonal protein band	Distinctive features
MGUS	Paraprotein band <30 g/L	No evidence of end organ damage (aka anaemia, renal failure, hypercalcaemia)
		No evidence lytic lesions on X-ray
		<10% clonal plasma cell in bone marrow
Multiple myeloma	Paraprotein band ≥30 g/L	Presence of paraprotein in the urine
		Evidence of end organ damage: anaemia, hypercalcaemia, renal disease, osteoporosis
		Evidence of lytic lesions on X-ray
		≥10% clonal plasma cell in bone marrow
	IgA ≥15 g/L	Note that IgA myeloma may present with lower paraprotein band, cut will still need to meet the CRAB criteria
	No or trace paraprotein, abnormal light chain ratio	Light chain myeloma (will still need to meet the CRAB criteria); rule out cyroglobinaemia (false negative) if clinical suspicion high (keep sample warm); a secretory myeloma, diagnosis still relies on the CRAB criteria for the other features
Smouldering myeloma	Paraprotein band ≥30 g/L	No evidence of end organ damage (aka anaemia, renal failure, hypercalcaemia)
	Urine monoclonal protein ≥500 mg/24 h	No evidence of lytic lesions on X-ray
		10–60% clonal bone marrow plasma cells
Waldenstrom macroglobulinemia	IgM paraprotein	Evidence of hyper-viscosity, enlarged spleen and/or liver, anaemia, and swollen lymph nodes

MGUS, monoclonal gammopathy of unknown significance; CRAB, Calcium elevation, Renal dysfunction, Anaemia and Bone disease.

that particularly hyperparaproteinaemia can cause a pseudohypophosphataemia and pseudohyponatremia sometimes seen in multiple myeloma patients (3,4,176).

Generally, there are more drugs that are associated with hypogammaglobulinaemia than hyperglobulinaemia. Drugs associated with hyperglobulinaemia include amiodarone, intravenous immunoglobulins, and drugs that can induce a drug-related autoimmune disease (177,178). Biological drugs such as daratumumab can be picked up as a monoclonal band on serum electrophoresis. As more biological antibody drugs are introduced this could become a larger problem. Good communication between the clinicians and the laboratory are required to avoid misclassifying the patient's therapeutics as new monoclonal bands (179).

Special states, e.g., pregnancy and childhood

Albumin in pregnancy

Haemodilution can also occur as part of normal physiological processes such as pregnancy, where plasma volume on average expands by 45% (33). During pregnancy and lactation there is also a chronic but mild inflammatory response increasing the vascular permeability and reducing albumin concentration (180,181). Hypoalbuminaemia is a common finding in pregnancy during the third trimester and should not be over investigated (182).

During breast feeding there does not seem to be a serum albumin difference between mothers who may be assumed to be malnourished compared to those considered to be well nourished (183). Studies have not demonstrated any effect of lactation in a large systematic review (184).

Albumin in childhood

In prematurity albumin is associated with gestational age and children born under 32 weeks have an average of albumin of 30.6±4.7 g/L (184). From early infancy over childhood albumin concentrations rise (185,186). Therefore, age-related reference intervals should be employed such as those from the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) database (186).

Immunoglobulin in pregnancy

During human pregnancy there is a shift from TH1 immune

response into a TH2 immune response and dynamic changes in the B-cell development of immunoglobulin profile are thought to occur. There is a significant decrease in the concentration of transitional B-cells within whole blood during pregnancy (187). IgG starts to mildly decrease during the second trimester due to haemodilution. IgM, IgA and IgE concentrations vary between individual pregnancies and have been observed to stay consistent as well as increase and decrease (27,188,189).

Immunoglobulins in childhood

Immunological changes within infancy throughout childhood to adolescence are diverse and complex. A full review is outside the scope of this paper, but the summarised expected changes within immunoglobulin concentrations are as follows: IgG concentration increases 18% per year until the age of five, where the rate of increase slows to 2% per year. IgA is on average higher in females than male infants, IgA increases by about 27% per year for the first 7 years of life before plateauing. IgM rapidly increases during the first year of life but tapers off past this point (190-192). Therefore, age-related reference intervals should be employed such as those from the Canadian Laboratory Initiative on Paediatric Reference Intervals (CALIPER) database (186).

Conclusions

Protein, albumin, and globulin is a useful screening test for detecting protein imbalances within the body, however the investigation can be challenging with numerous causes. Many factors need to be considered when interpreting these tests including acute phase/inflammatory response, hydration status and organ disease. A set of diagnostic algorithms have been created to guide the reader's approach and provide a systematic route of testing. Each patient is unique however and the clinical picture may direct the reader to skip steps or refer other algorithms within the series or in the literature. These algorithms are not a replacement for experience, expert opinion or local guidelines and should instead act as a diagnostic aid.

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Supplementary

Table S1 The search strategy summary

Items	Specification
Date of Search	December 2021
Databases and other sources searched	Medline, Google Scholar, OMIM
Search terms used. Examples include:	Total protein, protein, hypoalbuminemia, hyperalbuminemia, hypoproteinaemia, hyperproteinaemia, proteinuria, investigations, algorithms, guidelines, diagnosis, investigation, causes, aetiology, human
Timeframe	From database inception to 2023
Inclusion criteria	All papers and reviews were included restricted to English
Selection process	LE Duvall and AR Shipman conducted initial search, with refinement by all other authors to obtain consensus and agreement
Any additional considerations, if applicable	Seminal texts were also searched and the references of important articles and texts were obtained and checked for relevance

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