Serum Level of Osteopontin in Patients with CKD Stage 5 on Hemodialysis: An Updated Overview

Alaa M Nawar¹, Yasser A A Elhendy², Malak Nabil¹, May M. Sami³, Ahmed M Salah², & Hala M Allam²

¹Theodor bilhariz Research Institute, Cairo, Egypt.
²Department of Internal Medicine and Nephrology, Faculty of Medicine, Zagazig University, Alsharquia, Egypt.
³Department of Clinical Pathology, Faculty of Medicine, Zagazig University, Alsharquia, Egypt.

ABSTRACT

End stage renal disease (ESRD) is the fifth stage of renal failure necessitating dialysis or kidney transplantation. Chronic kidney disease leads to dysregulation of calcium (Ca²⁺), phosphorus (P), parathyroid hormone (PTH), and vitamin D metabolism, resulting in biochemical laboratory abnormalities, significant bone disease, and/or vascular calcification that define chronic kidney disease–mineral and bone disorder (CKD-MBD).

Even with careful monitoring of these markers, patients with ESRD on maintenance hemodialysis have poorer health outcomes related to mineral and bone disorder (MD, such as increased risk of developing cardiovascular disease and increased fracture risk, (KDIGO, 2017). While obtaining abdominal radiographs, computed tomography–based imaging, or dual-energy X-ray absorptiometry scans or performing a bone biopsy to help evaluate calcification and bone mineral density (BMD) status, the strength of recommendation of these practices are low.

Osteopontin (OPN) is a glycol-phosphoprotein found in bone, acute and chronic inflammatory cells, smooth muscle, epithelial, and endothelial cells, neurons and fetal renal tissue and is expressed in the thick ascending limb of the loop of Henle. Some of its functions include increasing macrophage and T-cell counts, perpetuation of inflammation, wound healing, tumor development and progression, roles in diabetes, and possible roles in the regulation of nephrolithiasis and nephrogenesis.

OPN was also found to promote angiogenesis, encourage growth and invasion of renal cancer, impact the development of lupus nephritis in patients with systemic lupus erythematosus, and potentially be useful as a marker of acute allograft rejection in kidney transplants. Local increases in OPN in vessel walls have been linked to atherosclerotic plaque formation, inflammation within arteries, and smooth muscle mineralization. The function of OPN in bone is defined by its ability to anchor osteoclasts via the α₅β₃ integrin.

Chronic Kidney Disease

Chronic kidney disease is becoming a major health problem worldwide, associated with significant morbidity and mortality for many patients, defined as abnormalities in the structure or function of the kidney that persist for> 3 months and have an impact on health. CKD can be
asymptomatic and early diagnosis of the disease is difficult (KDIGO, 2012). In undiagnosed and untreated people, CKD can gradually progress to end-stage kidney disease (ESRD), or end-stage CKD when expensive renal replacement therapy (RRT) through dialysis or a kidney transplant is necessary to save the patient's life. Can lead to various complications such as anemia and disorders of bone mineral metabolism and poor outcomes, including cardiovascular events, morbidity and mortality (Albalawi et al., 2018).

**Figure (1):** Prognosis of CKD by GFR and albuminuria category. (KDIGO, 2012).

**Epidemiology:**

Chronic kidney disease affects a large portion of the elderly, and the prevalence of chronic kidney disease has been increasing over the past two decades. Established risk factors for chronic kidney disease include age, gender, blood pressure, smoking, and diabetes. The increase in obesity, diabetes and hypertension is part of the reason for the increase in the prevalence of chronic kidney disease, but the epidemiological risk factors for chronic kidney disease have not been fully explained (Coresh et al., 2007).

Progressive renal disease affects people of all ages. The prevalence of CKD in patients under the age of 16 ranges from 1.5 to 3 per million. The prevalence of ESRD in elderly patients is 20 per million. (Locatelli et al., 2002).

**Causes:**

Kidney disease can be caused by any condition or disease that damages blood vessels or other structures in the kidneys. (Levey et al., 2003).

**The most common causes of chronic kidney disease are:**

**Diabetes:**

Diabetes causes about 35% of all chronic kidney disease. High blood sugar from diabetes can destroy the blood vessels. Over time, the kidney isn’t able to do its job as well. Later it may stop working completely. This is called kidney failure (Levey et al., 2003).

**Hypertension:**

[www.turkophysiotherrehabil.org](http://www.turkophysiotherrehabil.org)
High blood pressure damages the blood vessels in the kidneys. If the blood pressure remains high, the kidneys' function will gradually deteriorate. Another 30% of all kidney disease is caused by high blood pressure (hypertension). Because blood pressure commonly rises with chronic renal illness, high blood pressure can worsen kidney function, even if the disease was caused by another medical issue. The most common cause of chronic kidney disease, which leads to renal failure, is high blood pressure. High blood pressure may also speed up the progression of chronic kidney disease in someone who already has the disease (Levey et al., 2003).

Kidney diseases and infections:
Polycystic kidney disease, pyelonephritis, glomerulonephritis, or a kidney condition that has been present since birth, such as a constricted or blocked renal artery. (Yu, 2003).

Long-term use of medicines:
Long-term use of medicines that can damage the kidneys. Examples include Non-steroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen and celecoxib and certain antibiotics (Pinter et al., 2004).

Autoimmune causes of CKD:
Systemic Lupus Erythematosus (Lupus Nephritis):
In idiopathic systemic lupus erythematosus (SLE), renal involvement is prevalent; an abnormal urine analysis with or without an elevated plasma creatinine concentration is evident in a high number of patients at the time of diagnosis and may develop in up to 75% of cases. Proteinuria is the most commonly encountered abnormality. In SLE, there are several forms of renal disease that are usually distinguished by a renal biopsy, the most prevalent of which are immune complex-mediated glomerular disorders. (Kelley and Saurders, 2000).

Systemic Vasculitis:
Involvement of the kidneys is frequent in all kinds of systemic vasculitis. Classic polyarteritis nodosa, Wegener's granulomatosis, microscopic poly-arteritis, Churg-Strauss disease, and hyper-sensitivity vasculitis are just a few of them. (Savage, 2001).

Mixed Cryoglobulinemia (MC):
Glomerular disease may occur in those patients. The most common patterns are membranoproliferative glomerulonephritis (McGuire et al., 2006).

Sjögren's Syndrome:
Characterized histologically by an interstitial infiltrate that can invade and damage the tubules. Glomerular involvement is much less common than interstitial nephritis in Sjögren's syndrome. Membranoproliferative glomerulonephritis and membranous nephropathy are the most common (Kim et al., 2008). The pathogenesis of glomerular disease is unknown, including its possible etiologic relationship to Sjögren's syndrome, but it may be related to the deposition of circulating immune complexes. (Goules et al., 2000).
IgA Nephropathy:

The most common lesion found to cause primary glomerulonephritis is IgA nephropathy. (D’Amico, 2004). The mesangial deposition of IgA, which is predominantly polymeric IgA of the IgA1 subclass, is the initiating event in the pathogenesis of IgA nephropathy (polymeric IgA1-containing J chain). Codeposits of IgG and complement (C3 but usually not C1q) are also commonly seen and may contribute to disease severity. Mesangial deposition of secretory IgA has also been reported, but the pathogenic significance of this is unclear (Oortwijn et al., 2007).

Patients with IgA nephropathy typically present in one of three ways:

Approximately 40 to 50 percent present with one or recurrent episodes of gross hematuria, usually following an upper respiratory infection. Another 30 to 40 percent have microscopic hematuria and usually mild proteinuria and are incidentally detected on a routine examination.

Less than 10 percent present with either nephrotic syndrome or acute rapidly progressive glomerulonephritis picture characterized by edema, hypertension, and renal insufficiency as well as hematuria. (Donadio and Grande, 2002)

Antiphospholipid Syndrome (APS):

One of the organs that can be harmed by Antiphospholipid antibodies is the kidney. Renal complications caused by thrombotic events caused by these antibodies include glomerular disease, large vessel renal involvement, and coagulation issues associated with dialysis and renal transplants. (Joseph et al., 2001).

Pathology and Pathogenesis:

Development of chronic renal failure:

Chronic renal disease pathogenesis differs greatly from acute kidney disease pathogenesis. Whereas acute kidney injury causes death and sloughing of tubular epithelial cells, which is often followed by regeneration and re-establishment of normal architecture, chronic injury causes irreversible loss of nephrons. (Block et al., 2004). As a result, fewer nephrons bear a greater functional burden, which manifests as an increase in glomerular filtration pressure and hyperfiltration. For unknown reasons, this compensatory hyperfiltration, which can be thought of as a form of hypertension at the nephron level, predisposes to fibrosis and scarring (glomerular sclerosis). As a result, the nep rate has increased. (Go et al., 2004).

Pathogenesis of uremia:

Chronic renal failure is caused in part by a toxic effect of retained products normally excreted by the kidneys (e.g., nitrogen-containing products of protein metabolism), or normal products such as hormones now present in increased amounts, or loss of normal kidney products (e.g loss of erythropoietin) (Szczech, 2004). Excretory failure results also in fluid shifts with increased intracellular Na+ and water with decreased intracellular K+. These alterations may contribute to alterations in function of a host of enzymes transport systems and so on (Go et al., 2004). Finally, uremia has a number of effects on metabolism that are currently not well understood including a decrease in basal body temperature and diminished lipoproteins lipase activity with accelerated atherosclerosis (Block et al., 2004).
Signs and symptoms of CKD:

CKD is initially without specific symptoms and is generally only detected as an increase in serum creatinine or protein in the urine. As the kidney function decreases:

Because of fluid overload and the production of vasoactive hormones by the kidney via the renin-angiotensin system, blood pressure rises, increasing the risk of developing hypertension and/or suffering from congestive heart failure. (Hruska et al., 2008).

Urea accumulates, causing azotemia and, eventually, uremia (symptoms ranging from lethargy to pericarditis and encephalopathy). Because of its high systemic circulation, urea is excreted in high concentrations in eccrine sweat and crystallizes on skin as the sweat evaporates ("uremic frost") (Honda et al., 2007).

Potassium stays in the blood (hyperkalemia with a range of symptoms including malaise and potentially fatal cardiac arrhythmias). Hyperkalemia usually does not occur until the glomerular filtration rate falls below 20-25 ml/min/1.73 m2, at which point the kidneys' ability to excrete potassium is compromised.

Acidemia (which causes extracervation) can aggravate hyperkalemia in CKD. (Hruska et al., 2008). Erythropoietin synthesis is reduced, resulting in anemia. (Hruska et al., 2008).

The symptoms of fluid volume overload can range from mild edema to life-threatening pulmonary edema. (Faul et al., 2011).

Hyperphosphatemia, which occurs as a result of decreased phosphate excretion after a decrease in glomerular filtration, is associated with increased cardiovascular risk, as it is a direct stimulus to vascular calcification. Furthermore, as renal capacity for phosphate excretion declines, circulating concentrations of fibroblast growth factor-23 (FGF-23) rise. However, in CKD patients, this adaptive response may contribute to left ventricular hypertrophy and increased mortality. (Gutiérrez et al., 2008).

Hypocalcemia as a result of 1,25 dihydroxyvitamin D3 deficiency (caused by FGF-23 stimulation and decreased renal mass) and resistance to the calcemic action of parathyroid hormone. Osteocytes are responsible for increased FGF-23 production, which is a powerful inhibitor of the enzyme 1-alpha-hydroxylase (which converts 25-hydroxycholecalciferol into 1,25 dihydroxyvitamin D). Later, this progresses to secondary hyperparathyroidism, renal osteodystrophy, and vascular calcification that further impairs cardiac function. An extreme consequence is the occurrence of the rare condition named calciphylaxis (Bacchetta et al., 2012).

The term chronic kidney disease-mineral bone disorder (CKD-MBD) refers to a broader clinical syndrome caused by CKD that manifests as a systemic disorder of mineral and bone metabolism manifested by either one or a combination of: calcium, phosphorus (phosphate), parathyroid hormone, or vitamin D metabolism abnormalities, abnormalities in bone turnover, mineralization, volume, linear growth, or strength (renal osteodystrophy); and vascular or other soft-tissue calcification. CKD-MBD has been associated to poor hard outcomes (Brandenburg et al., 2011).
Metabolic acidosis is a common condition associated with progressive kidney function loss. The kidneys' declining ability to maintain acid–base homeostasis results in acid accumulation, which causes a variety of complications such as nutritional impairment, worsened uremic bone disease, and an increased risk of death. Aside from the adverse consequences associated with acid retention, metabolic acidosis may also cause kidney damage, possibly by stimulating adaptive mechanisms aimed at maintaining acid-base homeostasis in the face of declining kidney function. Recent clinical trials have suggested that correcting or preventing metabolic acidosis through alkali administration can reduce kidney damage and slow the progression of chronic kidney disease, and thus may offer an effective, safe, and cost-effective renoprotective strategy. (Moe et al., 2006).

Although anemia in CKD can be caused by a variety of mechanisms (iron, folate, or vitamin B12 deficiency; gastrointestinal bleeding; severe hyperparathyroidism, systemic inflammation, and shortened red blood cell survival), decreased erythropoietin synthesis is the most important and specific cause of CKD-associated anemia. Erythropoietin is a glycoprotein secreted by kidney interstitial fibroblasts that is required for red blood cell growth and differentiation in the bone marrow. Tubular atrophy in CKD causes tubule interstitial fibrosis, which impairs renal erythropoietin synthesizing capacity and results in anemia. (Ratcliffe, 1993).

People with CKD have accelerated atherosclerosis and are more likely than the general population to develop cardiovascular disease. Patients with CKD and cardiovascular disease have significantly worse prognoses than those with only cardiac disease. (Damman et al., 2014).

Sexual dysfunction is particularly common in CKD patients, both men and women. The majority of men have decreased sex drive, difficulties getting an erection, and achieving orgasm, and these issues worsen as they become older. (Vecchio et al., 2010).

Complications:

Cardiovascular Disease in ESRD:

Cardiovascular disease is a major source of morbidity and mortality in maintenance dialysis patients, accounting for about half of all deaths. Kidney function is inversely and independently related with cardiovascular morbidity and death, particularly with estimated glomerular filtration rate (eGFR)5 ml/min per 1.73 m2. (Matsushita et al., 2010). Patients with CKD stages 3-5 and known vascular/valvular calcification should be considered at highest cardiovascular risk, according to the KDIGO work group. (Katrin et al., 2010).

Compared to the general population, dialysis patients have a higher prevalence of various types of cardiac disease. For example, arrhythmic mechanisms or sudden cardiac arrest are the single most common specific cause of death, accounting for approximately 60% of all cardiac deaths. Nevertheless, dialysis patients have a higher rate of death from myocardial infarction and a higher incidence of coronary artery disease (CAD) than those who do not have kidney disease. (USRDS, 2010). CAD shows a diffuse multi-vessel involvement pattern with coronary calcification According to small angiographic studies, this incidence exceeds 50% in unselected CKD 5D patients. (Ohtake et al., 2010).
Mineral and Bone Disorders:

CKD is almost constantly associated with a systemic disorder of mineral and bone metabolism (Levin et al., 2007).

**Chronic kidney disease-associated haematological disorders:**

**Anemia:**

Anemia is defined as a decrease in one or more of the major red blood cell measurements, which are haemoglobin concentration, haematocrit, or red blood cell count. Anemia is defined by the World Health Organization as a haemoglobin level of less than 13 g/dL in men and postmenopausal women, and less than 12 g/dL in premenopausal women. Anemia is defined by the NKF as hemoglobin levels of less than 13.5 g/dL in men and less than 13.5 g/dL in women. And less than 12.0 g/dL in women (KDOQI, 2006).

Anemia in chronic kidney disease increases morbidity and mortality from cardiovascular complications (angina, left ventricular hypertrophy, and worsening heart failure), which may lead to further deterioration of renal function and the establishment of a vicious cycle known as the "cardio renal anemia syndrome." Anemia is an independent predictor of death in patients with stable coronary artery disease and CKD. (Muzzarelli and Pfisterer, 2006). The target level of hemoglobin (Hb) in patients with CKD has changed as more studies have been reported.

Normalization of haemoglobin levels is no longer considered the goal of therapy because these target levels have been linked to increased mortality. The Correction of Haemoglobin and Outcomes in Renal Insufficiency trial looked at the effects of anemia treatment in over 1400 CKD patients (eGFR between 15 and 50 mL/min per 1.73 m2) with a haemoglobin of 11 g/dL at the start. Enrolled subjects were assigned at random to erythropoietin therapy treatment protocols aimed at achieving target haemoglobin levels of 13.5g/dL (n=715) or 11.3g/dL (n=717). The study was closed due to higher mortality rates and adverse events in the group with higher targeted Hgb levels. (Singh et al., 2006).

Platelet function changes in chronic uraemia patients include decreased sensitivity to platelet agonists and adhesion to strange surfaces, decreased procoagulant activity and thromboxane A2 synthesis, decreased platelet clot retraction time, and decreased platelet glycoprotein Ib expression and increase of cyclical adenosine monophosphate (cAMP). Platelet dysfunction is caused, at least in part, by an excess of some toxins, including urea and other components such as guanidine succinic acid and phenols. (Rios et al., 2010).

**Immunological Alterations:**

Chronic kidney disease is associated with severe immune system alterations. Infections are a major cause of death in haemodialysis patients, and vaccination is mostly ineffective, especially in advanced stages of chronic kidney disease. Although vaccine advancements may reduce nonresponse rates in haemodialysis patients, whenever possible, it is preferable to vaccinate the patients in the early stages of renal disease, when the primary immune response has not been sensibly diminished (Kong et al., 2007). In HD patients, acquired immunity disturbances primarily affect the T-lymphocyte and the antigen-presenting cell (APC). Patients with chronic renal failure have a faulty costimulation function derived from APCs, which leads to impaired activation of effector lymphocytes. (Eleftheriadis et al., 2007).
Nutritional Issues:

In renal diseases, malnutrition and wasting are common. An expert panel recommends the term protein–energy wasting to describe the loss of body protein mass and fuel reserves in patients with chronic kidney disease and end-stage renal disease. (Fouque et al., 2008).

Osteopontin (OPN)

Osteopontin (OPN) is an extracellular matrix protein first identified in bone tissue and has pleiotropic functions due to its common expression in the main organs and apparatuses; also known as secreted phosphoprotein 1 (SPP-1), uroprotein or early T lymphocyte activation-1 (Eta-1), it is a glycoprotein that is highly phosphorylated, composed of 314 amino acids. (Icer & Gezmen-Karadag, 2018). OPN is a protein from the small integrin-binding ligand N-linked glycoprotein (SIBLING) family that was discovered in 1985 as a major sialoprotein of bone that is involved in biomineralization and remodelling. (Kruger, Miller, Godwin, & Wang, 2014).

OPN has a “double-faced” role in the kidney as well, especially for its dual role in physiological and pathological signalling. (Z. Xie, Singh, & Singh, 2004). Most of studies have shown that OPN mRNA and protein levels are elevated in a variety of renal disorders, including interstitial fibrosis, stone formation, acute ischemic renal injury, and others. (Bostan Gayret et al., 2018).

Significantly, all of these studies show a link between OPN protein levels and fibrosis, macrophage infiltration, proteinuria, and decreased creatine clearance, implying that OPN could be used to treat a variety of renal disorders. Indeed, OPN is pathologically present in various types of acute ischemia renal injury, with a significant rise in distal tubular cells, which are the primary cell type injured during tubulointerstitial nephritis. (B. J. I. R. Kaleta, 2019).

Osteopontin, which is released by osseous cells, also influences the actions of monocyte-derived cells, such as phagocytic macrophages, which are engaged in the resorption process. Moreover, it was discovered that OPN reduces hydroxyapatite accumulation and crystal development; as a result of these findings, OPN is regarded a biomineralization inhibitor. (Giachelli & Steitz, 2000).

Multiple research in recent years have found that OPN is not only linked to bone metabolism, but also works as an immune response regulator. (Murugaiyan, Mittal, & Weiner, 2008). OPN stimulates antibody synthesis by B cells, regulates nitric oxide generation, and increases interleukin (IL)-17 production by T helper (Th) 17 cells, as well as enhancing the Th1-mediated inflammatory process. (Murugaiyan et al., 2008). Apoptosis, angiogenesis, and cancer progression are all influenced by this protein. (Rangaswami, Bulbule, & Kundu, 2006).

Osteopontin is a useful marker for autoimmune illnesses such as systemic lupus erythematosus, multiple sclerosis, rheumatoid arthritis, inflammatory bowel disease, asthma, and liver disease, according to numerous studies. (Konno et al., 2011). Studies performed in
recent years provided new insights into the role of OPN in the pathogenesis of various kidney diseases.

Osteopontin: gene, structure, signaling

The human OPN gene is located on chromosome 4 (4q21–4q25), and it has seven exons and six introns.

The 5′-untranslated (5′-UTR) region contains exon 1, The 3′-UTR region consists of the last part of exon 7 and includes three polyadenylation sequences. Exons 2–7 contain coding sequences. Exon 7 contains the arg-gly-asp (RGD) motif and the central thrombin cleavage site. (Clemente et al., 2016)

Structurally, OPN protein, contains two fragments: N-terminal (which includes integrin receptor binding zones) and C-terminal (which binds two heparin molecules and CD44 variants). (Standal, Borset, & Sundan, 2004). OPN protein is composed of 314 amino acids. The molecular weight of OPN is between 41 and 75 kDa. (Clemente et al., 2016). It can be explained by multiple post-translational modifications, including serine and threonine phosphorylation, O-linked glycosylation, transglutamination, sialylation and tyrosine sulfation, these modifications affect OPN size and its functions. (Kazanecki, Uzwiak, & Denhardt, 2007), to date, three forms of human OPN transcript were identified: OPN-a (the full-length), OPN-b (without exon 5) and OPN-c (without exon 4). (Gimba & Tilli, 2013).

OPN acts as a regulator of the immune response, in addition to bone metabolism. Indeed, this protein has chemotactic properties for macrophages, dendritic and T cells, thanks to its interactions with multiple surface proteins localized in this cell’s targets (Clemente et al., 2016). The main interactions occur through integrin receptor binding that directly activates the nuclear factor kappa B (NFkB) in the intracellular space, OPN also can regulate the immune system through surface interaction with the CD44 receptor, able to stimulate T-cell chemotaxis, adhesion, and inhibition of interleukin (IL)-10 release by the macrophages. (Kiefer et al., 2008)

These interactions are principally mediated by the two different terminal zones (the N and C terminal, respectively), aimed to bind different ligands. (Herum et al., 2020). The C terminal interacts with two heparin molecules or CD44 variants, whereas the N terminal is involved in the interaction with integrin receptors. The C-terminal fragment is involved in macrophage chemotaxis. (Icer & Gezmen-Karadag, 2018).While the N terminal is especially implicated in the regulation of hematopoietic progenitor cell homing and in the secretion of interferon (IFN)-γ by the T cells. (Gomez-Ambrosi et al., 2007).
Figure (6): Structure of osteopontin

Structural features of the osteopontin (OPN) full-length secreted isoform with six translated exons (314 amino acids). OPN isoforms include the following functional domains: Arginine- glycine- aspartic acid (RGD) domain (159–161), serine-valine- valine- tyrosine-glutamate-leucine-arginine (SVVYGLR) domain (162–168), thrombin cleavage site, calcium binding domain (216–228), and heparin binding domain. Integrin binding occurs at RGD and SVVYGLR sequences. CD44 variant receptor binding occurs near the C-terminus and N-terminus. Phosphorylation sites exist across the precursor-mRNA (pre-mRNA). All OPN isoforms have thrombin and matrix metalloproteinase (MMP) cleavage sites.

Osteopontin in kidney physiology

OPN is expressed in both fetal and mature renal tissue. In the fetal kidney, OPN can be found in renal tubular epithelium, as well as in the ureteric buds and in some interstitial cells. Its expression is upregulated after 75–80 days of gestation. (Hudkins et al., 1999). In the normal adult kidney, OPN is expressed by the thick ascending limb of the loops of Henle. Moreover, OPN expression is observed in collecting duct epithelium, where it correlates with the number of macrophages in the tissue. (Y. Xie et al., 2001).

Multiple factors increase OPN production, including hormones (parathyroid hormone, PTH), calcitriol, calcium, phosphate, cytokines (tumour necrosis factor alpha, TNF-α), high-protein and high-fat diet. In contrast, estrogen, estradiol and progesterone are factors which inhibit OPN expression. (Y. Xie et al., 2001). As described earlier, OPN interacts with multiple surface proteins, which are localized in various tissues and organs, including human kidneys. (Green, Ludbrook, Miller, Horgan, & Barry, 2001). The ανβ3 and ανβ5 integrins are found in Bowman’s capsule and glomerular epithelial cells. β1 integrin is expressed in glomerular and tubular epithelial cells, Bowman’s capsule, as well as vascular epithelium. (Y. Xie et al., 2001).

The CD44 OPN receptor is expressed in a distal tubule (Hara et al., 1999). The role of OPN in normal human kidney is not fully understood, but some studies suggested that the protein is essential for tubulogenesis. (Hara et al., 1999). Multiple studies provided new insights into the protective role of OPN in renal stone formation.

It was found that OPN is able to inhibit the nucleation, growth, and aggregation of calcium oxalate crystals in vitro (Hoyer, Otvos Jr, & Urge, 1995). Moreover, it was demonstrated that patients with kidney stones have lower urinary excretion of OPN than healthy controls, in addition, it was suggested that some functional polymorphisms in the OPN
gene may predispose to urolithiasis formation. (Liu et al., 2010). Therefore, it was proposed that single nucleotide polymorphism (SNiP) genotyping, together with determination of urinary OPN concentration, could be helpful in detection of kidney stones formation. However, in contrast to these reports, some studies revealed that urine OPN levels did not differ between healthy controls and patients with renal stones (Tsuji et al., 2007)

Biomineralization

OPN is one of the most abundant non-collagenous proteins in bone. Because of its abundance in bone, OPN has been studied as a regulator of biomineralization. OPN is a potent inhibitor of mineralization, prevents ectopic calcification, and is an inducible inhibitor of vascular calcification. OPN binds hydroxyapatite and calcium ions thereby physically inhibiting crystal formation and growth in. OPN also plays a role in osteoclast differentiation and osteoblast recruitment and function. OPN functions in osteoclast migration to sites of resorption and is crucial for normal resorption and bone turnover (wei et al., 2017).

OPN appears also to be an important regulator of vascular calcification and is associated with mineralized deposits in humans. Vascular calcification is now recognized as a marker of atherosclerotic plaque burden as well as a major contributor to loss of arterial compliance and increased pulse pressure seen with age, diabetes, and renal insufficiency. These findings suggest that OPN may be an important inhibitor of arterial mineral deposition under conditions of injury and disease, and that strategies to replenish OPN might be useful to prevent or treat ectopic calcification, including vascular calcification (Bozic et al., 2018).

Kidney cancer

Multiple studies suggested that OPN plays an important role in the growth and invasion of human renal cancer. (Funakoshi, Lee, & Hsieh, 2014). OPN is associated with increased proliferation and invasion of human renal cancer. (Liu et al., 2010). The expression of OPN in normal renal tissue and in clear cell renal cell carcinomas (CRCCs). The group demonstrated a strong correlation between protein overexpression and progression of CRCC. (Matusan et al., 2006).

High OPN expression is associated with poor progression-free survival in patients with CRCC. (Rabjerg et al., 2016). It is likely that OPN promotes carcinogenesis and metastasis by induction of matrix metalloproteinase (MMP)-2, 3 and urokinase-type plasminogen activator (uPA). (Rangaswami et al., 2006). In addition, OPN inhibits apoptosis of cancer cells, promotes formation of new blood vessels and enhances macrophage infiltration. (Wai, Kuo, & Reviews, 2008). OPN can bind to transformed cells via RGD domain and thus can enhance their survival by inhibition of nitric oxide synthesis (Lund, Giachelli, Scatena, & signaling, 2009).

Immunoglobulin A nephropathy

It also known as synpharyngitic glomerulonephritis or Berger’s disease. The most characteristic feature of this disorder is deposition of IgA in the glomerular mesangium (Magistroni, D’Agati, Appel, & Kiryluk, 2015). The pathogenesis of IgAN is not fully explained. There are reports suggesting that OPN is involved in the development of this nephropathy, however, some studies yielded opposite results. OPN and CD44 receptor are highly expressed in tubular cells and interstitial infiltrating cells in areas of tubulointerstitial injury. (Sano, Kitazawa, & Sugisaki, 2001). Another study conducted in children with IgAN
showed that the urine level of OPN was higher in patients than in healthy controls and was associated with high OPN/creatinine ratio (Wasilewska, Taranta-Janusz, Kuroczycka-Saniutycz, & Zoch-Zwierz, 2011).

It was found that during IgAN development, increased OPN mRNA expression correlates with macrophage infiltration. (Thomas, Harris, Walls, Furness, & Brunskill, 2002), (Gang et al., 2001) reported that the N-half (trombin cleaved) OPN in urine of IgAN patients correlates with albuminuria. Urinary OPN excretion and OPN mRNA expression in proximal tubules in a group of IgAN patients. (Kaimori et al., 2002). However, no association between OPN mRNA expression or OPN urine level and clinical data or pathological findings in glomeruli and tubulointerstitial regions was found. Furthermore, the concentration of two forms of OPN (full and N-half) in plasma and urine of IgAN patients and healthy controls was measured in a study of (Kitagori et al., 2016), but the authors found no significant difference in OPN levels between these two groups.

**Minimal change disease and focal and segmental glomerulosclerosis**

Minimal change disease (MCD) is one of the most prevalent glomerular diseases in children (Wenderfer & Gaut, 2017). It is characterized by selective proteinuria, hypoalbuminemia, hypercholesterolemia, and absence of glomerular immune deposits or cellular infiltrates in the biopsy. In MCD, the podocytes injury is observed. The disease may evolve to focal and segmental glomerulosclerosis (FSGS). FSGS affects both children and adults and can be distinguished from MCD by steroid resistance, non-selective proteinuria, loss of podocytes number and progressive kidney damage. (Bertelli, Bonanni, Caridi, Canepa, & Ghiggeri, 2018). Despite intensive research, the pathogenesis of MCD and FSGS is only partly understood.

The association between OPN and these pathologies was investigated in several studies, (Wasilewska et al., 2011) demonstrated that urinary OPN/creatinine ratio in children with MCD and FSGS was higher than in the control group. Moreover, in FSGS patients, the ratio was higher than in MCD group and correlated with interstitial changes and mesangial expansion. In another study, a positive correlation of OPN mRNA expression in proximal tubules and urinary OPN excretion was shown in MCD patients. (Gang et al., 2001). There was no significant difference in urine OPN levels between MCD patients and healthy controls. (Kitagori et al., 2016). However, the plasma OPN concentration was higher in the MCD group. (Gang et al., 2001) obtained opposite results and showed that urinary excretion of OPN in patients with MCD did not differ significantly from healthy controls. Furthermore, the group analyzed the size of urine OPN fragments. A 34-kD fragment (N-half) was detected in MCD patients, but not in controls.

The N-half OPN in urine correlated positively with albuminuria. In another study by Mezzano et al., no OPN expression in renal biopsy specimens from MCD patients was found (Mezzano et al., 2001). Due to few studies, evidence for the role of OPN in the pathogenesis of MCD and FSGS is still inconclusive. Therefore, further analyses are necessary.

**Membranous glomerulonephritis**

Membranous glomerulonephritis (MGN) is the second most common (after FSGS) cause of nephrotic syndrome in adults. It is a slowly progressive disease of the kidneys. (Cattran &
In the MGN course, proteinuria and edema (with or without renal failure) are observed, however, some patients may be asymptomatic. The primary MGN is a disorder of autoimmune origin, but the disease can be also secondary, associated with tumors, infectious or autoimmune diseases and drugs. (Hoxha, von Haxthausen, Wiech, & Stahl, 2017).

The pathogenesis of MGN is associated with formation of immune complexes in sub epithelial sites, which initiates complement activation and glomerular damage. (Glassock, 2009). In most cases, deposits develop as a result of binding of circulating antibodies to endogenous antigens expressed on podocytes, or circulating pathogenic antigens planted in the sub epithelial sites. Moreover, it is possible that autoantibodies bind to podocyte membrane antigens and lead to sub epithelial deposition of immune complexes. (Couser, 2012). However, the autoimmune basis of MGN is not fully understood.

A wide spectrum of immune mediators is currently investigated in the context of MGN. One of them is OPN. Two recent studies demonstrated that patients with progressive and nonprogressive MGN had an overexpression of OPN in the proximal tubules (Mezzano et al., 2001). Moreover, a strong correlation between the mRNA and the protein was found.

High expression of OPN in kidneys was associated with increased infiltration of macrophages, as well as CD4+ and CD8+ T cells. In addition, OPN activates NFκB, which results in increased expression of proinflammatory cytokines (including transforming growth factor β (TGF-β)), which can contribute to glomerular damage. (Mezzano et al., 2000). Thus, it was suggested that OPN could be a potential predictor of primary MGN progression. However, only these few studies were conducted to investigate the role of OPN in MGN, therefore further investigation in this field is indispensable.

Lupus Nephritis

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, caused by endogenous and exogenous factors. Deregulation of the immune system leads to production of autoantibodies, which form complexes with antigens and are deposited in various organs.

Lupus nephritis (LN) is one of the most serious manifestations of SLE. (Ikeuchi et al., 2016). Many studies demonstrated that OPN level is increased in the plasma and urine of SLE patients and may correlate with disease pathogenesis and clinical manifestations (reviewed in (B. J. A. i. e. t. e. Kaleta, 2014). In a study of Wong et al. (Wong, Lit, Tam, Li, & Lam, 2005), it was found that plasma OPN concentration is significantly higher in SLE patients with renal impairment than in patients without LN and in healthy controls. Moreover, increased OPN correlated positively with SLE Disease Activity Index (SLEDAI), as well as with IL-18 level. Similar findings were reported in children with SLE (Afify, Mohamed, El-Maboud, & Abdel-Latif, 2009).

Another study was conducted in LN patients, (Kitagori et al., 2016) measured the urine and plasma concentration of the full and N-half OPN but found no difference in urine full OPN concentration between patients and healthy controls. However, the levels of plasma full and N-half OPN were significantly higher in LN patients. Moreover, urine N-half OPN correlated positively with urine thrombin activity. In addition, the study revealed that plasma and urine full and N-half OPN concentrations were not associated with SLEDAI and estimated glomerular filtration rate (eGFR).
The authors suggested that urine N-half OPN is associated with renal inflammation and may be a good prognostic marker for LN. Another study showed that OPN expression in LN patients was higher than in healthy controls and correlated positively with intrarenal macrophage infiltration. (Ma, Jiang, Li, Sun, & Wei, 2016). Moreover, the level of OPN was higher in active LN than in the inactive LN. Increased serum OPN level in LN patients was also demonstrated by (Salimi et al., 2016).

Few studies demonstrated that some OPN SNiP may be associated with LN. A study of (Forton, Petri, Goldman, & Sullivan, 2002), showed that the T allele of the rs1126616 polymorphism is associated with renal insufficiency in SLE patients. Similarly, (Salimi et al., 2016) genotyped the rs1126616 SNP in SLE patients and showed that the frequency of CT and TT genotypes was higher in SLE patients with LN compared to those without LN. This suggests that the T allele of this polymorphism is a risk factor for renal damage in SLE patients. In another study, (Xu, Liang, Lü, Li, & Wang, 2007) demonstrated that 9250 C>T OPN gene polymorphism is associated with the susceptibility to LN (the TT genotype was lower in SLE patients with LN). In conclusion, a large body of data indicates that OPN and some of OPN gene polymorphisms have an impact on LN development in SLE patients.

Diabetic nephropathy

Diabetic nephropathy (DN) or diabetic kidney is a serious complication of diabetes. It is characterized by the presence of albuminuria, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) (Lim & Disease, 2014). (Kitagori et al., 2016) measured the urine and plasma concentration of the full and N-half OPN in patients with DN. The study demonstrated that the level of the full OPN in plasma was significantly higher in patients than in healthy controls. However, there was no difference in urine OPN levels.

Yamaguchi et al., (2004) evaluated whether plasma and urine OPN concentrations in patients with type 2 diabetes (T2D) are associated with DN. They found that plasma OPN level is increased during DN progression, especially in the stage of renal failure, but in contrast, urinary OPN was not associated with renal disease in diabetic patients. The aim of another study was to assess possible associations between OPN (full and N-half) and nephropathy in patients with T2D. (Yan et al., 2010).

Similar to study of Yamaguchi et al., plasma levels of the full OPN were significantly higher in diabetic patients compared with controls. In addition, there was a higher frequency of moderate renal insufficiency and lower eGFR in patients with type 2 diabetes mellitus. Furthermore, an inverse correlation of OPN level and eGFR was observed. Moreover, the group found a significant association of the full OPN, but not N-half, and the severity of DN. Serum OPN concentrations were also measured in adult patients with type 1 diabetes (T1D). (Gordin et al., 2014). It was showed that OPN was independently associated with the development of microalbuminuria and was a strong predictor of incipient DN. The aim of another study, conducted in pediatric patients with T1D was to evaluate serum OPN concentrations and its role in renal failure. (Talat et al., 2016).

Diabetic patients had higher OPN levels than healthy controls. Moreover, OPN concentrations were higher in patients with microalbuminuria compared to those with normal albumin excretion. In addition, OPN levels correlated positively with higher systolic and
diastolic blood pressure, body mass index (BMI) and lower high-density lipoprotein (HDL) concentration. Most of the results indicate that plasma full length OPN, but not urine or N-half OPN, might be a good marker for the susceptibility to DN. In addition, recent studies demonstrated that some OPN gene polymorphisms might be associated with a higher risk of kidney failure in diabetic patients, (Cheema et al., 2012) examined the association of OPN gene promoter polymorphism C-443T (rs11730582) with DN in Asian Indians. The group found a higher risk of DN among carriers of T allele and TT genotype. Moreover, the T allele correlated with higher proteinuria and lower eGFR. These results are in agreement with the study of Nicholas et al. in which increased OPN levels were associated with proteinuria as well as DN. It was showed that rs11730582 polymorphism affects OPN expression (Nicholas et al., 2010). These results indicate that OPN gene polymorphisms and their haplotypes might be a good marker of the susceptibility to DN. However, further studies are needed to increase our understanding of the OPN gene’s role in disease pathogenesis.

Renal allograft rejection

Kidney transplantation is the most effective treatment for end-stage renal disease (Giacopelli et al., 2004), but the graft rejection still affects kidney viability and patient survival. Therefore, there is growing interest in finding non-invasive and early biomarkers for monitoring immune status of transplant recipients. One of the analysed markers is OPN. OPN mRNA and protein were found to be elevated in renal transplant biopsies from patients with acute allograft rejection. (Alchi et al., 2005). Moreover, it was demonstrated that OPN CD44 receptor was upregulated during rejection episodes. In addition, higher levels of serum OPN were associated with the lower probability of rejection-free survival of patients. (Rouschop et al., 2006).