

Clinical indications for plasma protein assays: transthyretin (prealbumin) in inflammation and malnutrition¹⁾

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Abstract

A large number of circumstances are associated with reduced serum concentrations of transthyretin (TTR), or prealbumin. The most common of these is the acute phase response, which may be due to inflammation, malignancy, trauma, or many other disorders. Some studies have shown a decrease in hospital stay with nutritional therapy based on TTR concentrations, but many recent studies have shown that concentrations of albumin, transferrin, and transthyretin correlate with severity of the underlying disease rather than with anthropometric indicators of hypo- or malnutrition.

There are few if any conditions in which the concentration of this protein by itself is more helpful in diagnosis, prognosis, or follow up than are other clinical findings. In the majority of cases, the serum concentration of C-reactive protein is adequate for detection and monitoring of acute phase responses and for prognosis. Although over diagnosis and treatment of presumed protein energy malnutrition is probably not detrimental to most patients, the failure to detect other causes of decreased concentrations (such as serious bacterial infections or malignancy) of the so-called visceral or hepatic proteins could possibly result in increased morbidity or even mortality.

In addition to these caveats, assays for TTR have a relatively high level of uncertainty ("imprecision"). Clinical evaluation – history and physical examination – should remain the mainstay of nutritional assessment.

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Introduction

The question of how best to diagnose, quantify, and follow protein energy malnutrition (PEM) in both acute and chronic medical situations has been a matter of debate for many years (1–6). Many investigators feel that clinical evaluation (history and physical examination, with or without anthropometric measurements) is the best and easiest method, particularly since this is part of good patient care (7). Others prefer direct, objective measurements, such as anthropometric measurements or bioelectrical impedance (BI) (8). However, many clinicians and laboratorians prefer a quick and easy laboratory means of diagnosis and follow-up. For this purpose, assays of various plasma proteins (sometimes referred to as hepatic, visceral, or serum secretory proteins) have been to the forefront in recent years, with albumin, transferrin, and prealbumin (transthyretin; TTR) advocated most commonly.

Many reports in the literature make the assumption that low concentrations of one or more of these three proteins indicate PEM and poor prognosis (4–6, 9). Conversely, several reports and reviews suggest that the specificity of reduced concentrations is too low for the diagnosis of PEM, especially if other confounding conditions are not considered (1, 2, 10, 11). C-reactive protein (CRP; as a marker of inflammation) and body mass index are probably better prognostic aids in most cases, as has been shown to be the case in patients with end-stage respiratory failure (12).

This paper is a brief summary of factors that influence plasma TTR concentrations and how they may or may not relate to PEM, with recommendations for the use (or non-use) of TTR assays in diagnosis. This discussion focuses on TTR because it is the most commonly used protein for this purpose; however,

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similar comments are also appropriate for albumin and transferrin (1–3). The reader is referred to these and other recent reviews for additional discussion and references for and against TTR assay for assessment of nutrition. The December 2002 issue of *Clinical Chemistry and Laboratory Medicine* (CCLM) is completely devoted to papers on various aspects of TTR, with presentations for both sides of the argument.

Biochemistry and physiology of transthyretin

Structure

TTR is a globular, non-glycosylated protein with a molecular mass of 54.98 kDa (13). With one complexed molecule of retinol-binding protein (RBP; 21 kDa) (14), the total mass is approximately 76 kDa, which is still small enough to diffuse out of the vascular space as readily as albumin (66.3 kDa) or transferrin (79.6 kDa); slightly less than 50% of each of these proteins is normally intravascular as a result (15).

Function The protein migrating anodal to albumin on non-sieving, routine serum electrophoresis at pH ~8.6 was initially noted to bind thyroxine (T_4) and was thus given the name thyroxine-binding prealbumin, or TBPA (13, 16). However, it was subsequently shown to bind triiodothyronine (T_3) and holo-retinol-binding protein (RBP with retinol, or vitamin A) as well, and the name was changed to *transthy(roxin)retin(ol)* to denote its dual transport function (17). TTR is a tetramer of four identical subunits. Although each of the four monomers has a binding site for RBP, the tetramer binds only one molecule of RBP with high affinity and possibly a second with lower affinity (18). The binding affinity for apo-RBP (RBP without retinol) is very low, and the loss of retinol (e.g., uptake by tissues) results in the separation and renal excretion of free apo-RBP, accounting for the very short biological half-life of RBP of ~3.5 h (19). Each TTR monomer also has two binding sites for thyroid hormones, but binding of one molecule of T_3 or T_4 significantly reduces the affinity of the second site. Binding affinity for T_3 is lower than that for T_4 . The TTR-RBP complex normally transports approximately 20% of circulating thyroid hormones (70% is transported by thyroxine-binding globulin or TBG, the rest by albumin) and 90%–95% of retinol/vitamin A. The complex is more important for retinol transport than for thyroid hormones.

Synthesis Essentially all plasma TTR is synthesized by the hepatic parenchymal cells or, in fetal life, the

yolk sac endothelium (20). Control of synthesis by the liver is dependent on C/EBP nuclear factor, which is homologous to interleukin-6 (IL-6) nuclear factor. When stimulated by acute phase cytokines such as IL-6 that *increase* synthesis of the positive acute-phase reactants, including CRP, serum amyloid-A, α_1 -antitrypsin, and the like, TTR mRNA is significantly *down*-regulated, resulting in *decreased* synthesis and plasma concentrations, as is also the case for albumin and transferrin (21). Small amounts of TTR are also synthesized by the choroid plexus, pancreas (22), and retina (23), but these probably do not affect the serum protein concentration.

Catabolism

TTR is catabolized primarily by the liver and by excretory loss via the kidneys and gastrointestinal tract. Its biological half-life is approximately 2.5 days (24) and is not altered by stress or acute inflammation (25).

Genetic aspects

The gene coding for TTR is located on chromosome 18q (26). There are over 100 known genetic variants, including a few with increased or decreased binding affinities for thyroid hormones but clinical euthyroidism. Many of the genetic variants are associated with deposition of amyloid in tissues, resulting in a group of autosomal dominant hereditary amyloidoses (27–29). Plasma concentrations of TTR are essentially normal in these disorders and are not helpful in diagnosis; however, some variants do show altered electrophoretic mobility.

Reference intervals

Serum concentrations of TTR are very low in the fetus and neonate, rise slowly to reach a maximum in the fifth decade of life, and then decline slowly. Most studies have shown lower concentrations in premenopausal females, probably due to the effect of estrogens. Selected reference intervals by age and gender, with values traceable to CRM 470 (30), are given in Table 1.

Clinical associations with high or low plasma TTR concentrations

Serum concentrations of TTR are influenced by a large number of factors, as summarized below:

A) Increased concentrations

1. Increased synthesis: exogenous corticosteroids or anabolic steroids; non-steroidal anti-

Table 1 Selected reference intervals for transthyretin by age and gender.

	Newborn	1–2 years	10–20 years	20–35 years	35–60 years	> 60 years
Males	0.07–0.17	0.11–0.26	0.16–0.40	0.22–0.44	0.22–0.45	0.16–0.40
Females	0.08–0.17	0.12–0.25	0.15–0.32	0.16–0.35	0.18–0.38	0.14–0.37

Reference intervals presented are approximately the 5th–95th centiles in g/L, modified from Ritchie et al. (30).

inflammatory agents (NSAIDs); insulin-like growth factor-1 (IGF-1), either endogenous or exogenous.

2. Decreased catabolism: chronic renal failure; renal tubular damage.
3. Distributional or hydrational changes: standing position prior to blood sampling; acute dehydration.

B) Normal concentrations

1. Absence of disease (and of other factors altering concentration).
2. Some forms of malnutrition: anorexia nervosa; restriction of protein alone; isolated vitamin A deficiency.

C) Decreased concentrations

1. Age: infancy, childhood, and advanced age (Table 1).
2. Decreased synthesis: acute phase response (infection, inflammation, trauma, malignancy, etc.); administration of IL-6; estrogens (endogenous or exogenous); acute total starvation; moderate to severe liver disease (may be due in part to inflammation and/or estrogen); thyroid disease, especially endemic goiter.
3. Distributional changes: increased vascular permeability; ascites or pleural fluid; recumbent position prior to blood sampling (e.g., in bedridden patients); acute hemodilution.
4. Increased loss/catabolism: acute blood loss; protein-losing enteropathy; nephrotic syndromes.

Transthyretin, inflammation, and PEM

Historical aspects

The suggestion that plasma protein assays might be useful in evaluating malnutrition originated from studies of children with kwashiorkor and marasmus in third-world countries. Kwashiorkor is secondary to intake of almost exclusively carbohydrate energy sources, whereas marasmus refers to general malnutrition – i.e., of all energy types (31). As might be expected, rates of protein breakdown are higher in marasmus than in kwashiorkor (32). In both cases, albumin concentrations may be very low; however, a very large percentage of such patients, and perhaps all, have infectious diseases, parasitic infestations (both associated with acute phase responses), gastrointestinal protein loss [in marasmus or marasmic kwashiorkor, but not typical kwashiorkor; (33)], or combinations of these. Kwashiorkor is also associated with edema and ascites, which alter the distribution of proteins.

Albumin has a relatively long plasma half-life [~ 19 days; (34)]. The assumption having been made that PEM was associated with decreased hepatic production of proteins, a search began for shorter-lived

proteins with concentrations that were also decreased along with those of albumin and which thus more nearly reflected the patient's status at the time of sampling. Concentrations of such proteins should hypothetically more rapidly reflect any decrease from poor protein intake or increase following institution of therapy. First, transferrin was utilized; however, iron deficiency and estrogens (among other things) are associated with increased synthesis and plasma concentrations of this protein, reducing the sensitivity of concentrations to decreased protein intake. Prealbumin (TTR) then became the protein of choice because of its even shorter half-life of ~ 2.5 days (35). RBP has also been considered, but it is more difficult to assay and, because of its binding to TTR, is usually present in concentrations proportional to those of TTR. RBP/TTR ratios are low in acute inflammation; therefore, the use of plasma retinol and RBP concentrations for evaluating nutrition in patients with inflammatory processes could result in over diagnosis (36).

Several studies have found that as many as 50% – or even more – of hospitalized patients have low serum concentrations of TTR and suggest that low concentrations may be associated – at least in some studies – with poor prognosis (but see below). The presence of low concentrations in the elderly, in particular, has been cited by some as evidence that PEM is very common in this group, leading to poor prognosis (37). However, the low concentrations in this age group are more likely related to age either alone (30) or associated with acute phase responses (37). The reduced concentrations are due in part to an age-related decrease in IGF-1 concentrations, resulting in decreased hepatic synthesis of several proteins (38).

Acute phase response

Plasma concentrations of all of the “nutritional” proteins are also affected by many additional factors. The most significant and most common of these is the so-called acute phase response (APR). In response to any of a large number of insults, such as inflammation (infectious, autoimmune, or otherwise), trauma (including surgery), malignancy, and tissue necrosis, the body responds by synthesizing a large number of cytokines. These include interleukins and tumor necrosis factors that induce (increase) synthesis of the positive acute phase reactants (CRP, serum amyloid A, procalcitonin, α_1 -antitrypsin, α_1 -antichymotrypsin, α_1 -acid glycoprotein, haptoglobin, and many other proteins) and down-regulate, or decrease, synthesis and plasma concentrations of others, including albumin, TTR, and transferrin (39). As previously noted, the administration of IL-6 is also associated with down-regulation of synthesis and reduced serum concentrations of these proteins (21, 40). Patients with severe sepsis or multiple injuries often have very low TTR concentrations that are inversely related to CRP concentrations and directly proportional to IGF-1 concentrations, which also fall in severe APR (41). Administration of IGF-1/BP-3 (binding protein-3) complexes leads to a rapid rise in TTR, RBP, and transferrin in these patients (42). Serum concentrations in APR may

be further reduced by extravasation from the vascular space, hemodilution, and possibly increased consumption in association with inflammation or trauma.

Protein energy malnutrition

A large number of studies have shown a presumptive correlation of TTR concentrations with the level of protein energy nutrition (3–6). However, a major problem with many, if not most, studies using TTR alone to diagnose PEM is that they did not evaluate other causes of low concentrations, especially for APR, or factors that may increase concentrations, such as anti-inflammatory or anabolic drugs. Even when the positive acute phase reactants (43) or inflammatory cytokines (44) are assayed and found to be elevated, low TTR concentrations are considered to be due to nutrition and not to APR. In addition, many studies use circular arguments – using TTR concentrations as a major factor in “diagnosis” of PEM, then considering the same concentrations to be the result of PEM.

Reported studies using ratios, including CRP/TTR (45) and the prognostic inflammatory and nutrition index, or PINI (46), consider high ratios as indicative of both inflammation and PEM; however, by far and away the predominant component of very high ratios in either case is elevated serum CRP. TTR alone can account for at most a two- or three-fold increase in these ratios, while even mild inflammation may be associated with a 10-fold or greater increase in CRP concentration and in the index value. Very high ratios (e.g., >20) are highly indicative of APR rather than PEM. There are no studies indicating that PEM alone (i.e., without inflammation) influences CRP concentrations – they are primarily, if not exclusively, raised by proinflammatory cytokines associated with APR.

Increases in concentrations of the “nutritional proteins” during treatment of inflammation or recovery from trauma or surgery, often credited to nutritional replacement, correlate with normalization of serum concentrations of CRP and therefore with decreased APR (47–49). In addition, treatment with corticosteroids or many non-steroidal anti-inflammatory agents (e.g., aspirin) is associated with increased concentrations of TTR (21), as is treatment with anabolic steroids (50).

Generalized malnutrition

As noted above, kwashiorkor and marasmus, particularly in third-world countries, are usually associated with infections and parasitic infestations, especially of the gastrointestinal tract. Thus, significant APR is nearly always present, often in association with gastrointestinal protein loss, and it is unclear how much of the hypoproteinemia observed with these disorders is due to individual factors.

In developed countries, anorexia nervosa is the most common cause of “marasmus,” with profound generalized malnutrition, but usually not with other confounding conditions such as inflammation (51). Several recent studies have shown that the three pro-

teins mentioned above (albumin, transferrin, and TTR) are present in normal concentrations in the majority of patients with anorexia nervosa, even in the presence of severe cachexia (51); in one study, significant alterations were only observed for C3, C4, and transferrin concentrations (52). In addition, long-term dietary protein restriction is associated with normal concentrations of these proteins in the absence of inflammation.

Surgery is a potent stimulus for APR. Postoperatively, TTR concentrations fall, regardless of optimal caloric intake (53). Conversely, in acute total starvation, plasma concentrations of TTR and RBP may fall by 30%–40% over the first 2–4 days, followed by concentrations of albumin and transferrin after approximately 4 days (39, 54). However, one study found that only transferrin concentrations fell with starvation alone, whereas transferrin and prealbumin concentrations fell with starvation plus stress or APR (55). The response observed with starvation is similar to that observed with injection of IL-6 (39), one of the cytokines involved in induction of APR.

Institutionalized patients with Alzheimer’s disease and malnutrition but normal inflammatory markers show no change in serum concentrations of albumin and TTR during therapy (9); “low” concentrations of these two proteins may be due to age alone in these patients. Nutritional therapy in such patients results in increased body mass index (BMI), arm muscle circumference, and triceps skin-fold thickness, but no increase in concentrations of albumin, prealbumin, or homocysteine (56).

Vitamin A deficiency Apo-RBP synthesis is not affected by vitamin A deficiency, but lack of the retinol ligand results in intrahepatic accumulation of RBP and lower serum concentrations (57). However, vitamin A deprivation does not decrease synthesis or release of TTR by hepatic parenchymal cells.

Renal disease Serum TTR concentrations have long been used to follow patients with chronic renal failure (CRF), especially those on hemodialysis. CRF is typically associated with increased serum TTR, presumably due to decreased tubular uptake and degradation of RBP, but concentrations fall with inflammation or malnutrition (58). Serum RBP also increases with renal damage (59). Concentrations have been shown to correlate inversely, but strongly, with concentrations of CRP, IL-6, and procalcitonin (60), suggesting that low levels are primarily the result of inflammatory disease. Low levels over-predict the number of patients at risk of malnutrition per se by at least 1/3 as compared to standard dietetic assessment (61). In addition, markers of inflammation alone (e.g., CRP and IL-6) are stronger predictors of outcome than the so-called “nutritional markers” (62).

Clinical prognosis and treatment

Although some investigators have suggested that decreased concentrations of the three “nutritional” proteins in hospitalized patients reflect nutritional

status and prognosis, others feel that they reflect disease severity instead. As noted above, in acute inflammatory or traumatic conditions TTR concentrations fall by as much as half or more within a few days, with the degree of decrease showing a strong negative correlation to positive acute phase reactants, such as CRP and orosomucoid (α_1 -acid glycoprotein). Not unexpectedly, clinical prognosis also correlates highly with these APR markers – and thus with disease severity rather than malnutrition (48). A recent symposium report states that changes in “nutritional markers,” including TTR, do not predict clinical outcome (11).

A few studies have evaluated multiple methods of nutrition assessment: medical history and questionnaires, clinical examination, anthropomorphic measurements, and BI measurements, in addition to plasma protein concentrations (3, 11). Multiple regression analysis has shown that, after correction for APR and/or anthropomorphic measurements, concentrations of albumin, prealbumin, transferrin, or combinations of these indicate an inflammatory response and are not significantly associated with either nutritional status or prognosis (49). The latter study concluded that concentrations of the proteins commonly associated with malnutrition are reduced only by preterminal starvation in the absence of inflammation, and that the decreased synthesis observed with inflammation explains both the low concentrations of these proteins and the increased cardiovascular risk in patients (49).

Assay of TTR followed by increased protein caloric intake for those patients with low concentrations has been reported in some studies to shorten the length of hospital stays; however, there are also conflicting reports that found no association between response of the TTR concentration and length of stay. If no independent assessment of protein nutritional adequacy is utilized, TTR concentrations probably reflect illness severity rather than malnutrition, and treatment of the underlying disease is more likely to be the cause of shortened stays (3, 63). A recent study in Japan did not find TTR to be a sensitive indicator of either nutrition or prognosis in critically ill patients (64). Sullivan and coworkers stated that there is little convincing evidence that any form of nutritional intervention significantly affects morbidity and hospital course (65), in part because the markers used to evaluate nutritional status reflect disease severity instead (53).

Other potential plasma markers for PEM

More recently, it has been suggested that serum concentrations of IGF-1 or sex hormone-binding globulin (SHBG) may be better indicators of PEM than are concentrations of TTR. SHBG concentrations are elevated in kwashiorkor and anorexia nervosa, but not in marasmus, acute inflammation, or renal failure; concentrations are inversely correlated to those of IGF-1 (66). Because of the very low concentrations of these analytes, however, quantification is more difficult and expensive than is the case for TTR. Plasma fibronectin

concentrations may be helpful in evaluating response to total parenteral nutrition (TPN); Sandstedt and coworkers found that only concentrations of this protein, among several others, showed a response to TPN in malnourished patients – most of whom also had evidence of inflammation (67).

Serum pseudocholinesterase concentrations are low in acquired nutritional deficiency, but may also be due to genetic deficiency (68). When it is readily available, BI can be used to detect shifts in fluid balance and body composition, as well as PEM (8). However, clinical impression alone may still be a better way to assess nutrition, at least in patients with gastric carcinoma (69). The value of assaying concentrations of zinc, vitamins, or other specifically nutritional analytes for clinical evaluation and dietary history remains to be assessed.

Laboratory assays of transthyretin

Although most immunochemical methods can be used to assay TTR, the methods most commonly used at present are immunonephelometry (IN) and immunoturbidimetry (IT). A new international reference material for serum proteins (CRM 470) was introduced in 1993–1994 in part because of wide differences in serum protein values used by various manufacturers. The concentration value for TTR in CRM 470 was assigned from highly purified and highly characterized TTR, using a very accurate and precise value transfer method (70, 71). It was hoped that among-manufacturer and among-laboratory assay values would converge following the introduction of this material; however, national and international quality control programs continue to show significant variation (21). A recent international quality control survey showed among-laboratory coefficients of variation (CV) in the range from 12% to >20% (Whicher JW, Milford Ward A, Johnson AM, unpublished data); thus, an assayed concentration may be as much as 50% or more above or below the true value. This high level of uncertainty of values relates in part to the relatively low serum concentrations of TTR, differences in antiserum specificity and reactivity, and inaccurate transfer of values to calibrators and controls by some manufacturers. Whether these problems will ever be adequately addressed remains to be seen.

Preanalytical factors may also influence plasma concentrations of proteins and other macromolecules. Of particular importance is body position; individuals who have been standing or recumbent for long periods of time have higher or lower concentrations, respectively. It is generally recommended that blood specimens for assay of plasma proteins be drawn after approximately 15–20 min in the sitting position if possible. Otherwise, concentrations must be evaluated with consideration of the position – e.g., lower concentrations are to be expected in bed-ridden patients.

Summary and recommendations

1. Serum TTR concentrations are affected by many factors, including age, gender, and blood-drawing methods, as well as factors influencing distribution or rates of synthesis and catabolism (see section on clinical associations).
2. Assay of serum TTR concentration is recommended by some investigators as a screening marker for inflammation, malnutrition, or both. There is little question that concentrations are low in some instances of PEM, but even severe, long-term malnutrition may be associated with normal concentrations. Low concentrations are more often associated with the acute phase response than with PEM, especially in developed countries. Therefore, the sensitivity and specificity of TTR concentrations for diagnosing PEM are low.
3. It is difficult if not impossible to sort out, on an individual patient basis, whether a low TTR concentration is in part due to PEM unless other causes, especially including inflammatory diseases such as infection, are ruled out and the overall clinical evaluation supports the diagnosis of PEM. The latter may include clinical evaluation, plasma assays of essential nutrients such as zinc or vitamins, nitrogen balance studies, or BI.
4. If a patient with a significant infection, malignancy, or trauma were diagnosed as having "only" PEM as the cause of a low serum TTR concentration, obviously treatment with nutritional supplementation alone – without treatment for the cause of the APR – could be disastrous. At the same time, patients with inflammatory processes, especially chronic ones, may need nutritional supplementation in addition to treatment of the underlying disease because of their hypermetabolic state, whether or not true PEM is present.
5. Over diagnosis of PEM using TTR concentrations alone is probably not deleterious to patient care per se, as long as other causes of low concentrations are recognized and treated appropriately. However, over diagnosis may result in significantly increased healthcare costs because of unnecessarily increased payments to hospitals, physicians, and laboratories.
6. Assays for TTR continue to show significant imprecision, with high among-assay, within-laboratory, and among-laboratory CVs, rendering their use for clinical purposes questionable unless concentrations are very low or very high.
7. Although the use of ratios such as CRP/TTR may slightly increase the detection of acute phase responses, the contribution of TTR is small and of questionable cost effectiveness. Assay of CRP alone is usually adequate for this purpose.

Increasing serum concentrations of TTR during treatment in general coincide with decreasing concentrations of CRP and other positive acute phase reactants. Therefore, it is often – if not usually – impossible to state with certainty that the increases

are related to nutritional therapy. This places in question the common use of sequential TTR assays to determine response to nutritional therapy.

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