Pentraxins in nephrology: C-reactive protein, serum amyloid P and pentraxin-3

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ABSTRACT
Every clinician uses C-reactive protein (CRP) levels as a biomarker for systemic inflammation in acute disorders. Nephrologists also consider CRP levels as a predictor for overall mortality in patients with chronic kidney disease or end-stage renal disease. But what is the biological function of CRP? CRP is a member of the family of pentraxins, which are small pentameric innate immunity effector proteins. Pentraxins are absent or weakly expressed during homeostasis. However, the pro-inflammatory cytokines interleukin (IL)-1, IL-6 and tumour necrosis factor induce CRP and serum amyloid P (SAP) in hepatocytes, whereas the long pentraxins, such as pentraxin (PTX)-3, are produced in peripheral tissues and monocytic phagocytes. Pentraxins opsonize pathogens or other particles such as dead cells, for their phagocytic clearance or induce pathogen killing in extracellular compartments. In this review, we discuss the immunoregulatory properties of the different members of the pentraxin family. We discuss the evolving evidence demonstrating their roles in acute and chronic forms of kidney disease and the significance of SAP and PTX3 as additional biomarkers of innate immune activation and systemic inflammation.

INTRODUCTION
Every clinician is used to assessing infectious or non-infectious types of systemic inflammation from C-reactive protein (CRP) levels in the peripheral blood. Nephrologists recognize CRP levels also as a predictor of long-term outcome potentially because CRP marks persistent inflammation that contributes to complications in the chronic kidney disease (CKD) or end stage renal disease (ESRD) population. But what is the biological function of CRP? Why is it not expressed under normal conditions? Why do its levels rise during inflammation? What does it mean that CRP is a pentraxin? And what should nephrologists know about the other members of the pentraxin family, i.e. serum amyloid P (SAP), and the long pentraxins? This review provides answers to these questions and focuses on the latest developments in pentraxin research in view of kidney medicine.
focused on the quality of long pentraxin PTX3 as a biomarker and demonstrated plasma PTX3 levels to also correlate with a higher risk of cardiovascular mortality [16–18]. Surprisingly, PTX3 levels were superior to CRP or troponin levels in more reliably predicting mortality after myocardial infarction. This may also relate to the time of blood sampling because PTX3 reaches the maximal plasma concentration already within 6–7 h after myocardial infarction, while CRP peaks not before 24 h [19]. In 871 patients with acute chest pain, PTX3 levels most reliably predicted long-term all-cause mortality [20]. In the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) and GISSI-HF trials, 2690 patients with chronic heart failure displayed a positive correlation between PTX3 levels and an advanced New York Heart Association (NYHA) class, and changes in PTX3 plasma level predicted fatal outcomes independently of CRP and NT-proBNP [21]. Consistent with these findings, the Heart and Soul study reported 986 patients with a stable coronary heart disease in which PTX3 plasma levels correlated with the risk for overall mortality independently of other markers of systemic inflammation [22]. Discrepancy data between the two markers may relate to their different secretion sites, i.e. the liver for CRP and leukocytes and peripheral tissues for PTX3 [23]. These positive correlations then also apply to other surrogate parameters of cardiovascular morbidity. CRP and PTX3 levels correlate positively with the body mass index but negatively with HDL cholesterol [24].

CRP plasma levels divide CKD patients into different subgroups concerning their long-term prognosis [25, 26]. Higher CRP levels indicate persistent inflammation and predict overall mortality in this population [27, 28]. CRP levels are elevated in IgA nephropathy and seem to correlate with a progression of the disease [29]. Serum PTX3 levels were shown to be an independent marker of disease activity because PTX3 is induced by tumour necrosis factor (TNF-α) at sites of inflammation and within adipose tissue [8, 30–32]. Interestingly, in ESRD, patients show an inverse relationship between PTX3 levels, fat body mass and abdominal obesity, while CRP and SAP levels positively correlate with these biomarkers [32]. However, CKD and ESRD are still associated with higher PTX3 plasma levels. This correlation is still significant after adjustment for comorbidities, interleukin (IL)-6 and demographics and predicts mortality independently of the CRP level and stage of cardiovascular disease [33, 34]. Furthermore, PTX3 levels positively correlate with endothelial dysfunction and proteinuria in stage 5 CKD patients and in patients with type 2 diabetes [35]. Inhibition of angiostatin-converting enzyme in patients with stage 1 diabetic CKD normalized proteinuria along with plasma PTX3 levels [36]. PTX3 levels are also associated with haemodialysis-induced systemic inflammation, cardiovascular disease and protein wasting as well as with overall mortality [37, 38]. Another study reported that PTX3 and CRP predict future cardiovascular events in CKD patients [39, 40]. Obviously, the biomarker effect of the pentaxins is linked to inflammation, but what are the native biological functions of the pentraxins?

**PENTRAxINS CONTRIBUTE TO HUMORAL INNATE IMMUNITY IN THE EXTRACELLULAR SPACE**

The superfamily of pentraxins is evolutionary conserved from insects to lower vertebrates and mammals. Pentraxins are characterized by a cyclic multimeric structure [41] and belong together with ficolins and collectins to the humoral arm of innate immunity. The immune system is a complex network of different elements that defend the host to external or internal types of dangers to homeostasis. Traumatic, infectious or toxic insults that disrupt homeostasis need to be detected in all body compartments, i.e. inside or outside of the cells. For example, the sensing and neutralization of intracellular pathogens requires different innate and acquired recognition receptors and effector elements than those needed for the control of extracellular pathogens or dying cells (Figure 1). The functions of pentraxins are conceptually similar to those of antibodies, i.e. the humoral products of adaptive immunity (Figure 1). Pentraxins are acute-phase proteins, which are weakly or not at all expressed during homeostasis, while the proinflammatory cytokines IL-1, IL-6 and TNF rapidly induce their expression during infections or sterile forms of tissue damage (Figure 2). While CRP and SAP are exclusively expressed by hepatocytes, the long pentraxins are produced by various cell types inside the peripheral tissues (Figure 2). Interestingly, IFN-α selects CRP transcription in hepatocytes [42]. This can explain why serum CRP levels poorly correlate with disease states that are associated with IFN-α signalling, such as viral infections or systemic lupus/lupus nephritis [43, 44].

Within the long pentraxins, the biggest subgroup is build by neuronal pentraxins, such as NPI/NPTXI and NP2/NPTXII, which are constitutively expressed in the central nervous system. Mutations in these genes lead to age-dependent neurodegeneration [45, 46]. They were found to be induced during physiologic synaptic activity [47] as well as during ischaemic injury of the brain [46, 48–51]. Unlike neuronal pentraxins, the expression of PTX3 was shown to be very remarkable in the heart and skeletal muscle, where vascular endothelial cells and smooth muscle cells seem to be its main producers [52, 53]. Also human airway smooth muscle cells produce increased amounts of PTX3 in bronchial tissues of allergic asthmatic patients. PTX3 expression is likely to be also related to the processes of matrix deposition, angiogenesis and tissue repair [54, 55]. It is expressed in an ovary by cumulus cells, where it is responsible for the stabilization of extracellular matrix and involved in processes of blastocyst implantation [56].

**THE PENTRAxIN GENE FAMILY**

Pentraxin genes encode for proteins that are characterized by a structural motif, the so-called pentraxin domain [57–59], which is located at the C-terminus of the pentraxin domain, with an eight amino acid long conserved pentraxin signature...
(HxCxS/TWxS, where x is any amino acid; Figure 3). In contrast, in the amino-terminal domain, less homology is found (only 10–38% similarity) [60]. All pentraxins have an oligomeric structure with protomers linked to each other by disulphide bonds [60]. The amino acid sequences include two conserved cysteines, which are involved in the forming of disulphide bonds. Short pentraxins are located on chromosome 1 and are organized in two exons. They are evolutionarily younger and most probably originate from single gene duplication [61, 62]. Despite high homology in mammals, they show differences in serum basal levels. CRP is known to be the main acute-phase protein in humans [61]. In contrast, mice seem to produce rather SAP during the acute inflammation phase [63], whereas in humans it is constitutively present in serum at a concentration of 30–50 mg/L.

The family of the long pentraxins consists of neuronal pentraxins, PTX3 and PTX4. They differ from short pentraxins with the presence of the long N-terminal part and share the same general organization described for PTX3. The human Ptx3 gene is organized in three exons and is located on human chromosome 3q25.6. PTX3 has a predicted molecular weight of 40 165 Da and consists of leader peptide, N-terminal domain (both encoded by the first two exons) and pentraxin domain (encodes by the third exon). As mentioned above, the 174-amino acid long N-terminal domain is not present in short pentraxins [52]. The C-terminal domain contains the conserved pentraxin signature and has been described to bind to C1q and activate the complement. This whole process and the potency of binding depend on a single N-glycosylation site on Asn 220, which indicates that the glycosylation status of pentraxins might contribute to their biological functions [64].

**THE BIOLOGY OF CRP AND SAP AND THEIR ROLES IN KIDNEY DISEASE**

Pentraxins act as ‘antibodies’ of innate immunity, which opsonize and neutralize pathogens in an antigen-unspecific manner (in contrast to antigen-specific adaptive/acquired immunity maintained by antibodies). They tend to be variably glycosylated [65], which appears to be crucial for conformational changes of protein structure [66, 67] and play a role in affinity to known ligands [68]. CRP- or SAP-mediated
opsonization of, for example, bacteria, means that they get marked for rapid clearance by phagocytes, again similar to the opsonization effect of antibodies that bind to specific antigens on the pathogens surface. As such, the rapid induction of CRP and SAP under inflammatory conditions supports the clearance of pathogens from the sites of infections. They also bind other particles such as oxidized LDL or apoptotic cells and facilitate their clearance by phagocytes. The recognition of these by the phagocytes involves surface Fcγ receptors (FcγR) which then triggers the phagocytic uptake. In addition, CRP and SAP bind to the globular recognition domains of C1q to activate the classical complement pathway as well as to ficolins, mannoside-binding lectins and factor H to regulate the alternative and lectin-dependent complement pathways. This way the short pentraxins contribute to lytic complement activation to kill pathogens as well as to complement-mediated pathogen removal by phagocytes. Particle opsonization and activating complement and FcγR pathways the pentraxins share with antibodies, i.e. the innate (antigen-unspecific) and adaptive (antigen-specific) elements of humoral immunity in extracellular compartments, respectively, which enforces particle clearance and host defence.

However, the diverse immunoregulatory functions of the short pentraxins can elicit different outcomes in specific (disease) contexts. The short pentraxins have an established role in lupus nephritis. Systemic lupus erythematosus (SLE) is often characterized by an impaired clearance of apoptotic lymphocytes which enforces the exposure of endogenous nuclear material to the immune system [69]. CRP or SAP deficiency, suppression of CRP expression in hepatocytes by interferon-α or anti-CRP antibody production further impair the clearance of dead cells which increases the activity of SLE and lupus nephritis [42, 70–73]. Vice versa, a single injection of recombinant CRP was sufficient to suppress SLE and lupus nephritis of MRL-lpr mice and in NZB/NZW mice even when initiated after the disease onset [74, 75]. Even though the effect of CRP and even SAP could not be replicated in NZB/NZW mice by another group [76]. CRP injections elicited anti-proteinuric effects in mice with nephrototoxic serum nephritis when given before or even after the onset of glomerulonephritis [74]. As CRP injections did not reverse proteinuria in IL-10-deficient mice with serum nephritis, it appears that CRP is also needed to trigger the anti-inflammatory cytokine IL-10 which keeps a balance during renal inflammation [74]. The capacity of the short pentraxins to trigger IL-10 was shown to regulate renal fibrogenesis. Work from Jeremy Duffield’s lab demonstrated that recombinant human SAP can prevent renal interstitial fibrosis at days 7 and 15 after renal ischaemia-reperfusion injury and at days 7 and 14 after unilateral ureteral obstruction [77]. SAP did not

**FIGURE 2:** Pentraxins in innate immunity. Liver-derived short pentraxins (CRP and SAP) and tissue expressed long pentraxins (PTX3) are produced in response to microbial sensing and inflammatory cytokines (IL-1, IL-6 and TNF). Short pentraxins ligands: C1q, Factor H, L-ficolin, M-ficolin, phosphorylcholine, C4b-binding protein, LDLs, amyloid fibrils, DNA, proteoglycans, laminin, collagen IV, fibronectin, PC, LPS, bacteria, fungi and viruses; long pentraxins ligands: C1q, Factor H, L-ficolin, TSG-6, FGF2, inter-α-trypsin inhibitor, KpOmpA, apoptotic cells, bacteria, fungi and viruses.

**FIGURE 3:** Structural organization of short and long pentraxins. Comparison in sequence homology between CRP, SAP and pentraxin 3 (PTX3). HxxCxS/TWxS, pentraxin sequence where x represents any amino acids; Asn220 is an N-linked glycosilation site localized in the PTX domain.
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THE BIOLOGY OF THE LONG PENTRAXINS AND THEIR ROLES IN KIDNEY DISEASE

PTX3 is the only member of the family of long pentraxins whose expression was detected in renal tissues [30]. It was formerly identified as an IL-1-inducible gene in endothelial cells or as a TNF-stimulated gene (TSG-14) in fibroblasts [52, 79]. Later, also dendritic cells (myeloid but not plasmocytoid) and macrophages were shown to be prominent producers of PTX3 in response to either the inflammatory cytokines IL-1α and TNF-α or Toll-like receptor ligands [52, 79-81]. PTX3 shares functional similarities with short pentraxins, but unlike these, it is acting locally at the site of inflammation [19, 82]. It is more selective than short pentraxins and involved in host defence against infections with some pathogens (in particular Aspergillus fumigatus) [83]. Ptx3−/− mice were susceptible to invasive pulmonary aspergillosis and treatment with recombinant PTX3 showed a protective effect [84]. Mice overexpressing the murine Pttx3 gene showed increased resistance to LPS toxicity [85], but increased inflammatory response to intestinal ischaemia reperfusion injury [86]. Just like short pentraxins, PTX3 binds to apoptotic cells and, to a lesser extent, to necrotic cells. However, human dendritic cells fail to internalize dying cells in the presence of PTX3, which suggest that PTX3 regulates the maturation of dendritic cells [87] and may move phagocytic activities in favour of macrophages [64, 83]. Inhibition of the internalization of dying leukocytes by dendritic cells, which was described above, might be involved in the onset of systemic autoimmunity. Thus, PTX3 might also inhibit the removal of apoptotic materials leading to the impairment of autoantigen clearance and, in turn, potentially contributes to autoantibody formation and tissue inflammation. Indeed, there is a correlation between anti-Ptx3 antibody production and protection from renal immunopathology in SLE patients. Patients with SLE were shown to have higher levels and prevalence of anti-PTX3 antibodies and anti-PTX3-related peptide antibodies than patients with other autoimmune rheumatic diseases or healthy controls. Anti-PTX3 antibodies were not associated with disease activity but with the absence of glomerulonephritis [88].

PTX3 also binds to the complement component C1q [64, 89]. However, interaction of C1q with PTX3 may induce or inhibit activation of the classical complement pathway, by binding to membrane bound- or fluid phase-PTX3 (competitive blocking), respectively [64]. Increased levels of PTX3 have been observed in some autoimmune disorders [7, 8]. Also in mice models, PTX3 was found to be increasingly expressed in the kidney and lungs along SLE progression. Lack of PTX3 aggravated autoimmune lung disease, whereas parameters of lupus nephritis remained unaffected [90]. PTX deficiency also aggravated septic lung disease or myocardial infarction in mice, which was found to relate to an inhibitory effect on leukocyte recruitment by blocking the binding sites of P-selectin E [91-93]. This particular function may also explain why post-ischaeemic renal inflammation and kidney injury are strongly aggravated in PTX3-deficient mice (own unpublished data). These data implicate that local PTX production limits tissue inflammation. PTX3 mRNA was also shown to be constitutively expressed in the human kidney. Expression and production of PTX3 was shown for primary mesangial cells, primary tubular epithelial cells and renal fibroblasts and is strongly enhanced by the previously described stimuli, such as IL-1 or TNF-α [30]. In addition, activation of tubular cells with IL-17 and CD40L, but not with IL-6 or IL-4, results in increased production of PTX3, whereas granulocyte macrophage-colony-stimulating factor inhibits PTX3 production. PTX3 may play also an important role in the modulation of glomerular inflammation. PTX3 expression is increased in the IgA, type I membranoproliferative, diffuse proliferative lupus glomerulonephritis and in membranous glomerulonephritis and focal segmental glomerular sclerosis. PTX3 is remarkably present in the mesangial, endothelial areas and inflamed interstitium in renal biopsies obtained from patients with these glomerulonephritis. Furthermore, exposure of mesangial cells to PTX3 leads to cell contraction and synthesis of the platelet-activating factor, which is a lipid mediator of inflammation [30]. The latest study showed that serum PTX3 is increased after successful renal transplantation [94].

reduce renal macrophage numbers but bound to macrophage FcγR which enhanced the phagocytic uptake of dead cells and IL-10 production. This process was associated with less intrarenal fibrogenesis in the post-obstructive kidney and obviously relates to defining the phenotype of intrarenal macrophages. Interestingly, transgenic mice that overexpress human CRP display increased intrarenal inflammation, tubular atrophy and interstitial fibrosis in the early phase (3d) but not in the late phase (14d) in post-obstructive kidneys [78]. The authors referred this effect to CRP’s capacity to induce pro-inflammatory mediators in macrophages (which could be a complement-dependent effect) and increased tumor-growth factor-β/Smad signalling. Even though both studies did not analyse the same time points, the data on CRP and SAP in the same model of post-obstructive renal fibrosis are discrepant and the reasons remain unclear. One possible explanation can be different binding affinities of CRP and SAP to the various stimulatory or inhibitory FcγRs [77].

Together, the short pentraxins CRP and SAP act like other humoral opsonins, whether they are antigen-unspecific like mannose-binding lectin, ficolins and complement or antigen-specific like antibodies. All these humoral components of immunity bind to extracellular particles such as pathogens, dead cells or dead cell debris and support their rapid clearance by phagocytosis, a process mediated by binding to FcγRs. This effect involves complement activation and other mechanisms of host defence. Therefore, the short pentraxins support host defence during infection but they also contribute sterile tissue inflammation in certain settings, conceptually similar to complement factors. In lupus nephritis, however, their protective role in dead cell clearance is often impaired, either by genetic variants, by interferon-γ-mediated suppression of transcription in hepatocytes or by specific autoantibodies that deplete CRP/SAP protein.
SUMMARY AND PERSPECTIVE

The family of the pentraxins contribute to the innate and antigen-unspecific humoral immunity which binds to circulating foreign or abnormal endogenous particles such as pathogens or apoptotic cells for their rapid clearance by phagocytes. In addition to this well-described opsonizing effect, the pentraxins have numerous additional regulatory functions during homeostasis and, most importantly, during infectious and sterile forms of inflammation. This explains their low expression under normal conditions and their massive induction by IL-1, IL-6 and TNF, the three central cytokines that trigger the systemic inflammatory response as general mechanisms of danger control. Beyond their expanding importance as biomarkers of systemic inflammation, the pentraxins evolve as important regulators of renal immunopathology. We have only started to learn about their functional contributions for acute and chronic forms of kidney injury. Therefore, focusing research efforts on CRP and SAP, but especially on the recently recognized long pentraxins, PTX3 and PTX4, offers the chance for unexpected discoveries.

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CONFLICT OF INTEREST STATEMENT

None declared.

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When to suspect a genetic disorder in a patient with renal stones, and why

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ABSTRACT

Nephrolithiasis is a common disorder, with a rising prevalence in the general population. Its pathogenesis is still unclear, but a role for genetics has long been recognized, especially in cases of the more common calcium nephrolithiasis. Although relatively rare, monogenic causes of hypercalciuria and nephrolithiasis do exist and their timely recognition is important from a prognostic and therapeutic viewpoint. This article reviews the clinical and laboratory findings characterizing inherited causes of nephrolithiasis with a view to helping clinicians to recognize and manage these rare conditions.

INTRODUCTION

Nephrolithiasis is a common disorder, affecting ~10% of individuals in Western countries [1], with a recurrence rate of 50% at 5–10 years; it requires the related frequent need for urological treatments [2] and it is a significant cause of morbidity.

Although ‘common’ forms of calcium oxalate nephrolithiasis and idiopathic hypercalciuria are complex polygenic disorders, with several genes contributing to their pathogenesis in as high as 50% of cases [3, 4], there are also a few infrequent or even very rare Mendelian monogenic renal stone conditions that are worth identifying because they carry an unfavourable prognosis (renal failure) and risk receiving an incongruous treatment. It is also important to identify these conditions in order to avoid patients being identified only after the disease has recurred in a transplanted kidney [5] (Table 1).

The diagnosis of these hereditary diseases can be challenging due to their rarity, shortcomings in physicians’ knowledge of inherited nephrolithiasis and the variability of the clinical phenotype, and also because their manifestations may be shared by different disorders, including an overlap with the much more frequently encountered common forms of nephrolithiasis. This delays the diagnosis of even very severe inherited conditions; for instance, Type 1 primary hyperoxaluria (PH1) is diagnosed on average 5 years after the initial onset of symptoms [6]. It is self-evident that a delayed diagnosis of such a severe condition is likely to have dramatic effects on the patient.

The aim of the present article is to discuss the clinical and laboratory findings that should alert clinicians to the possibility of a renal stone former having an inherited disease responsible for their lithogenesis.

PREVALENCE OF INHERITED RENAL STONES

Data on the prevalence of these rare conditions in the general population or among stone formers are vague. There could...