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Narrative Review

β-Trace Protein: A Marker of GFR and Other Biological Pathways

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β-Trace protein (BTP), also known as lipocalin prostaglandin D_2 synthase (L-PGDS; encoded by the *PTGDS* gene), is a low-molecular-weight glycoprotein and an emerging novel marker of glomerular filtration rate. BTP is an important constituent of cerebral spinal fluid and is found in much lower concentrations in blood. Its serum origin and renal handling remain poorly understood. Unlike serum creatinine, BTP is not physiologically inert. It possesses both ligand-binding and enzymatic properties. BTP catalyzes the conversion of prostaglandin H_2 (PGH_2) to PGD_2. PGD_2 is an eicosanoid involved in a variety of important physiologic processes, including platelet aggregation, vasodilation, inflammation, adipogenesis, and bone remodeling. Several studies now have documented BTP's strong association with glomerular filtration rate, end-stage renal disease, cardiovascular disease, and death in a variety of different patient populations. This review provides an overview of the biochemistry, physiology and metabolism, biological functions, and measurement of BTP; summarizes the evidence for BTP as a marker of both kidney function and cardiovascular disease; and then considers the interplay between its biological properties, serum concentration, and patient outcomes.

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INDEX WORDS: β-Trace protein (BTP); lipocalin prostaglandin D_2 synthase (L-PGDS); *PTGDS*; renal function; estimated glomerular filtration rate (eGFR); filtration marker; kidney disease progression.

While the kidney performs a wide range of essential physiologic functions, its filtration capacity or glomerular filtration rate (GFR) generally is considered the best indicator of overall kidney function.[1] Measuring GFR is time consuming, costly, and limited in availability and hence rarely is used in clinical practice. Serum concentrations of endogenously produced and renally excreted analytes are used almost exclusively instead. An ideal endogenous marker of GFR is produced at a constant rate, freely filtered at the glomerulus, and neither secreted nor reabsorbed in the tubules and has no extrarenal routes of elimination.[2] Creatinine, the most commonly used marker of GFR, does not satisfy all these criteria. It is secreted variably by the tubules[3] and, in advanced chronic kidney disease (CKD), undergoes elimination by the gastrointestinal tract.[4] Furthermore, marked interindividual variability in creatinine generation exists due to variables such as diet, synthetic liver function, muscle mass, and turnover.[2] As a result, a serum creatinine concentration of 1.2 mg/dL may represent normal kidney function in one individual and markedly reduced kidney function in another.

During the past decade, a number of alternative markers, including cystatin C, β_2 microglobulin, and β-trace protein (BTP), have emerged as alternative novel markers of GFR.[5] More recent epidemiologic studies have documented stronger associations between these markers and non–kidney disease outcomes such as mortality and cardiovascular disease than serum creatinine level and estimates based on serum creatinine level.[6-9] This has led to the question of whether this is because the novel markers are: (1) simply more accurate measures of GFR than serum creatinine level and therefore better capture the risk associated with CKD or (2) mechanistically linked to underlying pathophysiologic processes that influence serum concentration and adverse outcomes independently of GFR (Fig 1). Unlike serum creatinine, these markers are not physiologically inert but are enzymatic components of important biological pathways. Improved understanding of these markers and their biological functions is required to better understand the role of these novel markers in GFR estimation and outcome prediction.

HISTORY

BTP was identified first in 1961 by immunoelectrophoresis as 1 of 2 proteins found in human cerebral spinal fluid (CSF) and not in blood.[10] In 1985, a novel glycoprotein was extracted in purified form from rat brain and named lipocalin prostaglandin D_2 synthase (L-PGDS).[11] It subsequently was recognized that

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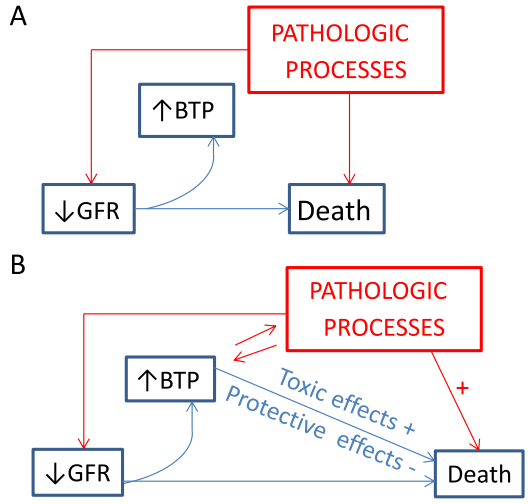

Figure 1. β-Trace protein's (BTP's) stronger association with death reflects either (A) improved accuracy as a marker of glomerular filtration rate (GFR) and therefore better captures the risk associated with decreased kidney function or (B) pathophysiologic processes that influence both its serum concentration and outcomes independently of GFR. BTP either directly or indirectly could contribute toxic or protective effects.

Box 1. Properties of BTP

Molecular structure: Heterogeneous monomeric glycoprotein with multiple isoforms
Molecular weight: 26-29 kDa
Serum origin: Unknown
Function: Catalyzes conversion of PGH_2 to PGD_2/ligand binding
Renal handling:
Filtration: unlikely to be freely filtered
Secretion: unknown
Reabsorption: partial (urine BTP present in healthy persons)
Extrarenal elimination: Unknown
Assays: ELISA and nephelometric
Reference methods/materials: None
Non-GFR determinants:
Age: likely
Sex: likely
Race: unknown
Muscle mass: possibly
Genetic factors: yes
Pregnancy: yes
Inflammation: possibly
Cardiovascular disease: yes
Liver disease: unknown

Abbreviations: BTP, β-trace protein; ELISA, enzyme-linked immunosorbent assay; GFR, glomerular filtration rate; PG, prostaglandin.

BTP and L-PGDS were identical compounds.[12,13] In 1997, Hoffmann et al[14] first noted BTP levels to be elevated in serum of patients with decreased kidney function and suggested that this protein could become a useful novel diagnostic marker in kidney disease. The association between decreased kidney function and elevated serum BTP levels was confirmed by Melegos et al[15] in 1999. Over the next 10 years, BTP was investigated as a marker of GFR in many, but small, populations. In the past 3 years, interest in BTP has increased substantially, with data emerging documenting its association with kidney disease and non–kidney disease outcomes in large population-based and study cohorts.[6-9,16]

BIOCHEMISTRY, PHYSIOLOGY, AND METABOLISM

BTP Structure

Encoded on chromosome 9, BTP is a heterogeneous monomeric glycoprotein with 168 amino acids[14,17] (Box 1). Its microheterogeneity is the consequence of post-translational N-glycosylation resulting in different glycoforms of varying molecular weight (23-29 kDa).[14,18] These glycoforms are found in differing relative amounts in different fluid compartments. The smaller "brain" type isoforms predominate in the CSF and have truncated oligosaccharide side chains with absent sialyation.[14] The larger "serum-compatible" glycoforms predominate in serum and urine and have more fully sialylated oligosaccharide chains.[14] The functional significance of the different isoforms and relative concentrations of different isoforms in different health and diseased states is not known. The presence of multiple isomers certainly has implications for BTP measurement, which is discussed further.

BTP Tissue Distribution

BTP has been identified in many human fluids and tissues.[18] BTP localizes to the leptomeninges, arachnoid cells, choroid plexus epithelial cells, and oligodendrocytes of the central nervous system (CNS).[18] Certain cochlear and ocular cells, testicular Sertoli and Leydig cells, and epithelial cells of the epididymis and prostate also contain BTP.[18] In the heart, BTP has been localized to myocardial cells, atrial endocardial cells, synthetic state intimal smooth muscle cells, and fibrous plaques of atherosclerotic stenosed coronary arteries (but not within normal coronary arteries).[18,19] BTP has been described in vascular endothelial cells,[20] skin melanocytes and keratinocytes,[21,22] gastric mucosal epithelial cells,[23] bone osteoblasts,[24] and adipocytes.[25] Within the monkey kidney, BTP localizes to cells of the proximal tubules, loop of Henle, and glomerulus.[26] In most reports, BTP protein expression is accompanied by evidence that its messenger RNA (mRNA) is present, suggesting local BTP cell production. It therefore is less likely that the intracellular detection of BTP protein is a result of its sequestration from the extracellular milieu. The postulated and possible functions of BTP within these tissues are described in greater detail later.

BTP represents almost 3% of the total CSF protein[10] and is found in smaller concentrations in serum, breast milk, aqueous and vitreous humor, seminal fluid, and amniotic fluid.[27] Its high CSF concentration has made it attractive as a diagnostic marker of CSF leaks.[28]

BTP Function

The physiologic role of BTP remains incompletely understood, although it is thought to have a number of functions (Fig 2). Groups that have conducted studies in BTP knockout mice do not report abnormal growth and development, suggesting that it is not an essential housekeeping type of protein.[29-32]

BTP is a member of the lipocalin superfamily, which consists of a group of secretory proteins that bind and transport lipophilic molecules.[18] In vitro, BTP binds with high affinity to a variety of lipophilic substances such as retinoids, thyroid hormones, bilirubin, and amyloid β.[33-35] Studies examining its biological role as a binder and transporter in vivo are lacking. BTP knockout and knockin mice experience increased and decreased amyloid β deposition, respectively, compared with wild-type mice after intraventricular injection of amyloid β peptide, raising the question of whether disturbances in BTP are involved in the pathogenesis of Alzheimer disease.[32]

BTP also possesses enzymatic activities (Fig 2). It catalyzes the conversion of arachidonic acid derivative prostaglandin H_2 (PGH_2) to PGD_2.[18] PGD_2 is involved in a wide range of physiologic functions such as sleep induction and regulation, nociception, bronchoconstriction, adipocyte differentiation, nitric oxide release and induction of vasodilation, inflammatory mediator modulation, and inhibition of platelet aggregation.[36] Recent studies also suggest a role for BTP/PGD_2 in maintenance of skin homeostasis,[21] bone remodeling,[24] hair growth inhibition,[22] and immune system modulation in inflammatory conditions of the gastrointestinal tract.[23,37]

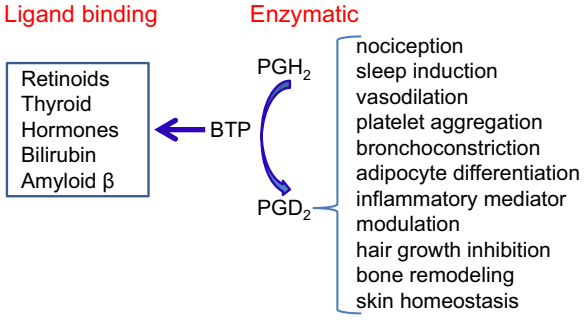

Figure 2. Dual functions of β-trace protein (BTP). BTP binds various lipophilic molecules and catalyzes the conversion of prostaglandin H_2 (PGH_2) to PGD_2. PGD_2 has a variety of physiologic functions.

PGD_2 is metabolized further to other prostanoids such as J series prostaglandins, identified to date only in vitro and therefore with a controversial biological role, and 9α11β-PGF_2, a mediator of bronchoconstriction.[36] Two PGD_2 receptors have been identified. The PGDR (DP1) receptor appears to be more widely expressed, whereas CRTH2 (DP2, or GPR44) is localized mostly to inflammatory cell types and skin.[36,38,39]

BTP (L-PGDS) must be differentiated from hematopoietic PGDS (H-PGDS). While both catalyze the conversion of PGH_2 to PGD_2, they are structurally and biochemically distinct and localize in different compartments.[36] H-PGDS is found in mast cells, T helper cells, Kupfer cells, dendritic cells, and microglia and appears to be more involved in allergic and inflammatory responses.[36]

Whether BTP effects on tissues are mediated by only locally produced BTP or also by circulating BTP is unknown and merits study.

Biosynthesis and Metabolism

Origin of Serum BTP

BTP generally is thought to have a constant production rate, but this has not been established using serial BTP urinary excretion studies. The origin of serum BTP has been hypothesized to result from diffusion of CNS BTP.[14] Oligosaccharide structural analysis reveals that the glycosylation patterns of the glycoforms seen in both serum and CSF are most typical for CNS-produced glycoproteins as opposed to hepatically produced glycoproteins.[14] According to this theory, CSF-produced BTP would diffuse from the CSF into plasma. The liver then would rapidly eliminate the nonsialylated "brain type" glycoforms by specialized receptors, leaving a predominance of the larger "blood/urine" sialylated glycoforms (Fig 3). This hypothesis is supported by canine studies in which intrathecally administered recombinant BTP was recovered from serum and urine.[40] However, in the only metabolic study of humans, the authors conclude that only 12% of serum BTP is derived from the CNS, using data from 2 patients with neurologic injuries who underwent intraspinal administration of radiolabelled BTP.[41] These data have not been reproduced using highly purified/recombinant BTP in healthy individuals.

Others have postulated that there is a significant cardiac source of plasma BTP based on autopsy studies revealing BTP expression in a variety of cardiac cell types, BTP mRNA expression in the human heart, and differential serum BTP concentrations across stenotic coronary lesions.[19] The induction of BTP mRNA expression in vascular endothelial cells in response to fluid shear stress in vitro[20] has led to

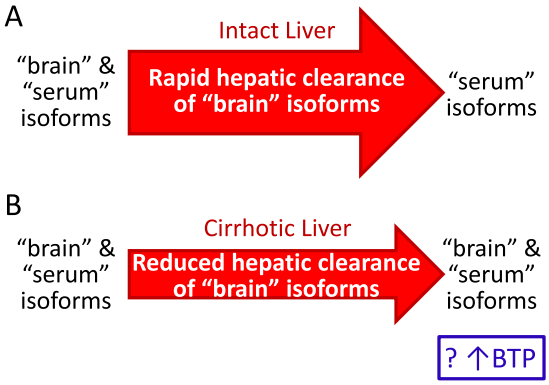

Figure 3. According to the central nervous system (CNS) origin theory, "brain" and "serum" isoforms are produced in the CNS and then traffic to the serum, where the liver rapidly metabolizes the smaller brain type isoforms, leaving the larger serum type intact. (A) Intact liver with only serum isoforms; (B) cirrhotic liver with accumulation of brain isoforms along with serum isoforms, potentially leading to higher serum concentrations depending on what epitopes the assay antibodies recognize. Abbreviation: BTP, β-trace protein.

the proposal of an endothelial source of serum BTP.[42] It is possible that serum BTP hails from a number of different cell types. Studies quantitating the amount of BTP that is spilled over into the circulation from different tissues have never been conducted. Given its biological functions, it is likely that production rates and spillover may vary over time and across disease states.

Renal Handling

Renal handling of BTP is uncertain. There are no studies comparing simultaneous urinary BTP and inulin clearance or any micropipette studies with direct sampling of differing nephron segments. These would help delineate renal BTP trafficking.

Despite this, almost all clinical reports state that BTP is freely filtered. BTP is much larger (26-29 kDa) than serum creatinine (113 Da) or cystatin C (13.4 kDa). It also is larger than myoglobin (17.8 kDa), a molecule that is poorly filtered. In a study performed in dogs (n = 5), only 10% of intravenously injected BTP was recovered in urine.[40] Conversely, in the only metabolic study of humans, 90% to 100% of radiolabelled BTP injected intravenously in 3 patients was recovered in urine, indicating near-total renal elimination and little if any tubular reabsorption or nonrenal elimination/metabolism.[41] The reasons for the discordance between the 2 studies are not known. The canine study used recombinant human BTP from transfected Chinese hamster ovary cells. The studies of humans used BTP isolated from pooled CSF of patients with neurologic disorders. The BTP isoforms in the 2 studies therefore were different and could have different filtration properties.

Its large size as compared with creatinine and other secreted anions/cations argues against BTP undergoing significant tubular secretion. Unlike cystatin C, BTP does not appear to undergo complete reabsorption by tubular cells and is detectable in urine of healthy individuals.[14,42-44] BTP is produced de novo in monkey kidney in both glomeruli and loop of Henle cells,[26] so some urinary BTP possibly could be produced locally. Further investigations into BTP renal handling are required.

BTP MEASUREMENT

BTP Assays

Much discussion has occurred over the years with respect to differences in creatinine assays, which has led to international standardization of the assay using reference materials traceable to the higher order method of isotope-dilution mass spectrometry. Similarly, reference materials are now available for serum cystatin C through the Institute for Reference Materials and Methods. No reference materials have been made available for BTP.

A variety of methods, including nephelometric, immunodiffusion, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence, have been developed over the years to quantitate BTP in biological fluids.[18] Nephelometric (Siemens) and ELISA (Cayman Chemical) methods are commercially available.

Nephelometric

The Siemens nephelometric assay has been used almost exclusively in recent studies.[7-9,16,45,46] Polystyrene particles coated with polyclonal rabbit antibodies against human BTP agglutinate when mixed with samples containing BTP,[47] scattering light; the intensity of this scattering is measured by the nephelometer. Serum concentration is determined by comparison with dilutions of a standard of known concentration. The polyclonal antibodies are obtained by injecting human urinary BTP into rabbits and purifying the rabbit serum by affinity to CSF BTP. Calibration of the assay is performed using stabilized urinary BTP derived from urine. A supplementary reagent containing rabbit immunoglobulin is used to suppress interference by rheumatoid factors.[47] The need for this is concerning, and it is unknown whether this is sufficient to eliminate interfering substances across all disease states.

The assay/product monograph does not detail which epitopes are recognized by the polyclonal antibodies. It is possible that some of the polyclonal antibodies recognize glycoprotein epitopes and therefore may not bind to all BTP isoforms.

The coefficient of variation (precision) for the BTP assay is acceptable, and, when reported, is consistently <6.5%.[48-52]

Immunometric ELISA

Fewer studies have used a commercial immunometric ELISA.[20,53] In the assay, microplate wells are coated with monoclonal anti-BTP antibodies derived in mice.[54] The biological fluid sample is added to the wells and any BTP contained within binds to the fixed antibody. Biotin-conjugated anti-BTP antibodies then are added, and these in turn will bind to any fixed BTP-antibody complexes. The wells then are incubated with horseradish peroxidase–conjugated streptavidin, the latter of which binds to biotin. A colorimetric indicator is added and oxidized by any bound horseradish peroxidase, leading to a color change that can be measured spectrophotometrically. Thus, the intensity of the color is proportional to the amount of BTP in the original fluid sample.

Analytical Concerns

There are no higher order reference methods or materials available for BTP. The 2 commercial assays use different analytical techniques and different antibodies. There is a high likelihood that significant differences may exist between the 2 assay types.

Important inter- and intralaboratory over-time differences in BTP assays also may exist even when using the same assay made by the same manufacturer, as was documented for Siemens' cystatin C assay before the advent of the reference materials.[55-58] This is a particular concern given the presence of multiple glycoforms that reasonably could be thought to increase the risk of between-lot differences in reagent antibodies. Split-sample studies looking at interlaboratory differences in serum BTP measurement have not been conducted.

The laboratory aspect of BTP has yet to generate much interest in the nephrology community. As outlined, there are a number of concerns related to BTP measurement that need to be addressed before confidence in its measurement can develop.

Reference Intervals

Reference ranges (covering 2.5th to 97.5th percentiles) for BTP frequently are cited to be 0.402 to 0.738 mg/L, undifferentiated by sex. These were derived from 200 apparently healthy blood donors using the Dade Behring Nephelometric assay.[59] However, the absence of higher order reference materials and standardization of the assay precludes the use of universal reference ranges and requires that locally and serially derived reference ranges be determined for each laboratory. Their absence also makes comparison of results between studies challenging.

NON-GFR DETERMINANTS

Similarly to other endogenous markers of GFR, it is clear that non-GFR determinants of serum BTP exist, with patients with the same BTP concentrations having dissimilar measured GFRs (mGFRs; Fig 4). Less is known about non-GFR determinants (demographic, biometric, clinical, and biological) of serum BTP compared to cystatin C or creatinine. A noted limitation of many of the available studies is the absence of a reference standard measure of GFR.

Demographic and Biometric

Insufficient data are available to comment on the impact of race on serum BTP levels. In one study of more than 200 adult kidney transplant recipients, women had an 11% lower BTP concentration than men (vs 19% for serum creatinine) after controlling for mGFR. Age was not associated independently with BTP concentration.[60] Above the age of 2, BTP is thought to be independent of age.[50,52] However, reference intervals for children consistently have a much higher cutoff (>1.0 mg/L) than those for adults (<0.8 mg/L).[50,52,59,61] In one study in which BTP was measured in the same laboratory and GFR by the same technique, the pediatric cohort mean BTP concentration was higher than that of the adult cohort despite a higher mean GFR.[46] Together, these suggest some effect of age, with higher levels in younger patients.[50,52,59,61]

Limited evidence suggests that muscle mass may affect BTP concentrations. BTP and GFR were measured in 27 children with spina bifida, and very poor correlation was noted between the 2.[62] In the mentioned transplant cohort, body mass index was not associated independently with BTP concentration.[60]

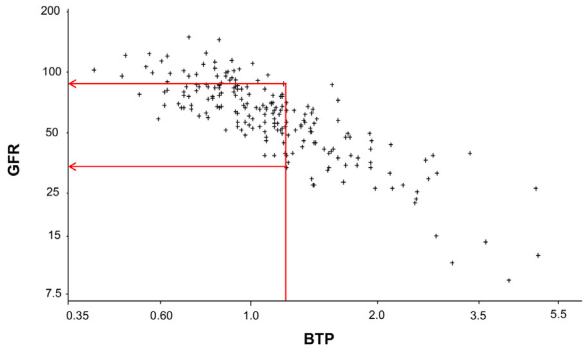

Figure 4. Glomerular filtration rate (GFR; mL/min) measured using ^{99m}Tc-DTPA in 200 kidney transplant recipients. Scatter is observed when β-trace protein (BTP; mg/L) is plotted against GFR on a logarithmic scale, as patients with the same BTP concentration have dissimilar measured GFRs, indicating substantial influence of non-GFR determinants on serum BTP concentrations. Adapted from White et al[60] with permission of the National Kidney Foundation.

Medications

A number of medications are known to affect serum creatinine levels through both biological and analytic mechanisms.[2] Very little is known about the effect of medications on BTP. In the transplant cohort, none of the commonly used immunosuppressive medications (low-dose prednisone, mycophenolate mofetil, cyclosporine, or tacrolimus) affected serum BTP.[60] The lack of impact of low-dose steroids on serum BTP levels was reported in another transplant cohort.[48] A dose-dependent reduction in serum BTP levels with relatively high doses of steroids has been described in pediatric patients after accounting for mGFR.[63] In rats, glucocorticoid exposure significantly increases BTP expression in cardiomyocytes, an effect also seen in vitro.[64] Serum levels were not reported in that study. In mice, administration of estrogen is reported to increase myocyte BTP expression; again, serum levels were not provided.[65]

Genetic Factors

Tin et al[66] conducted a genome-wide association study of plasma BTP in participants enrolled in the ARIC (Atherosclerosis Risk in Communities) Study (6,720 Americans of European ancestry and 1,734 Americans of African ancestry). They identified a significant locus upstream of the BTP gene that accounted for 5% and 6% differences in serum BTP levels in European Americans and African Americans, respectively.[66] The biological mechanisms by which the single-nucleotide polymorphisms found at the locus might lead to variations in serum BTP levels remain unknown to date.

Clinical Conditions and Factors

Few studies have examined the relationship between nonrenal systemic diseases and serum BTP levels. In vitro, recombinant BTP binds to both L-thyroxine (T_4) and 3,5,3′-triiodothyronine (T_3),[35] and its gene promotor is upregulated by T_3.[67] In developing rats, chemically induced hypothyroidism decreases CNS BTP mRNA levels.[68] Studies of humans of the impact of thyroid dysfunction and hormone supplementation on serum BTP levels have not been performed. Evidence of the effect of CNS disease on CSF BTP levels is not consistent and is altogether lacking with respect to serum BTP levels.

Of interest is the potential impact of hepatic dysfunction on serum BTP levels. If the liver clears nonsialyated "brain type" BTP isoforms, the presence of hepatic dysfunction would lead to these persisting in serum. Serum BTP concentrations would be higher than expected at any given GFR. This possibility has never been studied.

In one study, lower serum albumin levels were found to be associated independently with higher BTP concentrations, raising the question of whether inflammation influences BTP levels, as has been described for serum cystatin C.[60] This seems likely given that it catalyzes the production of PGD_2, which has a known role in immune system modulation. Further investigation into the role of inflammation on serum BTP levels is required.

The discovery that osteoblasts both produce PGD_2 and express PGD_2 receptors has led to interest in the role of BTP in bone remodeling.[24] In one study, serum BTP levels were higher in individuals (n = 35) 5 weeks postfracture (0.613 ± 0.06 mg/L) compared with age-, sex-, and weight-matched controls (0.536 ± 0.03 mg/L).[53] Serial BTP measurements were not done to examine the trend over time in individuals with fractures. In addition, no other measure of kidney function was reported to allow a comparison of kidney function between the 2 groups and a better understanding of the significance of the higher BTP levels in the fracture group. The authors postulated that BTP and PGD_2 are important modulators of bone anabolism.[53]

Serum BTP levels also have been reported to be higher (0.73 ± 0.06 mg/L) in patients with obstructive sleep apnea and excessive daytime sleepiness compared with healthy controls (0.62 ± 0.02 mg/L) and patients with obstructive sleep apnea but no excessive daytime sleepiness (0.58 ± 0.03 mg/L).[69] These observations led the authors to hypothesize that excessive production of PGD_2 and BTP is involved in the pathogenesis of excessive daytime sleepiness in obstructive sleep apnea. Again, no other marker of kidney function was provided in this study.

Curiously, serum BTP levels have been studied in the context of wireless telephone emissions.[70] Because electromagnetic fields in the radiofrequency range are associated with disturbed sleep in some studies and BTP/PGD_2 promote sleep induction, the authors queried whether disordered sleep in the presence of electromagnetic fields in the radiofrequency range was mediated through BTP. They found that serum BTP concentrations were associated negatively with number of years using a cellular telephone (β coefficient, −0.32). No other marker of kidney function was measured. A 30-minute exposure to electromagnetic fields emissions did not increase serum BTP levels.

Further discussion focusing on the relationship between BTP and cardiovascular disease is provided later.

CLINICAL AND DIAGNOSTIC UTILITY

BTP as a Marker of Kidney Function

A number of studies have examined the relative ability of BTP compared to creatinine in assessing GFR using a variety of different statistical techniques.

Early studies mostly performed receiver operating curve analyses to examine the ability of BTP to detect various different GFR cutoff points in a variety of patient groups, including kidney transplant recipients, children, and patients with CKD. Some found that BTP level was better at detecting reduced GFR than serum creatinine level,[52,71,72] whereas others did not.[48,59,61] One study of patients with early diabetic nephropathy reported higher serum BTP concentrations in patients with macroalbuminuria and normal kidney function (based on serum creatinine level) compared with those with microalbuminuria and normal kidney function. However, serum creatinine concentrations were similar in the 2 groups.[73] Similarly, Poge et al[48] demonstrated in kidney transplant recipients that BTP had a higher proportional increase above the upper limit of normal than serum creatinine at all levels of GFR. This was especially notable in early GFR decline, in which the creatinine curve remained flat while the BTP curve demonstrated an earlier increase in serum concentrations when GFR decreased to <60 mL/min/1.73 m^2.[48]

BTP Estimation Equations

Adults

Several equations (4 adult and 4 pediatric) have been developed to translate a serum BTP concentration into an estimate of GFR (Table 1).[49,51,63,74]

The adult equations were derived in kidney transplant recipients at 2 different centers.[49,51] Each center developed 2 equations that, in addition to BTP, included either serum urea or serum creatinine level (Box 1). Each center validated the equations in independent local populations who were not part of the equation development data set and found that their own equations were more accurate than the MDRD (Modification of Diet in Renal Disease) Study equation and the BTP equations developed at the other center[46,49] (Table 2). The equations also were evaluated in a small cohort of 36 patients with Fabry disease and well-preserved GFRs.[75] None of the BTP equations was superior to the MDRD Study equation.[75] The discrepancies between studies likely are the result of differences in calibration of the BTP assay, reference standard GFR measurement techniques, and patient populations. These studies highlight the importance of external equation validation.

At present, there is no clear advantage of the BTP equations over the traditional creatinine-based equations in adults. Equation imprecision remains suboptimal in all studies, suggesting substantial non–GFR-dependent influences on serum BTP concentrations, as similarly seen in creatinine-based equations. Furthermore, until BTP assay standardization is established, performance of any BTP equation likely will remain heterogeneous.

Children

In the pediatric population, GFR estimation is problematic. The traditional creatinine-based Schwartz equation is not very accurate.[76,77] An updated Schwartz equation has been developed[78] and showed similar accuracy (percent of estimates within 30% of mGFR) to the BTP White equation in a cohort of pediatric patients with CKD (Table 2).[46]

A BTP equation has been developed from 85 children not receiving steroids (Table 1).[63] The accuracy of this equation was reported to be much lower in an independent group on steroid therapy (66%) compared to an independent steroid-free group (82%; Table 3). This raises the question of a potential effect of steroids on serum BTP concentrations, as discussed previously.[63] This equation has not been externally validated.

Table 1. BTP Equations

Study	BTP Equations	Development Cohort
	Adult Equations	
Poge[49]	$GFR1 = 89.85 \times BTP^{-0.5541} \times urea^{-0.3018}$ $GFR2 = 974.31 \times BTP^{-0.2594} \times Scr^{-0.647}$	85 adult kidney transplant recipients; mean age, 50 (47-52) y; 60% men; mean GFR, 39 (35-42) mL/min/1.73 m^2
White[51]	$GFR1 = 112.1 \times BTP^{-0.662} \times urea^{-0.280} \times$ (0.880 if female) $GFR2 = 167.8 \times BTP^{-0.758} \times Scr^{-0.204} \times$ (0.871 if female)	163 adult kidney transplant recipients; mean age, 53±12 y; 67% men; 90% white; mean GFR, 59 ± 23 mL/min/1.73 m^2
	Pediatric Equations	
Abbink[63]	$GFR = -35.20 + (122.74 \times BTP^{-0.5})$	85 children (steroid free); mean age, 10±6 y; 67% male; mean GFR, 90±33 mL/min/1.73 m^2
Benlamri[74]	$GFR = 10^{(1.902 + (0.9515 \times \log(1/BTP)))}$	474 repeated measures in 367 children (steroid free); mean age, 11 ± 7 y; 66% male; median GFR, 105.5 mL/min/1.73 m^2

Abbreviations: BTP, β-trace protein; GFR, glomerular filtration rate; Scr, serum creatinine.

Table 2. Performance of GFR Estimation Equations in Independent Adults Who Were Not Included in Equation Development

Study	Mean Bias	Precision	Accuracy
4-Variable MDRD Study[109]			
Poge[a,49]	3.4	10.8	88%
White[b,46]	−9.0	12.1	76%
Rombach[c,75]	3.7	NA	78%
Poge BTP1[49]			
Poge[a,49]	0.43	9.5	80%
White[b,46]	−11.0	11.3	76%
Rombach[c,75]	−28.3	NA	42%
Poge BTP2[49]			
Poge[a,49]	−2.1	9.9	79%
White[b,46]	−13.3	12.4	65%
White BTP1[49]			
Poge[a,49]	9.4	10.8	61%
White[b,46]	−1.5	10.6	89%
Rombach[c,75]	−16.8	NA	71%
White BTP2[49]			
White[b,46]	−1.7	10.5	90%

Note: Bias was defined as the difference between estimated and measured GFR (estimated GFR − measured GFR). Precision was defined as the standard deviation of the mean bias. Both precision and bias were expressed as mL/min/1.73 m^2. Accuracy was defined as the proportion of estimates that were within 30% of measured GFR.

Abbreviations: BTP, β-trace protein; CI, confidence interval; GFR, glomerular filtration rate; MDRD, Modification of Diet in Renal Disease; NA, not available.

[a]Characteristics of validation cohort: 102 adult kidney transplant recipients; mean age, 50 (95% CI, 46-50) years; 60% men; mean GFR, 39 (95% CI, 35-42) mL/min/1.73 m^2.

[b]Characteristics of validation cohort: 92 kidney transplant recipients; mean age, 53 ± 12 years; 68% men; 95% white; median GFR, 51 (interquartile range, 28) mL/min/1.73 m^2.

[c]Characteristics of validation cohort: 36 adults with Fabry disease and repeated measures (total n = 136) over median of 3.1 years; median age, 46 years; 56% men; median GFR, 89 mL/min/1.73 m^2.

Table 3. Performance of GFR Estimation Equations in Children

Study	Mean Bias	Precision	Accuracy
Schwartz[110]			
White[a,46]	18.6	17.0	49%
Benlamri[b,74]	—	—	71%
Updated Schwartz[78]			
White[a,46]	−4.9	13.1	85%
Poge BTP1[49]			
White[a,46]	−18.4	14.2	50%
Poge BTP2[49]			
White[a,46]	−9.5	16.2	72%
White BTP1[51]			
White[a,46]	−8.6	14.4	78%
White BTP2[51]			
White[a,46]	−7.1	16.1	83%
Benlamri[74]			
Benlamri[b,74]	—	—	72%
Abbink[63]			
Abbink (steroids)[c,63]	—	—	67%
Abbink (no steroids)[d,63]	—	—	82%

Note: Bias was defined as the difference between estimated and measured GFR (estimated GFR − measured GFR). Precision was defined as the standard deviation of the mean bias. Both precision and bias were expressed as mL/min/1.73 m^2. Accuracy was defined as the proportion of estimates that were within 30% of the measured GFR.

Abbreviations: BTP, β-trace protein; GFR, glomerular filtration rate.

[a]Characteristics of validation cohort: 54 children; mean age, 10 ± 5 years; 69% male; median GFR 69 (interquartile range, 38) mL/min/1.73 m^2.

[b]Characteristics of validation cohort: 103 children with repeated measures (total n = 125); mean age, 10 ± 5 years; 61% male; mean GFR 96 ± 45 mL/min/1.73 m^2.

[c]Characteristics of validation cohort: 32 children on steroid therapy; mean age, 11 ± 5 years; 60% male; mean GFR 108 ± 37 mL/min/1.73 m^2.

[d]Characteristics of validation cohort: 76 steroid-free children; mean age, 9 ± 6 years; 75% male; mean GFR 87 ± 32 mL/min/1.73 m^2.

Additional pediatric BTP equations were derived from a heterogeneous cohort of 374 children with various kidney pathologies and then validated in a smaller independent cohort of 103 patients.[74] Separate equations were presented for males and females along with a third gender-neutral equation (Table 1). Only 72% of estimates using the gender-neutral formula were within 30% of mGFR, which was very similar to that of the Schwartz equation, for which the value was 71% (Table 3). The gender-specific equations did not confer an incremental benefit.[74]

BTP-based GFR estimation equations in children are not yet at a stage at which they can be recommended for clinical use. The same issues with respect to assay standardization apply for the pediatric equations as for the adult equations.

Residual Kidney Function

Residual kidney function is associated with better survival in both hemodialysis (HD) and peritoneal dialysis patients.[79,80] Its preservation is an important area of clinical research. The traditional methods of quantifying GFR are highly problematic in dialysis-dependent stage 5 CKD.[81] For both peritoneal dialysis and HD, protocols have been developed to estimate residual GFR using urine collections and calculating the average of urea and creatinine clearance over various times, but none is ideal or proved to be accurate.[81]

In the original report identifying elevated serum BTP concentrations in end-stage renal disease (ESRD), BTP was identified (although not quantitated)

in the hemofiltrate of HD patients, indicating some clearance using conventional HD.[14] The authors proposed hemofiltrate as a potential source of BTP.[14] Gerhardt et al[82] compared serum BTP concentrations pre- and post-HD using high-flux membranes (correcting for body weight loss) and found only a slight reduction ($-0.6\% \pm 16.1\%$) in serum concentrations after HD. BTP in the hemofiltrate was not measured. They conclude that because of BTP's higher molecular weight, it is not cleared to a great extent by conventional HD, making it an interesting candidate as a marker of residual kidney function.[82,83]

Two studies have examined the association between serum BTP concentrations and residual diuresis in HD patients.[16,82] Both demonstrated significantly lower serum concentrations of BTP in patients with higher residual urine output.[16,82] Neither study included measures of residual GFR. To the authors' knowledge, BTP clearance in peritoneal fluid has not been examined.

Pregnancy

BTP does not appear to be an appropriate marker of GFR in pregnancy. Despite hyperfiltration, levels are unchanged during the first and second trimesters and then increase during the third trimester.[84,85] It has been hypothesized that this could be due to reduced glomerular sieving for medium-sized molecules such as BTP with increasing GFR.[84] Dynamic alterations in glomerular permeability in response to alterations in GFR are supported by studies of both humans and rats.[86-88] Alternatively, this could reflect fetal/placental BTP production with spillover into the maternal circulation.

Marker of Tubular Dysfunction

Low-molecular-weight proteins such as cystatin C, β_2 microglobulin, and α_1 microglobulin are considered early urinary markers of tubular dysfunction. Freely filtered, these are reabsorbed and metabolized by proximal tubular cells and therefore are absent from urine in individuals without tubular dysfunction.[43] BTP also has been investigated as a marker of proximal tubular damage.[43,44,89] In one study, urinary BTP levels were found to be significantly higher in a CKD cohort than in an apparently healthy cohort (3.93 vs 2.02 mg/d).[43] Similar findings were seen in hypertensive patients without evidence of kidney disease and in patients with early diabetic nephropathy compared with healthy controls.[42,44]

It should be noted that unlike cystatin C, β_2 microglobulin, and α_1 microglobulin, BTP does not undergo significant tubular reabsorption in healthy tubules, which argues against tubular dysfunction as the sole explanation for increased urinary levels.[14,43] Increased glomerular permeability potentially could account for increased urinary levels if BTP is not freely filtered.[26,44] Also, BTP has been shown to be produced de novo in monkey kidney in both glomeruli and loop of Henle cells.[26] Increased urinary BTP could result from increased local production in the setting of physiologic stress, with spillover into the collecting system independent of changes in GFR or tubular function. Further, from a purely practical standpoint, unlike cystatin C, BTP is always present in urine, which diminishes its comparative utility.

Association With ESRD

Studies also have looked at associations of BTP with "hard" kidney outcomes such as ESRD or doubling of serum creatinine level. In one study of 177 nondiabetic patients with CKD followed up for up to 7 years, there was no difference between BTP and creatinine levels in predicting kidney function decline (defined by doubling of serum creatinine or ESRD) by area under the curve analysis, with C statistics of 0.899 and 0.888, respectively.[45] There also was no difference between the 2 in terms of correlation with mGFR and ability to detect reductions in mGFR at various cutoff levels. In a follow-up study of 816 participants enrolled in the MDRD Study (median follow-up, 16.6 years), the reciprocal of BTP concentration (hazard ratio [HR], 2.19) was associated less strongly with ESRD than the reciprocal of serum creatinine level (HR, 2.26).[9] Conversely, in the African American Study of Kidney Disease (AASK), ESRD was associated more strongly with serum BTP concentrations than either creatinine or cystatin C.[8]

Most of these studies have focused on patients with established CKD. One of the difficulties with serum creatinine has been its relative stability in the face of early GFR decreases attributed to increased creatinine secretion and decreased creatinine generation, referred to as the "creatinine blind region." The ARIC Study, a large observational cohort study of the US general population followed up for 10 years, sheds some light on patients with near-normal GFRs.[7] At baseline, patients were divided into quintiles by CKD-EPI (CKD Epidemiology Collaboration) estimated GFR (eGFR) and by BTP concentration. Patients in the fifth quintile then were subdivided further into tertiles. Overall, BTP level was associated more strongly with ESRD than serum creatinine level. In the group of patients with a CKD-EPI eGFR of $\sim$60 mL/min/1.73 m^2 (quintiles 5b and 5c), BTP level showed a stronger and more graded association with ESRD than did the CKD-EPI eGFR.[7] This is in keeping with earlier studies indicating a role for BTP in early recognition of declining GFR.[48,73] β_2 Microglobulin and cystatin C levels also were shown to be superior to serum creatinine level. The results of this study are of potential great interest given the

importance now placed on early detection as a means to reduce the burden and prevent the extreme morbidity and mortality of more advanced CKD.[90]

Beyond GFR: Cardiovascular Disease and Death

In recent years, increasing attention has been paid to associations between markers of GFR and non–kidney disease outcomes such as cardiovascular events and death.

In a study of 1,000 patients with angina who underwent diagnostic coronary angiography, BTP level was a stronger independent predictor of coronary severity score than any other risk factor or biomarker studied, including diabetes, hypertension, total cholesterol level, age, and smoking.[91] No other marker of kidney function was examined in the analysis, and patients with serum creatinine concentrations > 1.2 mg/dL were excluded. The authors concluded that BTP level is suitable to be used in the evaluation of the severity of ischemic heart disease, although the incremental benefit over serum creatinine level in this application was not demonstrated.[91] BTP concentrations also were examined in the context of atrial fibrillation and warfarin therapy and outcomes such as death, cardiovascular events, and bleeding in 1,279 patients.[92] Adding a point for high BTP level (defined differently for each outcome) was found to improve the predictive ability of the $CHADS_2$ and HAS-BLED scores for cardiovascular outcomes and bleeding, respectively, although C statistics remained suboptimal (0.63-0.69).[92] A comparative analysis was not done using serum creatinine instead of BTP level.

A number of recent epidemiologic studies have examined the association between BTP level and non–kidney disease outcomes. Astor et al[7] showed that BTP levels associated more strongly with coronary heart disease, heart failure, and death than the creatinine-based CKD-EPI eGFR in the ARIC Study described previously. Stronger associations with both all-cause and cardiovascular mortality were found for BTP compared with creatinine level in 6,445 adults enrolled in the Third National Health and Nutrition Examination Survey (NHANES III) study.[6] Shafi et al[16] measured serum BTP in 503 incident HD patients and found it to be associated with both all-cause and cardiovascular mortality. BTP, but not serum creatinine, also was reported to be associated with death and rehospitalization in 220 patients admitted with decompensated heart failure.[93] However, the discriminatory ability as determined by receiver operating curve analysis was not statistically different between the 2 markers (C statistic of 0.62 for BTP vs 0.58 for serum creatinine; $P = 0.43$).[93]

Two studies have attempted to delineate whether BTP level's stronger association with outcomes than creatinine level is a result of it providing a more accurate estimate of kidney function by examining associations between outcomes and BTP after adjusting for mGFR. In AASK, mortality was associated more strongly with serum BTP concentrations than any of the other markers studied, including serum creatinine and cystatin C, and this persisted after correction for mGFR (determined using urinary iothalamate).[8] Interestingly, in unadjusted analyses, BTP level was correlated even more strongly with mortality than mGFR.[8] In the follow-up to the MDRD Study, BTP level (HR, 1.33) was associated more strongly with mortality than serum creatinine level (HR, 0.82) after adjustment for mGFR (determined using renal iothalamate clearance).[9] Similarly to the previous study, the unadjusted association with death was stronger for the reciprocal of BTP concentration (HR, 1.33) than was mGFR (HR, 1.27).[9]

Together, this evidence suggests that the increased risk associated with BTP is beyond that which can be explained by the known association between GFR and poor outcomes. Alternate determinants of serum BTP, such as pathophysiologic processes associated with adverse events, likely exist. Further examination of the biological functions of BTP may help clarify the relationship between BTP level and nonkidney outcomes.

BACK TO BIOLOGY

BTP is an enzyme involved in diverse important physiologic pathways (Fig 2). From a cardiovascular perspective, BTP promotes vasodilation,[94] inhibits platelet aggregation,[95,96] and modulates inflammation[23,97] and adipogenesis.[98] As such, it and its downstream elements reasonably could be postulated to be upregulated in pathophysiologic processes associated with adverse outcomes. There are at least 2 possible and opposing mechanisms to consider. BTP, through its biological activity, may be pathogenic and contribute to tissue injury or, conversely, might confer protection against ongoing tissue damage, with elevated levels reflecting a beneficial response to injury (Fig 1). A number of in vitro and in vivo studies have shed some light on this. Unfortunately, most of these studies do not provide serum BTP concentrations, focusing instead on tissue-level production, which hampers our ability to draw firm conclusions about the relationship between serum levels and biological processes.

BTP expression is observed in human atherosclerotic lesions.[19,99] Angiographic studies reveal higher serum BTP concentrations in the great cardiac vein than at the orifice of the left main coronary artery in patients with significant stenosis; this is not observed in patients without stenosis, suggesting increased local production by diseased coronary arteries.[19] This

could be an adaptive protective response in stenotic lesions to prevent thrombosis by impeding platelet aggregation, improve perfusion by promoting vasodilation, or reduce a pathogenic inflammatory response. Alternatively, the increase itself might contribute to the genesis of the lesion through unclear mechanisms.

In mice, chronic hypoxemia results in a 2-fold increase in myocyte BTP mRNA production and BTP protein expression.[100] Intravenous administration of dexamethasone also increases murine myocyte BTP production.[64] In ischemia-reperfusion experiments, dexamethasone reduces infarct size in wild-type mice, but not in BTP-deficient mice, suggesting that the protective effect of glucocorticoids in hypoxia is mediated through BTP.[64] Together, these data suggest a protective role of BTP in hypoxic injury. The authors did not advance a mechanistic theory, although modulations of the acute inflammatory response or other mechanisms such as vasodilation are attractive candidates.

In one in vitro study, there was a 3-fold elevation in BTP production in endothelial cells in response to shear stress, with a resulting 3-fold elevation in PGD_2 levels.[20] Laminar shear stress is thought to stimulate endothelial responses that are considered atheroprotective.[101] These include alterations in coagulation, leukocyte and monocyte migration, smooth muscle growth, lipoprotein uptake and metabolism, and endothelial cell survival.[101] Conditions of low laminar shear stress, as seen in turbulent blood flow, are thought to contribute to atherogenesis.[101] Thus, BTP and PGD_2 may contribute to the protective endothelial adaptations seen in conditions of high shear stress.

In another animal study, mice exposed to 3 weeks of oral estrogen therapy were reported to have a 4-fold increase in cardiac BTP mRNA and a 3-fold increase in cardiac BTP protein production compared with control mice.[65] Conversely, oophorectomized mice were observed to have significantly lower cardiac BTP mRNA and protein levels compared with controls. Serum levels were not provided.[65] The authors identified an estrogen response element in the BTP promoter region that in vitro is upregulated by the estrogen β receptor. This receptor is found at high concentrations within blood vessels and cardiac cells. These data suggest that the presumptive cardioprotective effect of estrogen may be mediated in part through increased local BTP production.

Further important information is provided by studies using BTP knockout mice. When fed a high-fat diet, these animals develop increased subcutaneous and visceral fat tissue volume, increased aortic wall fat deposition, increased aortic root atherosclerotic plaque area and macrophage cellularity, and increased expression of proinflammatory cytokines compared with wild-type mice.[102] Another group has described accelerated insulin resistance, aortic thickening, glomerular hypertrophy and fibrosis, and GFR reduction in BTP knockout mice fed a high-fat diabetogenic diet.[30] Together, these animal studies directly implicate BTP and its downstream effectors as protective elements against cardiovascular disease development. The latter study also links BTP to the prevention of kidney injury.[30]

Ito et al[103] examined the effect of unilateral ureteric obstruction, performed to induce primary tubular epithelial injury, on intrarenal BTP production and interstitial fibrosis development in BTP knockout mice. They described increased intratubular cell expression of BTP in wild-type mice after obstruction. In knockout mice, BTP expression remained undetectable as expected and the mice had decreased interstitial fibrosis and decreased production of T-cell–derived cytokines compared with wild-type mice. Similar biopsy findings were seen when the wild-type mice were treated with a CRTH2 PGD_2 receptor antagonist. Here, the authors concluded that BTP contributes to interstitial fibrosis by activating T cells and the production of proinflammatory cytokines. The conflicting results of the 2 knockout studies[30,103] could be the result of differing mechanisms of kidney injury (hyperglycemia with primarily glomerular injury vs obstruction with primarily tubulointerstitial injury). Differences in responses also might be related to the relative importance of the 2 PGD_2 receptors (DP1 and CRTH2) in the local environment. That is, stimulation of the different receptors may exert different local effects. This has not been well studied.

THERAPEUTIC APPLICATIONS AND DRUG SAFETY

We are not aware of ongoing development of BTP potentiators as therapeutic agents, although this could be of interest, particularly in cardiovascular disease. However, an oral BTP inhibitor, AT-56, has been developed and proposed as a potential antisomnolence agent.[104] A number of PGD_2 receptor antagonists have been introduced or are currently under active development by a variety of pharmaceutical companies for the treatment of asthma, chronic obstructive pulmonary disease, and allergic rhinitis and to counteract the facial flushing experienced with niacin.[105] More detailed descriptions of these compounds have been reviewed elsewhere.[105] Topical PGD_2 inhibitors have been proposed as a treatment for male-pattern baldness.[22]

These compounds would have the potential to affect serum BTP levels, and this would need to be considered if their use becomes more widespread and BTP becomes more commonly used as a marker of GFR. From a safety perspective, these compounds

likely would affect not only the desired therapeutic pathway, but also the multiple other functions of PGD_2, leading to potential adverse side effects. In particular, cardiovascular safety will need to be given significant attention.

CONCLUSION

GFR estimation using endogenous markers is an evolving area of clinical practice and clinical research. Serum creatinine level, the historical favorite, has been replaced by creatinine-based GFR estimation equations. These in turn are likely to be supplanted by more accurate equations that include cystatin C level.[90] More recent interest in BTP and other novel endogenous markers likely will lead to consideration of their inclusion into multiple-marker equations to further reduce equation imprecision.[6]

In nephrology, as in all areas of medicine, the most appropriate diagnostic test is determined by the information the clinician or researcher desires. In some instances, such as clinical trials, CKD diagnosis when this is uncertain, and potential kidney donor assessment, accurate assessment of GFR is required. In others, when patient prognosis rather than an exact GFR is of interest, a marker with greater ability to predict outcomes may be more appropriate.[106] The research community has become increasingly interested in outcome prediction with incorporation of additional variables such as albuminuria, demographic characteristics, and other analytes into CKD patient risk assessment.[90,107,108] Predictive models may become refined further with inclusion of markers such as BTP and others.

The evidence accumulated to date consistently shows a stronger association between BTP levels and adverse outcomes than creatinine levels, which likely is related in part to its involvement in prostaglandin biosynthesis. Human, animal, and in vitro studies reveal that its production is influenced by a variety of pathophysiologic processes and much of the accumulated evidence suggests a protective role for BTP, with increased levels likely reflecting beneficial biological adaptations to injury. Thus, BTP should not be considered narrowly as a marker of GFR, but rather as an integrated marker of GFR and tissue injury. Whether elevated circulating BTP levels due to decreased renal elimination influences biological processes at the tissue level is an interesting concept that has not been explored.

A note of caution needs to be injected into discussions about BTP. Unlike serum creatinine and even cystatin C, knowledge about BTP's production and renal and nonrenal handling is rudimentary. Critically, significant issues with respect to its multiple isoforms, related measurement issues, and absence of higher order reference materials exist and need to be resolved. The nephrology community should use the lessons learned from the analytical difficulties it has experienced with creatinine and cystatin C before the marker becomes more widely applied. Much money and energy was expended on studying creatinine and cystatin C, with the resulting data in part difficult to interpret once analyte standardization came into force.

"Beta trace protein" is not yet a MeSH heading, and a MEDLINE search using it as a keyword fails to identify many articles in which L-PGDS is the preferred terminology. Until very recently, most renal epidemiologic and clinical studies mentioned only in passing (if at all) the biological roles of BTP. Conversely, most basic science studies fail to acknowledge the impact of decreased kidney function on serum BTP concentration. The former studies almost always use BTP, whereas the latter almost exclusively refer to the compound as L-PGDS, which further exacerbates the disconnect between the 2 research communities. Much remains to be learned about BTP (Box 2). Improved understanding of the clinical utility of BTP will require significant collaboration between basic scientists, clinical chemists, clinical researchers, and renal epidemiologists.

Box 2. Areas of Uncertainty and Related Questions

- *BTP production:* Where is circulating BTP produced and by what cell type(s)?
- *BTP renal handling:* How does the kidney handle BTP?
- *BTP measurement:* Are the 2 commercial assays equivalent across the spectrum of health and disease? What would be considered an appropriate reference standard?
- *Non-GFR determinants:* What demographic, biometric, clinical, and genetic factors and medications impact on serum BTP concentrations?
- *GFR estimation:* Can estimation equations that include BTP (alone or multiple marker) be developed that are more accurate than current equations?
- *Outcome prediction:* How can BTP be used to identify patients at risk of poor outcomes, renal and otherwise?
- *Early CKD detection:* Can/should BTP be used to identify early CKD?
- *BTP function:* What are the biological roles of BTP in pathogenic processes? Does decreased kidney function with decreased BTP excretion lead to modulation of biological mechanisms that are BTP dependent?
- *Therapeutics:* Could modulation of BTP production or function using pharmacologic agents impact on the development of cardiovascular or other disease?

Abbreviations: BTP, β-trace protein; CKD, chronic kidney disease; GFR, glomerular filtration rate.

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