

# $\beta$ -Trace Protein: From GFR Marker to Cardiovascular Risk Predictor

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## Summary

$\beta$ -trace protein, also known as Lipocalin type prostaglandin D synthase, is a low-molecular mass glycoprotein (between 23,000 and 29,000 Da depending on the degree of glycosylation) that converts prostaglandin H2 into prostaglandin D2.  $\beta$ -trace protein was initially isolated from cerebrospinal fluid and served as a marker of cerebrospinal fluid leakage; however, its cDNA and gene have been isolated in numerous human body tissues, including central nervous system, retina, melanocytes, heart, and male genital organs. In recent years,  $\beta$ -trace protein has emerged as a promising novel endogenous marker of GFR, representing a more sensitive marker for mild kidney dysfunction than serum creatinine. In this regard,  $\beta$ -trace protein has been proposed as an alternative marker to Cystatin C for measuring kidney function. Beyond its role for estimating renal function,  $\beta$ -trace protein is also emerging as a novel biomarker in cardiovascular risk. It has been associated with several cardiovascular disorders, playing a potential role for prognostic stratification in patients with acutely decompensated heart failure and acute coronary syndromes and being advocated as a novel marker for cardiovascular risk prediction.

*Clin J Am Soc Nephrol* 8: 873–881, 2013. doi: 10.2215/CJN.08870812

## Introduction

Prostaglandin D synthase (PGDS; prostaglandin H2 D-isomerase [EC 5.3.99.2]) catalyzes the isomerization of prostaglandin H2, a common precursor of various prostanoids, to produce prostaglandin D2 (PGD<sub>2</sub>) in the metabolism of AA (1). Two distinct types of PGDS have evolved from phylogenetically distinct protein families (1). The first is hematopoietic PGDS, which belongs to the  $\sigma$ -class of GSH S-transferases and requires glutathione for its function (2), and the second is a glutathione-independent enzyme called lipocalin-type PGDS, also known as  $\beta$ -trace protein (BTP) (2). Although hematopoietic PGDS and BTP catalyze the same reaction, they are quite different in terms of catalytic properties, amino acid sequence, tertiary structure, evolutionary origin (2), cellular localization, tissue distribution, and functional relevance (1). BTP is a monomeric glycoprotein with 168 amino acids and an estimated molecular mass between 23,000 and 29,000 Da, depending whether it is N-glycosylated at two positions, Asn51 and Asn78 (1).

Clinically, BTP was first isolated from rat cerebrospinal fluid and has been used as a marker of cerebrospinal fluid leakage, because it represents approximately 3% of total cerebrospinal fluid protein (3). More recently, BTP has been found to be expressed in the brain, retina, melanocytes, male genital organs (4), heart (5), and kidney (6), and it is secreted into various body fluids, such as cerebrospinal fluid, seminal plasma (4), plasma (5), and urine (6). BTP plays a role in the inhibition of platelet aggregation and induction of vasodilatation and bronchoconstriction, and it acts as an allergic and inflammatory

mediator (7). In addition, BTP binds with high affinity to various lipophilic compounds, such as retinoid acid, bilirubin, biliverdin, thyroid hormones, gangliosides, and amyloid- $\beta$  peptides (8), acting as an extracellular transporter of these compounds and serving as an endogenous amyloid- $\beta$  chaperone to prevent amyloid deposition *in vivo*. This way, BTP can be considered a dual function protein, acting as a PGD<sub>2</sub>-producing enzyme within cells and functioning as a lipophilic ligand-binding protein after its secretion.

Beyond these biologic roles, it has been shown that BTP might serve as an alternative endogenous marker for GFR (9); in this regard, BTP has been shown to be a more sensitive marker for small renal impairment than creatinine serum concentrations. Moreover, BTP has been proposed as an alternative marker to Cystatin C (Cys C) for estimating GFR in renal transplantation patients (9).

More recently, BTP is emerging as a novel biomarker in cardiovascular risk. Different studies in human and animal models have clarified a protective role of BTP in heart under hypoxia and ischemia (4,10); the stabilization of the atherosclerotic plaque (11,12); and stable coronary artery disease (13), hypertension (14), acutely decompensated heart failure (ADHF) (15), angina pectoris (5), coronary vasospasm (16), and acute coronary syndrome (ACS) (17), among others.

In this review, we will summarize recent advances in the understanding of BTP in patients with kidney dysfunction, its role in the development and progression of several cardiovascular disorders, and the

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clinical studies that support a potential role for measurement of BTP in patients with ADHF and ACS.

## Methods

Published data for this review were identified by search and selection in MEDLINE database and reference lists from relevant articles and reviews. A two-step approach was used. First, the effects of BTP on cardiovascular diseases were identified in a search with the keywords " $\beta$ -trace protein/Lipocalin type prostaglandin D synthase" and "cardiovascular disease". Second, the cardiovascular diseases that were identified with this search were used as keywords with the addition of the following keywords: "glomerular filtration rate," "cystatin C," "creatinine," "atherosclerosis," "hypertension," "endothelial cells," and "hypoxemia". Bibliographies of all selected articles and review articles about  $\beta$ TTP and/or cardiovascular disease were reviewed for other relevant articles.

## BTP as a Serum Biomarker of Renal Injury

The most commonly used indicator for estimation of GFR is serum creatinine. Measurement of creatinine is inexpensive and convenient, but it has several drawbacks (Table 1), most notably that it is increased only after moderate to severe reduction in GFR. Thus, serum creatinine is a poor marker of kidney function in the near-normal

GFR range (18). Also, a variety of nonrenal physiologic (*e.g.*, muscle mass, age, sex, and diet) and pathologic factors affect the circulating concentration of creatinine, and measurement of creatinine by the common Jaffe method is subject to numerous analytical influences (*e.g.*, certain antibiotics, bilirubin, and ketones). Moreover, creatinine is not only filtered at the glomerulus but excreted in the tubules, and thus, does not behave as an ideal marker of GFR. This way, current laboratory guidelines recommend the use of estimated GFR equations, which take into account serum creatinine and demographic and anthropomorphic variables (19). Several equations, such as the Cockcroft Gault (20) and the Modification of Diet in Renal Disease (MDRD) (19,21) for adults and the Schwartz and Counahan-Barratt (19) for children, have been developed in an attempt to improve GFR estimation from serum creatinine. However, these equations often give conflicting estimates of GFR and have not been completely validated in a large diverse population (22). More recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) has proposed three alternative equations, which apply different coefficients to the same four variables used in the MDRD Study Equation or add Cys C values (23). These new CKD-EPI Equations estimate GFR more accurately than the MDRD Study Equation (23,24). The use of equation for estimating GFR is also of special interest for kidney graft function monitoring after renal transplantation (9). As a result of more effective immunosuppressive regimens, clinical

**Table 1. Comparison of creatinine, Cystatin C, and  $\beta$ -trace protein as GFR markers**

	Creatinine	Cystatine C	$\beta$ -Trace Protein
Weight	113 Da	13,000 Da	23,000–29,000 Da depending on the degree of glycosylation
Structure	Amino acid derivative	Nonglycosylated basic protein	Glycosylated protein
Synthesis	Muscle mass, age, sex, diet, and pathologic factors can affect the circulating concentration of creatinine; lower in the elderly, women, and white people	Constant by all nucleated cells	Cerebrospinal fluid, brain, retina, melanocytes, heart, endothelial cells, and male genital organs; it is secreted into various body fluids, such as cerebrospinal fluid, plasma, seminal plasma, and urine
Presence in the serum	Increases at reduced GFR	Increases at reduced GFR	Increases at reduced GFR
Accuracy	Serum creatinine is increased only after a 50% reduction in GFR	Very accurate	Very accurate
Assay method	Colorimetric and enzymatic assays	Immunonephelometric assay	Immunonephelometric assay
Assay precision	Very good except in the presence of mild renal impairment	Precise throughout the range	Precise throughout the range
Advantages	Inexpensive and well characterized	More precise and accurate than creatinine	More precise and accurate than creatinine
Limitations	Not only filtered at the glomerulus, but it is also excreted in the tubules; low precision at low renal impairment	Affected by C-reactive protein levels and thyroid function, not precise in the third trimester of pregnancy, and higher levels in tumor progression and metastasis; corticosteroid treatment falsely increases Cystatine C levels;	Higher levels in meningiomas and hemangiopericytomas

presentation of acute rejection is often less apparent. Thus, detection of slight alterations in GFR is mandatory to improve patient outcome. In this context, a recent study including 187 consecutive patients after kidney transplantation showed that a BTP-based GFR equation was superior to the MDRD Study Equation, with a better bias and 10% more accuracy (25).

Because of the commented limitations of serum creatinine as a marker of GFR, new alternatives for detecting reduced GFR are under investigation. Serum concentrations of low-molecular weight proteins such as Cys C,  $\beta_2$ -microglobulin ( $B_2M$ ), and BTP could be useful alternatives. Compared with serum creatinine, all of these three low-molecular weight proteins, Cys C,  $B_2M$ , and BTP, have been reported to show a better diagnostic sensitivity for detection of impaired GFR (9,26,27). However,  $B_2M$  has the disadvantage of being increased in patients with several malignancies and infectious diseases, particularly lymphoproliferative disorders (28).

There is an intense interest to determine if BTP is a better marker of renal injury than Cys C. Although some authors have shown that BTP is a more suitable marker because of less extra renal interferences (25,26), some others have found that BTP was not superior to Cys C as a biomarker of GFR or did not find significant differences between them (27,29–32), although some of these latter analyses have the important limitation of small numbers of patients.

In one study with 865 African Americans with hypertensive CKD, 246 participants reached ESRD during a median follow-up of 102 months (26). The association between higher BTP level and ESRD was stronger than those associations for the other markers, including measured GFR and Cys C. In addition, it has been shown in a study with 503 participants, with a median follow-up of 3.3 years of a national prospective cohort study of incident dialysis patients, that the serum level of BTP is an independent predictor of death. BTP was used as an endogenous filtration marker of kidney function, because it is not removed during hemodialysis (29).

However, in one study with only 62 patients with liver or renal diseases, serum creatinine, Cys C,  $B_2M$ , and BTP were analyzed (31), and it was shown that Cys C was better than  $B_2M$ , BTP, and creatinine as an indicator of reduced GFR. A huge analysis of 9988 participants in the Atherosclerosis Risk in Communities Study (27), with a follow-up of approximately 10 years, showed that  $B_2M$  and BTP levels share the advantage of Cys C over the creatinine-based CKD-EPI Equation in predicting outcomes, including kidney failure. Another analysis, including 225 children with various renal pathologies, showed that BTP and Cys C had higher diagnostic accuracy than serum creatinine for identification of moderately impaired GFR in children (33). Moreover, BTP has been compared with Cys C and creatinine for its diagnostic and staging capacity and its value as a progression predictor of primary nondiabetic patients with CKD (34). It was revealed that all three clearance markers were almost equally strong predictors of CKD progression, even after adjustment for age, sex, GFR, and proteinuria in a cohort of 227 patients with different degrees of renal impairment (34).

It is important to remark that some advantages have been reported of BTP compared with Cys C (Table 1). It has been observed that serum BTP levels do not have a significant relationship with C-reactive protein and that they are unaffected by body composition (29). During the third trimester of pregnancy, BTP, but not Cys C, has been shown to adequately reflect the GFR. Unlike Cys C, thyroid function has not been reported to affect the concentration of BTP (29). Another possible advantage of BTP is found in renal transplantation patients (Table 2), in whom surveillance of GFR is necessary to identify worsening graft function (9). These patients are normally on corticosteroids, and this treatment has been observed to falsely increase Cys C concentrations, whereas BTP concentrations were not influenced (9,25,35,36). However, patients who suffered from brain tumors and related malignancies could increase BTP synthesis (Table 1). Thus, although promising, more data are needed regarding the use of serum BTP as a biomarker of renal dysfunction.

### BTP as a Urinary Biomarker of Renal Dysfunction

Albuminuria is a well known biomarker of kidney damage that occurs when glomerular function is normal, but the proximal tubules have a diminished capacity to reabsorb and catabolize proteins, causing an increased urinary excretion of proteins that usually pass through the glomerulus, including albumin. Much evidence suggests that albuminuria *per se* worsens the prognosis in various systemic diseases, increasing the incidence of cardiovascular events (37,38). Urinary BTP may detect renal injury earlier than albuminuria because of its molecular lower mass, its anionic property, its constant production rate, and its stability (37).

Urinary excretion of BTP has, thus, been used as a marker of renal dysfunction in animal models (39) and human studies (37,38,40,41). In a rat model experiment, a genetic model of spontaneous noninsulin-dependent diabetes mellitus accompanied by kidney dysfunction was used (39). In this study, it was observed that urinary BTP excretion in early diabetes correlated well with the subsequent alterations of glomerular integrity: the greater degree of BTP excretion in young rats and the greater proteinuria or glomerular lesions seen in elderly rats. These observations were confirmed in two studies analyzing the excretion of BTP in type 2 diabetes patients (37,38). In one study, the authors segregated the patients depending on the BTP concentration in urine. They observed that, in approximately 2 years, one fourth of the patients with levels of urinary BTP above the cutoff developed albuminuria, whereas in the case of patients with levels of urinary BTP excretion below the cutoff, only 5% exhibited albuminuria (37).

Subsequently, two different studies with patients suffering from CKD showed that BTP was a useful marker for the early detection of renal tubular damage and that a BTP urine-based test can predict a slight GFR impairment in CKD patients (40,41).

In aggregate, these results suggest that BTP is a promising alternative marker to creatinine, estimated GFR, or Cys C for kidney dysfunction, especially in the early stages.

**Table 2. Summary of  $\beta$ -trace protein clinical studies of renal impairment and cardiovascular risk**

Authors	Year	Ref.	Publication	Main Findings
<b>Renal transplantation</b>				
Pöge <i>et al.</i>	2005	9	<i>Clin Chem</i>	BTP levels are not influenced by corticosteroid administration
Pöge <i>et al.</i>	2008	25	<i>Am J Transplant</i>	
Risch <i>et al.</i>	2001	35	<i>Clin Chem</i>	
Risch <i>et al.</i>	2005	36	<i>Clin Chim Acta</i>	
<b>Renal injury in diabetes mellitus</b>				
Uehara <i>et al.</i>	2009	37	<i>Nephrol Dial Transplant</i>	BTP levels increase the power to detect the early stage of renal injury in type 2 diabetes
Yoshikawa <i>et al.</i>	2007	38	<i>Diabetes Res Clin Pract</i>	
<b>CKD</b>				
Dajak <i>et al.</i>	2010	40	<i>Clin Chim Acta</i>	Early detection of renal tubular damage
Donadio	2010	41	<i>Am J Physiol Renal Physiol</i>	
<b>Heart hypoxemia</b>				
Han <i>et al.</i>	2009	4	<i>Biochem Biophys Res Commun</i>	Adaptive mechanism to pressure overload
<b>Heart ischemia</b>				
Tokudome <i>et al.</i>	2009	10	<i>J Clin Invest</i>	Protect cardiomyocytes against cell death induced by ischemia
<b>Stabilization of atherosclerotic plaque</b>				
Cipollone <i>et al.</i>	2004	12	<i>Arterioscler Thromb Vasc Biol</i>	Higher BTP expression in asymptomatic plaques
Inoue <i>et al.</i>	2008	13	<i>Atherosclerosis</i>	Concentrates retinoids in the AP, inhibiting the proliferation of human coronary artery smooth muscle cells and atherosclerosis progression
Taba <i>et al.</i>	2003	45	<i>Am J Physiol Heart Circ Physiol</i>	Inhibits inducible nitric oxide synthase
Negoro <i>et al.</i>	2002	43	<i>J Hypertens</i>	Suppresses expression of proinflammatory mediator PAI-1
Negoro <i>et al.</i>	2005	46	<i>Life Sci</i>	Suppresses expression of vascular cell adhesion molecule 1
Rossi <i>et al.</i>	2000	48	<i>Nature</i>	Activation of the nuclear receptor PPAR $\gamma$ and inhibition of NF- $\kappa$ B activity
<b>Endothelial injury</b>				
Taba <i>et al.</i>	2000	42	<i>Circ Res</i>	Steady laminar shear stress stimulates BTP expression in endothelial cells, thereby inhibiting endothelial cell apoptosis
<b>Hypertension</b>				
Hirawa <i>et al.</i>	2002	14	<i>Hypertension</i>	BTP overexpression protects against cardiac overload
<b>Hypertensive CKD</b>				
Bhavsar <i>et al.</i>	2011	26	<i>Am J Kidney Dis</i>	Plasma BTP may be useful in evaluating risk of progression of kidney disease
<b>Vasospastic angina</b>				
Matsumoto <i>et al.</i>	2011	16	<i>Circ J</i>	BTP elevation is associated with the degree of coronary vasoconstriction (higher arterial shear stress)
<b>Acute decompensated heart failure</b>				
Manzano-Fernández <i>et al.</i>	2011	15	<i>J Am Coll Cardiol</i>	BTP predicts mortality, heart failure, hospitalization, and adverse cardiovascular events (These findings also apply to the next three studies.)
<b>Hemodialysis patients</b>				
Shafi <i>et al.</i>	2012	30	<i>CJASN</i>	
<b>General population</b>				
Astor <i>et al.</i>	2012	27	<i>Am J Kidney Dis</i>	
<b>Acute coronary syndromes</b>				
Manzano-Fernández <i>et al.</i>	2012	17	<i>Am J Cardiol</i>	

AP, apoptosis protein; BTP,  $\beta$ -trace protein; PAI-1, plasminogen activator inhibitor-1; PPAR $\gamma$ , peroxisome proliferator-activate receptor- $\gamma$ .

### BTP and the Biology of Cardiovascular Disease

Biologically speaking, BTP clearly has a role in the cardiovascular system. Among various human tissues examined, cardiac expression of the mRNA for BTP was most substantial (5). In the human heart, BTP is localized in myocardial cells, atrial and ventricular endocardial cells, both coronary arteries, smooth muscle cells, and atherosclerotic plaque (5). It is not surprising, therefore, that BTP levels are elevated in circulation of patients with severe coronary heart disease, and for that reason, recent studies have highlighted the implications of BTP in progression of several cardiovascular diseases (5,12–17). It is important to remark that prostaglandins derived from PGD<sub>2</sub>—and hence, extending from BTP—exert significant effects on the cardiovascular system, including 15-deoxy-D12,14-PGJ<sub>2</sub> (15d-PGJ<sub>2</sub>), the most effective endogenous activator for the nuclear receptor peroxisome proliferator-activate receptor  $\gamma$  (PPAR $\gamma$ ) (42). 15d-PGJ<sub>2</sub> promotes several processes in vascular cells, such as anti-atherogenic, antithrombotic, antiapoptotic, and anti-inflammatory effects (42) (Figure 1). In the same way, PGD<sub>2</sub> may suppress inflammatory processes and prevent platelet aggregation, and it decreases the marker of fibrinolytic system plasminogen activator inhibitor-1 (PAI-1) mRNA expression and PAI-1 synthesis in cultured bovine endothelial cells after incubation with IL-1 $\beta$  (43).

### Role of BTP in Heart in the Context of Hypoxia and Ischemia

Pulmonary hypertension caused by chronic hypoxemia can eventually lead to heart failure because of pressure

overload of the right ventricle. In a mouse model of chronic hypoxemia, expression of BTP mRNA and protein level in the heart correlated with hypoxic conditions, and BTP values tended to increase under hypoxia, reaching the highest levels after 14 days, compared with the levels in age-matched control mice kept under normoxia (4). The expression of BTP protein was analyzed using immunohistochemistry and was twofold higher in the heart of cases compared with wild-type mice. This increased expression in the heart may reflect an adaptive mechanism to pressure overload (Table 2). However, the protective role of BTP in rodent hearts from an ischemia-reperfusion injury model has also been shown (10). This effect is carried out through glucocorticoid receptor (GR), a member of the nuclear receptor superfamily of ligand-dependent transcription factors. In the absence of glucocorticoids, the GR is sequestered in the cytoplasm by a protein complex that includes heat shock proteins. On glucocorticoid binding, GR is released from this inactive complex and translocates to the nucleus. Within the nucleus, ligand-bound GR binds to specific promoters, thereby activating the transcription of target genes, including BTP (10). BTP overexpression stimulates PGD<sub>2</sub> synthesis, thus attenuating myocardial ischemia-reperfusion injury in isolated perfused hearts (Figure 1). Moreover, when a genetic deletion of BTP or knockdown of BTP was carried out, cardioprotective effect afforded by glucocorticoids to isolated perfused hearts was abrogated compared with wild-type mice (10). In addition, it was shown that glucocorticoids, such as dexamethasone, reduce infarct size in a BTP-dependent manner after ischemia-reperfusion injury *in vivo*. The

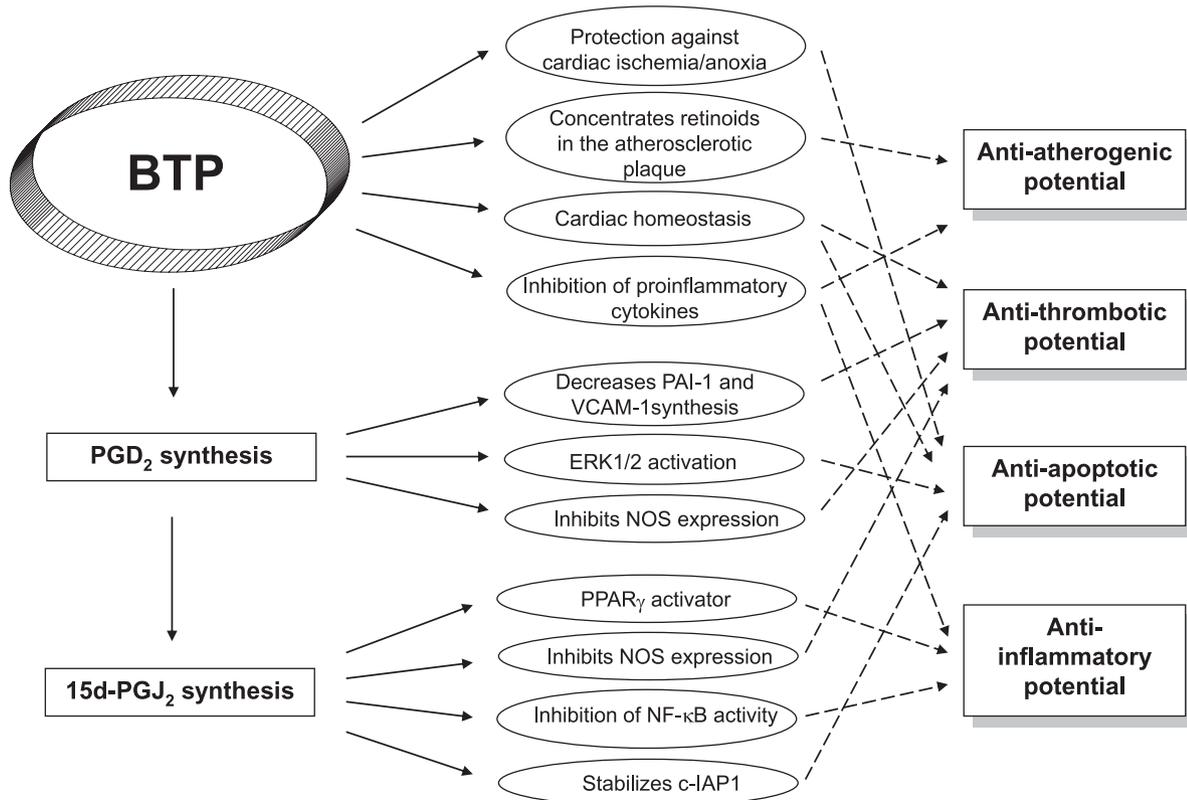


Figure 1. | Effect of  $\beta$ -trace proteins and their metabolites in cardiovascular processes.

activation of BTP-mediated PGD<sub>2</sub> biosynthesis may, thus, mitigate pathologic ventricular remodeling in the late phase of acute myocardial infarction (10).

Mechanistically, the cardioprotective effects of PGD<sub>2</sub> are likely mediated through the D-type prostanoid receptor, a G protein-coupled receptor. After PGD<sub>2</sub>-D-type prostanoid receptor binding, extracellular signal-regulated kinase 1/2 activation leads to cardiomyocyte protection (10) (Figure 1).

### Role of BTP in the Stabilization of the Atherosclerotic Plaque

It has been reported that serum BTP concentrations increase with aging, and such increases are associated with subclinical atherosclerosis as evaluated by the maximal intima-media complex thickness of the common carotid artery and increased aortic stiffness by the pulse wave velocity (44). In addition, single nucleotide polymorphism 4111A>C, found in the BTP gene in Japanese people, is associated with maximal intima-media complex thickness of the common carotid artery (44). These observations, among others, strongly support the importance of BTP in the pathogenesis of atherosclerosis (Table 2).

There are several studies showing the stabilizing role of BTP in the atherosclerotic plaque in mouse models and humans (11,12) and in vascular endothelial cells (42,45). It is widely known that the modulation of vascular remodeling mediated by inflammation is a key event in the progression of atherosclerosis and that BTP and its metabolites may have an important anti-inflammatory role in this milieu. For example, in a BTP knockout mouse model, lack of BTP tended to increase total and LDL cholesterol and triglycerides in plaque, and HDL cholesterol tended to decrease compared with wild-type mice (11). Moreover, comparison of macrophages infiltration in the atherosclerotic lesions in aortic root sections between wild-type and BTP knockout mice using anti-CD68 antibody showed an increasing in the number of macrophages in the latter group. Furthermore, the expression of proinflammatory cytokines such as monocyte chemoattractant protein-1 and IL-1 $\beta$  in aortic root sections were also markedly enhanced in BTP knockout mice (11).

In humans, the same protective role has been described. However, in this case, a balance between a proinflammatory enzyme Prostaglandin E (PGE synthase) and the anti-inflammatory BTP has been proposed (12). For example, immunohistochemistry of plaques from patients with either transient ischemic attack or stroke revealed strong PGE synthase immunoreactivity but only very weak staining for BTP. In contrast, BTP was the predominant isomerase in asymptomatic plaques. In addition, staining for matrix metalloproteinase-9 was significantly more abundant in the symptomatic than the asymptomatic lesions, and PPAR $\gamma$  expression was significantly higher in asymptomatic plaques, showing a stable concordance with BTP (12). The BTP enzymatic product PGD<sub>2</sub> inhibits inducible nitric oxide synthase in vascular smooth muscle cells (45) and suppresses the expression of proinflammatory mediators such as PAI-1 (43) and vascular cell adhesion molecule-1 (46) in endothelial cells (Figure 1). 15d-PGJ<sub>2</sub> has also been shown to suppress inflammatory responses inhibiting macrophage activation (47), favoring the production of

monocyte inflammatory cytokines and biologic functions of human natural killer cells by binding and activation of the nuclear receptor PPAR $\gamma$  and inhibition of NF- $\kappa$ B activity (48) (matrix metalloproteinase-9 [MMP-9] and cyclooxygenase-2 [COX-2] are two important targets of NF- $\kappa$ B). Moreover, 15d-PGJ<sub>2</sub> has also been shown to suppress inducible nitric oxide synthase expression (47). This way, PG isomerase profile may influence the proinflammatory or anti-inflammatory role of COX-2 in atherosclerotic plaques, and this finding is in agreement with the observation that COX-2 overexpression, when associated with low PGE<sub>2</sub> synthesis and high BTP levels, may contribute to the resolution of inflammation.

These findings suggest that, in the vascular wall, BTP deficiency facilitates atherogenesis caused by the lack of anti-inflammatory effects, and it could be a potential novel biomarker of atherosclerotic process and evolution. Indeed, in a multicenter study of 1013 patients, it was shown that serum BTP level was elevated in patients with stable coronary artery disease and that the level increased in association with the number of affected vessels (13). Both simple and multiple regression analyses showed that the BTP level could predict lesion severity and extent of plaques for the entire coronary artery system (13). Taken in aggregate, concentrations of BTP in atherosclerotic plaques could be related with a more diffuse, unstable, and active plaque; these observations raise the possibility of pharmacological stabilization of the atherosclerotic plaque using selective modulators of BTP synthesis.

Beyond atherogenic mechanisms of plaque formation, BTP may also play a role in endothelial injury, a pivotal step in plaque genesis (Table 2). Laminar fluid shear stress inhibits endothelial cell apoptosis, and a lack of shear stress triggers apoptosis. It is well established that steady laminar shear stress stimulates endothelial cells to express BTP, thereby stimulating the production of PGD<sub>2</sub> and 15d-PGJ<sub>2</sub>, and inhibiting endothelial cell apoptosis (42) through increase in the expression of the cellular inhibitor of apoptosis protein 1 (44,49). However, the role of 15d-PGJ<sub>2</sub> in apoptosis is controversial. It has been reported to induce apoptotic cell death in several other cell species, including vascular cells (50). Importantly, lending some explanation to this finding, 15d-PGJ<sub>2</sub> has been reported to have biphasic effects on endothelial cell apoptosis. In this way, low-micromolar concentrations show a cytoprotective effect by inducing glutathione expression, whereas higher concentrations induce apoptosis caused by oxidative stress and activation of mean arterial pressure kinase cascades involving reactive oxygen species (51).

### Role of BTP in Hypertension

It has been reported that serum and urinary BTP values are much higher in patients with essential hypertension than patients in normotensive subjects, even when patients with essential hypertension exhibit apparently normal renal function (14). Moreover, hypertension with renal injury was associated with further increased BTP concentrations in sera and urine (14,26). It has also been observed that the increase in serum BTP was associated with urinary excretion of BTP, and consistent with its role as a sensitive kidney function marker, urinary BTP preceded an increase

in urinary albumin excretion (14). This increase probably reflects injuries in the renal tubules and arterioles induced by hypertension (Table 2).

### Role of BTP in Stable Atherosclerotic Disease and Vascular Disease

BTP has intimate roles in the pathophysiology of vascular disease and atherosclerosis; thus, its testing in patients with coronary syndromes seems promising.

In patients with stable angina, plasma level of BTP was significantly higher in the cardiac vein than coronary arteries (5), suggesting production through the heart structures. BTP concentrations in the cardiac vein decreased after percutaneous coronary intervention (52), and either reduction in coronary release or improvement in myocardial ischemia triggered BTP secretion. Similarly, it has also been observed that an increase in serum BTP levels 48 hours after percutaneous coronary intervention correlates with restenosis rates (53).

Regarding vasomotor reactivity, coronary spasm plays an important role in the pathogenesis of ischemic heart disease. Currently, there are no established biomarkers that assess the presence of vasospastic angina (VSA), and endothelial dysfunction has been implicated as a crucial factor in the pathogenesis of coronary vasospasm in patients with VSA (54). Shear stress stimulates  $\text{PGD}_2$  synthesis by BTP expression in vascular endothelial cells in response to blood flow, and vasoconstriction can reduce blood flow, thus increasing arterial shear stress (54). It has been observed that serum levels of BTP were elevated in patients with VSA (Table 2), and they were associated with the degree of coronary vasoconstriction in response to acetylcholine, a pharmacological tool used to induce coronary vasospasms and evaluate endothelial function (17).

### BTP in ADHF and ACS

Given the multiple pathways that BTP is associated with, we thought it reasonable to examine the value of the biomarker for predicting adverse outcome in patients with HF and ACS. The rationale is easy to understand—beyond the links between BTP and coronary and heart muscle disease, kidney dysfunction is an exceptionally important adverse prognostic factor in patients with ADHF (15) and ACS (17). Accordingly, the identification of laboratory parameters capable of more accurately assessing renal function than conventional measures of renal function (*e.g.*, creatinine, estimated GFR, or BUN) may be particularly relevant for this population. As mentioned above, conventional measures of renal function have limitations; thus, the characterization of newer markers of renal dysfunction for application in patients with these clinical syndromes may be of considerable significance.

Along this line, in a recent study (15), our group described the prognostic importance of BTP in a hospitalized cohort of 220 patients with ADHF and compared it with the prognostic importance of Cys C and other conventional measures of renal function, including serum creatinine, estimated GFR, and BUN. The primary outcome of this study was the combination of mortality and/or HF hospitalization. All patients were clinically followed

during a median of 500 days. Over the study period, a total of 116 patients (53%) presented adverse clinical events. Compared with patients who did not have events, those patients who presented adverse clinical events had higher plasma BTP and Cys C. In receiver operating characteristic curve analyses, BTP and Cys C had comparable area under the curve (0.62 and 0.63, respectively), with overall performance characteristics that seemed similar if not slightly superior to the more conventional measures of renal function (15). Furthermore, in the overall renal function markers examined, only BTP and Cys C remained as significant predictors of adverse events, even in the presence of results for estimated GFR (Table 2).

Consistent with its role as a candidate marker for prognostic stratification in patients with acute heart failure, BTP may also have a role in predicting mortality among patients with ACS (17). Indeed, we found that plasma BTP concentrations were predictive of mortality in this setting. In this analysis, patients were followed for at least 1 year (median=859 days [interquartile range=524–1164]), and as we found in patients with ADHF, decedents ( $n=24$ , 10.6%) had higher concentrations of BTP and Cys C (17). Furthermore, BTP and Cys C were found to be significantly associated with all-cause death (Table 2), whereas estimated GFR and serum creatinine concentrations did not achieve statistical significance. Of note, both BTP and Cys C added complementary prognostic information to the Global Registry of Acute Coronary Events risk score.

### Future Research Directions

Additional research in different ethnic groups and persons with extremes of body size or diet is required to better understand BTP as a filtration marker, including its production as well as renal and nonrenal handling. As mentioned above, BTP may represent a potential alternative endogenous filtration marker to serum creatinine and Cys C. Most of the studies suggest that BTP is a renal function marker at least comparable with serum creatinine and Cys C; however, comparisons between BTP-based estimating equations and serum creatinine or Cys C-based GFR estimating equations are lacking. Moreover, there is a lack of validation studies for BTP-based estimating equations.

Given the potential intersection between the biology of BTP, vascular disease, and renal function, other factors to examine in future studies include whether the vascular role of BTP interferes with its interpretation relative to kidney function as well as the appropriate mode of interpretation of BTP in patients with and without arteriopathy. More studies in large cohorts are mandatory to define the prognostic value of BTP in various cardiovascular diseases, such as coronary artery diseases, hypertension, diabetes, arteriosclerosis, and others. It would be very relevant to determine whether serial measurements of BTP could add prognostic value. This way, in the future, pharmacological or biologic interventions targeting positive modulation of BTP production would be a possible strategy to prevent coronary artery disease. From a practical standpoint, these findings raise the possibility that drugs able to selectively modulate AA metabolism might provide a novel form of therapy for these cardiovascular diseases.

In situations in which creatinine-based estimates can only be applied with caution and Cys C is influenced by a nonrenal factor, such as renal transplantation, patients on steroids, or patients with thyroid disorders, BTP might, thus, serve as a valuable alternative marker of renal function. With the experience of creatinine in mind, efforts for standardization of assays should be initiated sooner rather than later.

## Conclusions

It has been shown that BTP might serve as an alternative endogenous marker for GFR, and it has been shown to be a more sensitive marker for renal impairment than creatinine serum concentrations, particularly because BTP can detect mild deterioration in renal function. This better accuracy and sensitivity is promoting the rise of BTP as a novel and promising maker for renal function. More recently, BTPs and their metabolites (PGD<sub>2</sub> and 15d-PGJ<sub>2</sub>) have been identified as novel markers in the development and progression of several cardiovascular disorders, because their plasma levels are found to be elevated in patients with atherosclerosis, angina pectoris, HF, and ACS among others. Furthermore, they have been shown to regulate several important biologic functions through their anti-inflammatory, antiapoptotic, antithrombotic, and anti-atherogenic effects, promoting a cardiovascular protective role. Future studies are necessary to elucidate a potential clinical role for BTP.

## Acknowledgments

E.O.-P. is supported by a postdoctoral contract from Fundación para la Formación e Investigación Sanitarias de la Región de Murcia, Murcia, Spain. J.L.J. is supported by the DeSanctis Clinical Scholar Endowment.

## Disclosures

None.

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