

SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

**Investigations into Haematological Disorders and Their  
Treatment in Kidney Diseases**

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## **Introduction**

Haematological diseases are pathologies of myeloid or lymphoid origin caused by a disturbance of normal haematopoiesis. We distinguish between malignant and premalignant diseases and those with a benign course. The most frequent malignant haematological diseases with high morbidity and mortality are lymphoma, leukaemia and multiple myeloma, which are the leading malignancies in the overall ranking of cancer diseases in terms of both incidence and mortality. It is also important to highlight the high prevalence of premalignant monoclonal gammopathy of undetermined significance (MGUS), which is a disease with a better prognosis that does not require haematological treatment. The incidence is increasing, but the mortality associated with these conditions is decreasing due to better diagnosis, early detection and earlier treatment. As the incidence increases, so does the prevalence of other organ involvement associated with the malignancies.

Renal involvement is an almost constant feature of haematological malignancies and its incidence is generally underestimated. The aetiology is usually multifactorial and may be associated with the patient (co-morbidities, medications), the tumour itself and its treatment. In about half of cases, kidney damage is an acute kidney injury (AKI). This is most often associated with treatment drugs or tumour lysis syndrome (TLS) or a prerenal cause unrelated to the underlying disease - volume depletion, sepsis. Damage may affect other structures of the kidney beyond the renal tubules. Rarely, a postrenal, obstructive origin is also suspected. In the other half of the cases, renal involvement is not associated with acute kidney injury, but may present as a nephrotic syndrome, chronic kidney disease (CKD). This includes, for example, paraneoplastic glomerulopathies, kidney diseases associated with MGUS.

Establishing the exact cause of renal damage in both AKI and CKD is important, often requiring renal biopsy, even in the case of known haematological diseases. Once this is known, patients can receive targeted treatment, thereby reducing the extent of kidney damage. The histopathological result can influence the choice of chemotherapy treatment, its dose and when to start. In its absence, the chance of renal remission may be reduced and mortality may significantly be increased. Acute kidney failure – even requiring dialysis, or the development of chronic/end-stage renal disease – leads to a deterioration in the long-term quality of life of patients and places a greater burden on the health care system, hence the importance of early detection and adequate treatment. For this reason, a multidisciplinary approach and haemato-onco-nephrology collaboration is essential.

## **Literature Review**

### **Epidemiology of Renal Diseases Associated with Haematological Disorders**

Renal impairment is an almost constant feature of haematological disorders. Accurate data are only available in relation to AKI. The largest study of cancer patients during follow-up measured the 1-year risk of acute kidney injury at 17.5% and the 5-year risk at 27%. AKI was most commonly associated with myeloma, leukemia and lymphoma, with a 1-year risk of AKI in these diseases of 31.8% - 27.5% - 18.8%, and a 5-year risk of AKI of 52.7% - 40% - 30.8%. In the study population, 5.1% of patients required dialysis within 1 year of AKI diagnosis. These data are confirmed by a study in which every tenth patients treated with cancer developed acute kidney damage, even requiring dialysis. Kitchlu *et al* showed that although they were not the leading tumour pathologies, multiple myeloma and leukaemia were most commonly implicated in the development of acute kidney injury. The mean incidence of AKI in the patient population was 6.7%, while an incidence of 21% was found in patients with myeloma. The 5-year cumulative incidence of AKI also showed a marked difference, with 7.8% in the study patient population compared to 26% in patients with myeloma.

There is lack of data on chronic kidney disease developing from acute kidney injury or associated with a specific haematological pathology. CKD is only reported as a comorbidity, a predisposing factor to AKI or a treatment-related factor in relation to renal impairment associated with cancer. There is little mention of direct or indirect (immunopathomechanism) damage in haematological pathologies. There are only case reports on paraneoplastic glomerulopathy, no major studies or epidemiological data are available. The prevalence of monoclonal immunoglobulin-related renal disease associated with MGUS is 2-10% among patients diagnosed with MGUS. In a meta-analysis study, 41% of renal biopsies for MGUS were found to be MGRS (monoclonal gammopathy of renal significance), while in another cohort study, monoclonal gammopathy was the cause of renal impairment in more than 50%.

### **Causes of Kidney Impairment in Haematological Diseases**

Kidney damage in cancer is generally underestimated. The aetiology is multifactorial, and the underlying cause of kidney disease may be associated with either the patient (co-morbidities, medications) and the cancer itself, or its treatment, either directly or indirectly; other damage (e.g. TLS) may occur during treatment. Most commonly, acute kidney damage associated with treatment or drugs used during TLS, or kidney damage of prerenal origin - volume depletion, sepsis - unrelated to the underlying disease. The effects of other drugs (RAS inhibitors,

NSAIDs) used in addition to prerenal renal impairment may also contribute to the development of renal disease.

Renal impairment may be caused by involvement of any structure of the nephron (blood vessels, tubulointerstitium, glomerulus). Rarely, a postrenal, obstructive origin is also suspected. Identifying the exact cause of kidney damage allows patients to receive targeted treatment, thereby reducing the extent of kidney damage. In 30-70% of cases, kidney damage is acute, even requiring dialysis. CKD is most often caused by diseases affecting the renal parenchyma (glomeruli, tubulointerstitium and blood vessels) that do not result in acute renal impairment, but may lead to long-term deterioration of renal function. Typical examples are paraneoplastic glomerulopathies and monoclonal gammopathy associated renal failure (MGRS). CKD can also be a consequence of previous AKI of any cause, but chronic kidney disease also significantly increases the risk of superimposed acute kidney injury.

### **Associated Renal Lesions and Their Risk Factors**

#### **Prerenal Causes Associated with Underlying Disease, Treatment**

Existing risk factors may be associated with additional risk factors due to haematological disease or its treatment. Dehydration, reduced fluid intake due to nausea as a side effect of chemotherapy treatment, is one of the most common causes of prerenal kidney damage. It is also negatively affected by pre-existing or reduced blood volume due to the use of cardiotoxic chemotherapy. The risk of sepsis in the context of anticancer treatment is increased, and may be related to neutropenia, a weaker immune response or the potential role of invasive devices as a focus. Possible hyperviscosity or vasoconstriction due to ion imbalances (hypercalcaemia) may also worsen renal perfusion. The development of acute kidney injury may also be contributed to by the frequently used NSAID and RAS inhibition mentioned above.

#### **Renal Causes Associated with Underlying Disease, Treatment**

##### **Vascular**

Vascular origin is most commonly associated with thrombotic microangiopathy (TMA). TMA is a microangiopathic haemolytic anaemia, often associated with renal damage, 'onion-skin-like' thickening of the arteriolar and capillary walls, fibrin thrombus, and consequent obstruction of small vessels, often with a histological picture characteristic of membranoproliferative glomerulonephritis. The presence of TMA in haematological pathology suggests a secondary origin. TMA may be caused by monoclonal immunoglobulins either

directly by damaging the endothelium or indirectly by their action on proteins regulating thrombosis. Drug-induced TMA is also not negligible and may occur in haematological disease, mainly during the administration of multi-receptor tyrosine kinase inhibitors, proteasome inhibitors.

Rarely, a hypercoagulable state may be observed in hyperleukocytosis, with increased thrombotic propensity and disseminated intravascular coagulation.

### **Tubulointerstitial**

Mainly B-cell lymphoproliferative pathologies may be associated with monomorphic cellular interstitial infiltration, occasionally with granuloma formation, which may be indicated by a preserved or rather increased kidney size despite deteriorating renal function. Interstitial infiltration may be focal or diffuse and it is usually correlated with renal function. Interstitial infiltration may coexist with glomerular involvement. Diseases with monoclonal immunoglobulin deposition in B-cell or plasma cell pathologies may lead to infiltration, but even without immunoglobulin deposition (TMA, C3GN), secondary to acute or chronic tubulointerstitial damage. It is important to highlight acute tubulointerstitial nephritis as a side effect of drugs (chemotherapeutic and immunomodulatory agents in addition to "traditional" antibiotics, NSAIDs), which is characterised by acute renal function impairment. The pathology is also increasingly described with the administration of immune checkpoint inhibitors, which are used with good results in the treatment of solid tumours and in haematological malignancies.

### **Tubular Necrosis**

Prerenal acute kidney injury with prolonged ischaemia may be associated with the development of acute tubular necrosis, but it may also occur as a side effect of medication. Nowadays, it is most commonly seen with the administration of vancomycin and aminoglycosides, but the importance of intravascular iodinated contrast agent studies is not negligible.

TLS is a spontaneous or drug-induced cell lysis in diseases with high proliferation rates, which may be associated with severe metabolic and ionic abnormalities (hyperuricemia, hyperphosphatemia, hypocalcaemia), multi-organ failure, acute kidney injury and high mortality.

Kidney damage may also be caused by chemotherapy drugs (methotrexate) or acyclovir used as prophylaxis, and crystalline tubulopathy caused by uric acid crystals in TLS. Crystal formation in the kidney induces inflammation, leading to fibrosis. In patients with myeloma,

calcium - phosphate precipitation may cause acute tubular damage, and in some cases acute phosphate nephropathy may also develop, based on our own experience.

### **Proximal Tubulopathy**

Damage affecting the proximal tubule can most often appear in the form of Fanconi's syndrome (hypouricaemia, hypophosphatemia, loss of amino acids, normoglycaemic glucosuria, acidosis-bicarbonate loss). Acquired Fanconi syndrome is a common consequence of haematological pathologies, with a high percentage of cases being due to light-chain proximal tubulopathy (LCPT) characterised by kappa light-chain deposition.

In clinical practice, lysosimuria causing proximal tubule damage is rarely encountered. Lysozyme is a bactericidal enzyme produced by monocytes and reabsorbed by the proximal tubule, but with a decrease in reabsorption capacity, filtered lysozyme appears in the urine as proteinuria (even nephrotic in extent), accompanied by acute renal damage, a clinical picture characteristic of proximal tubulopathy. The disease is most commonly associated with monocytic chronic leukaemia, but has also been described in association with myeloproliferative disease.

The most common complication of antiviral cidofovir treatment is proximal tubulopathy, which may also present as Fanconi syndrome.

### **Distal Tubulopathy**

Extreme amounts of toxic light chain secreted by B or plasma cells, due to exhaustion of reabsorption capacity, reach the more distal nephron section and bind to the physiologically present uromodulin (Tamm-Horsfall protein). The resultant cast causes obstruction of the distal tubule and acute kidney injury. Cast nephropathy is a probable cause of multiple myeloma, and according to the 2014 recommendation, a myeloma defining event is defined as cast nephropathy if the amount of involved free light chain is >1,500 mg/L. In myeloma, renal failure develops in at least 25% of patients. Other factors such as NF- $\kappa$ B (nuclear factor kappa B) activation through inflammation-inducing factors are also involved in the development of acute kidney injury, leading to fibrotic processes and ultimately to chronic kidney injury.

### **Glomerulopathy**

Glomerulopathies associated with haematological pathologies can be divided into two broad groups, those associated with monoclonal immunoglobulin and those associated with

paraneoplastic glomerulopathies, *i.e.* glomerulopathies that are secondary to the underlying disease. With few exceptions, these diseases present as chronic kidney disease, often preceding the clinical diagnosis of haematological disease.

Renal diseases associated with monoclonal immunoglobulin are most often associated with monoclonal gammopathy, plasma cell dyscrasia or other B cell disease secreting nephrotoxic monoclonal immunoglobulin, which do not exhaust the definition of myeloma or other B cell malignancy. Renal involvement associated with MGUS, which is haematologically premalignant, most commonly causes glomerulopathy (exceptions are LCPT and crystal storing histiocytosis). Renal disease of immunoglobulin origin can be clearly demonstrated by renal biopsy, with MGRS being confirmed in 40-50 % of renal biopsies performed in patients diagnosed with MGUS. The histopathological presentation of renal diseases associated with dysproteinemia, although the underlying disease is the same, is variable and may include crystallisation, aggregation and misfolding of aggregated proteins, determined by the specificity of the light chains. A distinction can be made between kidney damage with or without organised deposits, crystallisation and the lack of immunoglobulin deposition, but in associated with MGUS.

Plasma cell clones are responsible for 95% of light-chain amyloidosis (AL) and monoclonal immunoglobulin deposition disease, and clones of CLL (chronic lymphocytic leukaemia) origin for 50% of immunotactoid glomerulopathy (ITG). In proliferative glomerulonephritis with monoclonal immunoglobulin deposition disease (PGNMID), 50% of the clones are CD20+, half of which also show CD38 co-expression. Lymphoplasmacytic lymphoma clones are most common in type I cryoglobulinemia. The most common histological type of MGRS is amyloidosis of immunoglobulin origin (64%), followed by monoclonal immunoglobulin deposition disease (11%), cryoglobulinemia (6%), PGNMID (6%), with the remaining occurrence below 5%.

Recent studies on fibrillary GN (glomerulonephritis) have shown that paraffin-embedded tissue after pronase digestion, immunoglobulin depletion is mostly polyclonal rather than monoclonal, and therefore its classification as MGRS has been questioned. Paraneoplastic glomerulopathy is defined as glomerulonephritis associated with a pre-existing haematological disease and not associated with immunoglobulin deposition. Renal disease is often the first sign, and investigation reveals a pre-existing haematological origin. The development of the disease is independent of tumour mass and extension.

Haematological diseases of lymphoid origin are most often associated with minimal change nephropathy, secondary membranous nephropathy, and rarely with focal segmental

glomerulosclerosis, IgA nephropathy, AA amyloidosis. In CLL, the most common glomerulopathy is MPGN, but other rare glomerular pathologies such as ITG or cryoglobulinemic GN may also develop, but ANCA (anti-neutrophil cytoplasmic antibody) associated pauci immune crescentic glomerulonephritis has also been described.

TMA, which develops after stem cell transplantation and can be associated with GVHD (graft versus host disease), as well as *de novo* glomerulonephritis, which often manifests as protocadherin FAT 1 positive secondary membranous nephropathy, should be highlighted.

### **Postrenal Causes Associated with Underlying Disease, Treatment**

Obstructive uropathy, postrenal acute kidney injury may be caused by lymphoproliferative disease, ureteral compression due to retroperitoneal lymph nodes, which can be easily diagnosed with abdominal ultrasound. In the presence of retroperitoneal fibrosis (Hodgkin's lymphoma, non-Langerhans cell histiocytosis), the dilatation of the renal pelvis may not occur due to the embedded ureter. Renal function improves after treatment of the underlying disease, reduction of the degree of fibrosis and the cessation of urinary obstruction.

In the differential diagnosis of new-onset kidney stones, in addition to the more frequently occurring sarcoidosis and myeloma, lymphoproliferative and myeloproliferative diseases should also be considered. In the latter, increased uric acid level – due to chemotherapy, TLS – , its excretion and disturbances in calcium-phosphate homeostasis cause kidney stones in 5.5% of patients.

### **Prevention and Treatment of Kidney Injury Associated with Haematological Diseases**

Clarifying the cause of kidney damage is essential to reduce the extent of kidney injury through appropriate treatment. To prevent prerenal acute kidney injury, euvolaemia should be aimed at, and volume deficits should be corrected by parenteral fluid replacement. Fluid replacement, haemodynamic stabilisation and anaemia follow-up are of great importance in the prevention of AKI. In addition, attention should be paid to infection monitoring, replacement and timely removal of invasive devices as needed. In case of hyperviscosity, leukostasis, leukapheresis is necessary, which may improve perfusion of the kidneys and other organs.

Fluid replacement is also important in preventing tubular damage and TLS. Maintaining renal perfusion is the cornerstone of uric acid, potassium and phosphate excretion, which can be used to delay the introduction of renal replacement therapy. Uric acid-lowering treatment is indicated with all chemotherapeutic agents. In addition to TLS prophylaxis, uric acid lowering also

reduces the development of crystalline tubulopathy, renal calculi by reducing urate excretion, in which febuxostat has been shown to be superior to allopurinol.

The development of TLS was reduced with urate oxidase but was not different from the control group using allopurinol. Rasburicase also did not reduce the rate of death or the rate of initiation of renal replacement therapy.

To prevent TMA, it is necessary to choose the drugs with great care.

To prevent contrast induced nephrotoxicity, patients below 30 mL/min/1.73 m<sup>2</sup> eGFR should be pre-conditioned with iso-osmolar contrast material and repeat testing for 5-7 days is not recommended.

In the case of proximal tubulopathy, associated glomerulopathy, acute tubulointerstitial nephritis or other acute parenchymal renal injury, if a pre- or postrenal cause can be excluded, or if the acute tubular necrosis suspected to be the cause of AKI does not resolve after 2-3 weeks, renal biopsy is indicated. In case of drug-induced ATIN (acute tubulointerstitial nephritis), immediate discontinuation of the drug is necessary, with consideration of short-term steroid treatment to reduce interstitial inflammation.

In hyperviscosity syndrome, severe, life-threatening organ damage in type I cryoglobulinemia and in myeloma cast nephropathy, plasma exchange may be indicated. In cast nephropathy, high cut-off membrane haemodialysis is also an option to reduce the light chain burden. Plasma exchange is recommended in patients with an affected light chain >1,500 mg/L or if the light chain is <1,500 mg/L but the renal biopsy confirms myeloma cast nephropathy. Apheresis should aim for a light chain reduction of at least 50%, and if this fails, stopping plasma exchange then starting bortezomib-based chemotherapy with daratumumab is recommended.

The risk of developing AKI with newer CAR-T (chimera antigen receptor T cell) treatment is up to 50-90%, influenced by other renal abnormalities. Renal damage, most commonly tubular injury, is reversible in most cases.

Treatment of myeloma patients with bisphosphonate can reduce the likelihood of developing AKI, and the reduction of hypercalcaemia has an advantage over the potential development of bisphosphonate nephropathy.

In the case of postrenal kidney damage is necessary to ensure unobstructed urine flow, with a reduction in tumour mass (lymph node compression), the flow may also be resolved, and in the case of persistent flow disturbance, permanent urine diversion is indicated.

### **Renal Side Effects of Drugs used in Oncohematology**

In the treatment of haematological diseases, molecularly targeted therapy can be used in addition to classical chemotherapy. Over the last decade, advances in cell biology have led to the development of anti-tumour agents that target specific molecular pathways, inhibiting tumour progression by interfering with molecules involved in tumour growth and progression. Notable among these is ibrutinib, the first of the BTK (Bruton's tyrosine kinase) inhibitors, which has significantly improved prognosis in high-risk chronic lymphocytic leukaemia, mantle cell lymphoma and Waldenström's macroglobulinemia resistant to conventional chemo-immunotherapy.

However, most of the agents used are nephrotoxic and can cause both acute and chronic kidney injury, and should be used with caution, taking into account the potential side effects.

### **Renal manifestations of light chain amyloidosis**

Light chain amyloidosis is easily identified in the renal biopsy specimen. It is characterized by the deposition of PAS-negative, amorphous, acellular material, which shows a characteristic staining with Congo red. Congo red positive areas show an apple green birefringence under polarising microscopy, electron microscopy confirms the presence of amyloid fibrils (organised deposits 8-12 nm in diameter). The amyloidosis starts in the mesangium, so that glomerular amyloid deposition is most frequently confirmed in 97% of cases, followed by vascular amyloid deposition (85%) and tubulointerstitial involvement (58%). The least frequent involvement is in the tubular basement membrane (8%).

Glomerular deposition results in significant or nephrotic proteinuria. When the amyloid is predominantly deposited in the arteries and arterioles, proteinuria is lower and renal failure is more advanced. Interstitial fibrosis, tubular atrophy, as a consequence of massive glomerular and vascular amyloid deposition, play a major role in the development of renal failure, but tubulointerstitial and tubular epithelial cell lesions may also be a consequence of interstitial amyloid deposition. The study of tubule basement membrane amyloid deposition has received little attention.

## **Aims**

The aim of my work was to study the diverse renal lesions in haematological diseases with a scientific focus. I have made close professional relationships with haematologists in order to identify renal involvement in haematology patients and to identify possible chemotherapeutic treatment for patients presenting primarily with nephrology. Accurate evaluation of renal histological lesions and repeated comparison with clinical data were ensured by collaboration with a nephropathologist colleague. Thanks to the above, I have been able to publish two papers in internationally referred journals. In my PhD thesis I shall present these two studies.

### **Study 1: Examination of Amyloid Deposition in the Renal Tubular Basement Membrane in Patients with Light Chain Amyloidosis**

The glomerular involvement of amyloidosis is well known, but the examination of tubulointerstitium has received less attention so far. I aimed to establish the following:

1. How frequently is light chain deposition detected in different structures of the kidney?
2. Which factors may play a role in amyloid deposition in the tubular basement membrane?
3. What are the clinical consequences of amyloid deposition in the tubular basement membrane and does it affect the progression of renal disease?

### **Study 2: Examination of Acute Kidney Injury during Ibrutinib Treatment**

There was no previous report of severe AKI associated with the administration of ibrutinib. I aimed to establish the following:

1. What clinical signs should warrant ibrutinib induced AKI?
2. What is the histological pattern of ibrutinib-induced AKI?
3. What pathomechanism might play a role in adverse events?

## **Patients and Methods**

### **Study 1**

#### **Study Population**

A total of 630 patients underwent percutaneous renal biopsy between 2015-2022. Some of those patients who diagnosed with MGUS are highlighted during screening (proteinuria, urine sediment, kidney function) referred from Division of Haematology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen. Other part of the patients referred from nephrology outpatient and inpatient care sites to the Division of Nephrology, Department of Internal Medicine, Faculty of Medicine, University of Debrecen. Retrospective processing of these confirmed light chain amyloidosis in 12 cases. In my work, I collected and analyzed the data of these patients in detail.

#### **Methods**

##### **Kidney Biopsy and its Histological Processing**

Percutaneous, ultrasound-guided renal biopsy was performed, after informed consent of the patients. Histopathological processing of the specimen was performed at the Institute of Pathology, Faculty of Medicine, University of Debrecen. In addition to routine immunofluorescence administration, IgG-IgA-IgM-C3-C1q-fibrinogen-kappa staining, Congo red staining was performed in all cases, after which samples were also examined under polarizing microscope. Electron microscopic processing, detailed examination performed, the areas corresponding to light chain and Congo positivity were analyzed in the direction of organised deposits, amyloid deposition. This also provided an accurate picture of tubular basement membrane involvement, and other organized deposits of non-amyloid origin were identified.

##### **Haematological Tests**

To assess the light chain burden in the patients and to confirm the underlying disease (MGRS, myeloma or other B cell disease), bone marrow sampling was performed at the Division of Haematology, Department of Internal Medicine, University of Debrecen at the same time as the kidney biopsy. Further analysis of bone marrow samples was performed by flow cytometry, cytogenetics, FISH (fluorescein *in situ* hybridization) at Institute of Laboratory Medicine, University of Debrecen according to routine methods.

## **Assessment of Extrarenal Involvement**

In all cases, cardiac Troponin T, NT-pro-BNP and cardiac ultrasonography were performed in addition to a targeted medical history recording and physical examination to look for extra-renal symptoms (cardiac, gastrointestinal, neuropathy). In case of suspected neuropathy, ENG (electroneurography) was performed.

## **Statistical Methods**

Statistical evaluation was not performed due to the small number of patients.

## **Study 2**

### **Study Population**

In addition to the screening of chronic kidney disease associated with haematological conditions, we have continuously provided nephrology consultation for inpatient care of the Haematology Department. In all cases of suspected acute kidney injury, in addition to confirming the diagnosis, it was considered necessary to establish the underlying cause. If there was no pre- or postrenal origin, tumour lysis or other known cause, we considered the possibility of a possible drug side effect. In a 76-year-old patient with CLL treated with ibrutinib, AKI detected 4 weeks after the administration of treatment during an examination for lower limb purpura and foot pain was attributed to the provoking role of the drug. Such an adverse event has not been previously reported and it was therefore considered worthwhile to elaborate and report this case in detail. The patient's medical history is described in the results section.

## **Method**

### **Histopathological Examination**

A percutaneous ultrasound-guided renal biopsy was performed after the patient's information, consent and preparation. Histopathological processing of the retrieved specimen was performed using routine immunofluorescence assays IgG-IgA-IgM-C3-C1q-fibrinogen-kappa staining in addition to the routine immunofluorescence assays to assess B cell interstitial infiltration associated with the underlying disease, using CD3-CD20-CD68-CD117 surface marker labeling. In addition, electron microscopic processing was performed to assess ultrastructural lesions.

## **Results**

### **Study 1**

A retrospective review of our percutaneous renal biopsy database from 2015-2022 confirmed light chain amyloidosis in 12 patients (12/630=1.9%). Histopathological work-up revealed interstitial amyloid deposition in 8 of 12 patients in addition to glomerular amyloid deposition, four of them also had tubular basement membrane amyloid deposition.

Amyloidosis was diagnosed by haematological examination, bone marrow biopsy and the amount of M (monoclonal) component. In 8 cases MGRS, in 3 cases multiple myeloma and 1 case B cell lymphoma verified. Lambda light chain (LC) was overrepresented in our cohort; however, with respect to TBM involvement, the type of LC was not different (kappa/lambda ratio of 1/3 vs. 2/6).

In 3 out of 4 patients with TBM amyloid deposition, advanced glomerulosclerosis and tubulointerstitial damage were present at the time of diagnosis, with more severe renal disease: eGFR ranged from 9-19 mL/min/1.73 m<sup>2</sup> and renal failure progressed despite chemotherapy. However, in our fourth case sclerosis and fibrosis were negligible, and kidney function remained normal for 2.5 years without chemotherapy, only besides conservative treatment, even though the TBM was affected by both amyloid fibrils and non-amyloid, diabetic fibrillosis.

In our previously mentioned 3 cases had significant amyloid deposition in the arterioles as well as in the smaller arteries, which was absent in the fourth case. Among patients without TBM involvement, those with blood vessel amyloid deposition IFTA varied between 10 and 50%, but in those who did not have vascular involvement IFTA was less than 10%.

Glucosuria was observed only in one patient without TBM amyloid involvement, none of them had other symptoms were suggestive of Fanconi syndrome, polyuria or diabetes insipidus. One of our patients without TBM amyloid involvement had LC cast nephropathy, amyloid cast formation was excluded.

### **Study 2**

#### **Case Presentation**

The 76-year-old man had a history of depression, hypertension, previous regular smoking and alcohol consumption. In January 2018, close haematological monitoring was started due to high white blood cell count (42 G/L, predominantly lymphocytes 32 G/L). By peripheral flow cytometry, 77% of the B cells proved to be abnormal. In January 2020 white blood cell count

increased to 116 G/L, CT scan showed significant splenomegaly, paraaortic lymph node enlargement, serum creatinine (sCr) was normal (59  $\mu\text{mol/L}$ ) at that time. Prognostic markers included monosomy 13, TP53 (17p13.1) deletion, and borderline mutation status (homology: 97.28%) of the immunoglobulin heavy chain variable region. Due to progression, ibrutinib was started on 26 March at a daily dose of 420 mg. Four weeks later, on 28 April, he was admitted to our nephrology department with lower limb purpuras, burning pain in both feet and soles, significant Scr elevation (296  $\mu\text{mol/L}$ ). On the following day, despite preserved urinary output, Scr increased to 365  $\mu\text{mol/L}$ , with a significant proteinuria (urine protein to creatinin ratio: 70 mg/mmol), and microscopic haematuria. No other abnormalities than moderate serum uric acid (537  $\mu\text{mol/L}$ ), and phosphate (1.8 mmol/L) elevations, a slightly decreasing white blood cell count (98 G/L), and anemia (hemoglobin 113 g/L) were detected. Serum potassium, tCO<sub>2</sub>, calcium, platelet and eosinophil cell counts, liver and muscle enzymes, immunoglobulin and complement levels were normal. Urgent anti-neutrophil cytoplasmic antibody (ANCA) and antiglomerular basement membrane antibody test results were also negative. Ultrasonography showed normal sized kidneys, without urine outflow obstruction. Because of rapidly deteriorating renal function, parenteral methylprednisolone (MP) was initiated at a dose of 1 mg/kg body weight, and ibrutinib was omitted. Renal biopsy on day 4 of treatment confirmed acute interstitial nephritis (ATIN). MP treatment was continued, with which the patient's skin symptoms, lower limb numbness were completely resolved, on day 11 of treatment, Scr was reduced to 125  $\mu\text{mol/L}$ , when no proteinuria/haematuria was detectable. Apixaban was started due to atrial fibrillation occurring at this time and high stroke risk score. MP therapy was gradually tapered off over 3 months, and eGFR stabilized at 56 mL/min/1.73 m<sup>2</sup>. Due to further increase in white blood cell count (160 G/L), rituximab-venetoclax treatment was initiated in August 2020. Tumor lysis syndrome did not occur with rasburicase and allopurinol prophylaxis. Renal biopsy demonstrated clinically probable drug-induced ATIN. The glomeruli in the sample showed no crescent formation or other significant abnormalities, interstitial B-cell infiltration was not verified. Focal intense, interstitial infiltration of mixed inflammatory cells with some eosinophil cells were seen despite 3 days steroid treatment. Immunofluorescence revealed no immunodeposits in the glomeruli, the blood vessels, interstitium, tubules, and it was also confirmed by electron microscopy. In addition to the significant number of CD20 positive B cells, CD3 positive T cells and CD68 positive macrophages, the low number of CD117 positive mastocytes may have played a role in the development of tubulointerstitial nephritis.

## Discussion

### Study 1

A retrospective review of our percutaneous renal biopsy database from 2015-2022 confirmed light chain amyloidosis in 12 patients (12/630=1.9%). Histopathological work-up revealed interstitial amyloid deposition in 8 of 12 patients, four of them had tubular basement membrane amyloid deposition.

Interstitial involvement appeared to be similar to that observed in large cohorts. Fan *et al.* reported interstitial amyloid deposits in 63.7% (165/259) of LA cases. In a Mayo Clinic cohort of systemic (but 85.9% immunoglobulin amyloid type) amyloidosis, the interstitium was involved in 58% of the cases, whereas TBM deposition was found in only 8% (38/470) of the cases. Compared to patients without TBM involvement, 3 of our patients with TBM amyloid deposition were at a later stage of the disease, presenting with lower eGFR (9–19 ml/min/1.73 m<sup>2</sup>), and higher rate of both sclerotic glomeruli and IFTA. Late diagnosis probably contributed to the higher frequency of TBM involvement because the mean serum creatinine was only 1.3 mg% in the Mayo Clinic cohort.

In 3 of 4 patients with TBM amyloid deposition, advanced glomerulosclerosis and tubulointerstitial damage were present at the time of diagnosis, and renal failure progressed despite chemotherapy. However, in our fourth case, sclerosis and fibrosis were negligible, and kidney function remained normal for 2.5 years without chemotherapy, even though the TBM was affected by both amyloid fibrils and non-amyloid, diabetic fibrillosis. This indicates that amyloid deposition in the TBM alone does not play a significant role in tubulointerstitial damage. In contrast, there are opposing observations. In a case report of a patient with rheumatoid arthritis, TBM amyloid deposition with negligible glomerular and absent vascular involvement was proposed to be responsible for renal failure. Kuroda found a similar frequency of circumferential TBM amyloid deposition in rheumatoid arthritis and LA; however, its influence on renal function remains unclear.

In these 3 cases mentioned previously, there was significant amyloid deposition in the arterioles as well as in the smaller arteries, which was absent in our fourth case. Among patients without TBM involvement, those with blood vessel amyloid deposition, IFTA varied between 10 and 50%, but in those who did not have vascular involvement, IFTA was less than 10%. The narrowing and occlusion of vessels by amyloid deposition may contribute to advanced tubulointerstitial fibrosis. In a report from the Mayo Clinic, 12 of 234 patients with AL amyloidosis had vascular-limited renal amyloidosis. Compared with patients with diffuse renal

involvement, patients with vascular-limited amyloidosis have higher serum creatinine levels at the time of diagnosis and significantly shorter survival times. Besides localization, amyloid load is also an independent predictor of AL amyloidosis prognosis. The missing vascular and only focal and segmental glomerular amyloid involvement indicated a limited amyloid load in the fourth case which could also explain the favorable prognosis.

The fourth case had long-standing diabetes mellitus with histologically verified diabetic nephropathy; therefore, 'diabetic fibrillosis' emerged in the background as non-amyloid TBM fiber. These fibrils generally have a similar diameter (10 nm) as immunoglobulin-related amyloid fibrils (8-12 nm), especially in the expanded diabetic mesangium. They may represent a type of fibrillary, perhaps glycosylated, collagen cross-linked to other proteins, making it impossible for them to be properly catabolized. The N-glycosylation process also plays a major role in LC amyloidogenesis; therefore, the collagen fibrils may have helped in the precipitation of LC in our case. This can also explain the patient's carpal tunnel syndrome, which is common in both diabetes mellitus and LA, but its frequency is higher in LA, and can be the first warning sign of the disease. Subsequent surgery in our patient also confirmed light chain origin.

Apart from the uncertain effect on renal function, amyloid deposition in the TBM was not associated with other clinical consequences. Glucosuria was observed only in one patient without TBM amyloid involvement. No other symptoms were suggestive of Fanconi syndrome. This is typically observed in light chain proximal tubulopathy when the excess free light chain injures proximal tubular epithelial cells, sometimes with crystal formation in the cytoplasm. Very rarely, intracellular amyloid, termed amyloid proximal tubulopathy, has been detected in such cases. One of our patients experiencing LA without TBM amyloid involvement had LC cast nephropathy, but no amyloid casts were formed, which is not a rare phenomenon in such cases.

None of the patients had polyuria or diabetes insipidus, which have been reported in cases with predominant tubulointerstitial amyloid involvement. This is mostly observed when the medullary region is involved. In cases with non-significant glomerular deposition (resulting in less proteinuria), the clinical presentation could suggest acute interstitial nephritis, especially in patients with a predisposition, such as in Sjogren syndrome. Rarely, giant cell reactions provoked by interstitial amyloid deposits are observed, typically in AA amyloidosis, but they also occur in LA and LCDD.

Amyloid formation does not depend on the amount of light chains but on mutations. The lambda light chain is significantly more amyloidogenic than the kappa light chain. The variable region of the lambda light chain sequences has the most extensive non-conservative and total number

of mutations compared to all other sequence groups. Lambda LC was also overrepresented in our LA cohort; however, with respect to TBM involvement, the type of LC was not different (kappa/lambda ratio of 1/3 vs. 2/6).

There is substantial information about renal amyloidogenesis in LA; however, amyloid deposition within the TBM is not well established. Amyloid formation begins in the mesangium. The normal smooth muscle phenotype of mesangial cells changes to a macrophage phenotype upon recognition of precursor light chains. Amyloidogenic light chains are internalized in mesangial cells and transported to lysosomes for digestion. Here, their degradation remains unsuccessful due to the unique property and amyloid formation begins. This process is the same in the proximal tubular cells. They are able to reabsorb the immunoglobulin light chain through the cubilin-megalin receptors and by clathrin-dependent endocytosis, and amyloid may form in the tubular cells. Accordingly, during the EM examination of the TBM, it was established that in two cases, amyloid accumulated more on the cell side of the TBM; however, in one case, it was dominant on the interstitial side. This may suggest that amyloid deposition can also occur from the interstitium; although peritubular capillaries have a fenestrated endothelium, their basement membrane, like the glomerular basement membrane, is impermeable to amyloid.

Based on this small cohort study, we would like to raise awareness and emphasize the importance of finding amyloid deposition in the TBM. This usually represents a late-stage presentation of the disease with a heavy LC burden. However, it can occur in cases with a smaller amyloid load and does not in and of itself lead to the deterioration of kidney function or tubular dysfunction. Amyloid deposition in the TBM depends not only on the concentration and properties of folding immunoglobulins, but also on the local environment. ‘Diabetic fibrillosis’ may have served as a precipitating factor.

## **Study 2**

Significant progress has been made in the treatment of CLL over the past decade. In our patient, genetic tests clearly indicated a poor prognosis, thus according to the recommendations, ibrutinib was initiated at disease progression, after which, 4 weeks later, nonoliguric AKI occurred. Both pre/postrenal etiologies and tumor lysis syndrome were ruled out on the basis of the clinical picture, renal ultrasonography and laboratory tests. Proteinuria and microscopic haematuria raised the possibility of both glomerular and tubulointerstitial damage. Because serum creatinine, urine tests were not performed before starting treatment, it could not be ruled out that renal involvement may be a rare consequence of CLL.

Leukemic cell infiltration is usually associated with renal enlargement, while paraneoplastic glomerular diseases are often associated with nephrotic syndrome, which were all absent in our case. However, they can only be ruled out by kidney biopsy, which also enables us to distinguish among the various histological forms of involvement. In the Mayo Clinic's 10 years of practice, renal biopsy was required in 1.2% of CLL patients due to kidney failure or nephrotic syndrome. The coinciding skin symptoms and neuropathy a month after the initiation of ibrutinib made us think of its provocative role in the development of AKI. The discontinuation of a potentially life-saving treatment warranted a kidney biopsy. Ibrutinib inhibits the collagen-induced platelet aggregation by inhibiting platelet BTK (bruton tyrosine kinase) and it is generally recommended to hold it 3–7 days before and after and invasive interventions. Thrombocytopenia may have a role in the formation of small, non-palpable petechias usually developing after 2 months of ibrutinib treatment. In our patient, the prompt presence of dermatological symptoms within one month of therapy was more suggestive of vasculitis. Such early onset of palpable purpuriform lesions may suggest more severe disease and may require discontinuation of treatment, glucocorticoid therapy, and could recur upon repeated administration of the drug. Peripheral neuropathies due to various pathomechanisms are not uncommon in haematologic malignancies. Ibrutinib may ameliorate anti-myelin-associated glycoprotein mediated form but can also provoke it by an unknown mechanism. In our case, its simultaneous appearance with skin and kidney symptoms raised the possibility of vasculitis. The related clinical picture above made drug-induced interstitial nephritis higher on the differential over IgA or ANCA induced vasculitis. The rapid loss in GFR prompted us to start glucocorticoid immediately prior to any histological verification along with holding ibrutinib. Renal biopsy confirmed our suspicions. One prior case showed biopsy-proven ibrutinib induced AIN to date; however, in that case, chronic kidney disease and diarrhoea were also implicated in the AKI.

In the background of drug-induced AIN, a hypersensitive reaction is suggested, primarily characterized by the Th2 cytokine response. Its occurrence is unusual because ibrutinib can prevent cytokine release in CLL patients, decreases the elevated cytokine, chemokine levels and polarizes T cells in Th1 direction. Macrophage response shifts from M1 toward M2 based on mouse models and human studies, too. In our case, we did not examine cytokine levels. Recently serum and urine levels of several cytokines were found to be higher in ATIN patients compared to healthy ones, but cytokine levels characteristic of Th2 response did not differ. Because ATIN is mostly restricted to the kidneys, testing urine cytokine levels is more relevant.

Th2-specific TNF-alpha urinary level was higher in AIN compared to other AKI, but it can be mast cell origin, as indicated by higher IL-9 levels. Mast cells were present in our case biopsy specimen also.

With glucocorticoid treatment, proteinuria, haematuria resolved within 2 weeks, and renal function improved significantly. Glucocorticoid treatment efficacy is contentious in AIN, and many consider it only if no improvement in renal function is observed within 3-5 days despite omitting the responsible drug. This wait would certainly have resulted in lower GFR

later. In case of malignant diseases, we consider it important to better preserve kidney function also for the prevention of AKI that may be superimposed due to possible subsequent damages. Ibrutinib has minimal renal clearance. It can be given safely in patients with kidney injury, dose adjustments in patients with impaired renal function may help improve tolerability without reducing efficacy, and patients below 30 ml/min/1.73 m<sup>2</sup> eGFR should be monitored. Primary FSGS with nephrotic syndrome and acute tubular injury in association with ibrutinib have been reported, no case reports of ibrutinib-induced ATIN are available. There is no literature data on renal damage with other BTK inhibitors (nemtabrutinib, acalabrutinib, zanubrutinib).

With this case report, we would like to draw the attention to careful use of ibrutinib, which shall certainly be applied more widely. Publication of our case may also contribute to explore the pathomechanism of ATIN.

## **Conclusions, New Findings and their Clinical Relevance**

Data on renal diseases associated with haematological conditions are increasing, and this is the reason for the emergence of the concept of MGRS in 2012. However, some of these renal diseases are not related to the underlying disease but to its management. New, innovative drugs allow better treatment of the disease, but their side effect profile is not fully understood.

This thesis summarises the results of two studies. In the first, we investigated the involvement of TBM in light-chain amyloidosis associated with haematological diseases and its role in the progression of renal injury, while in the second, we identified a new etiopathogenesis of acute kidney injury during the treatment of a haematological disease.

Our investigations led to the following new findings:

### **Study 1**

1. In light chain amyloidosis associated with B-cell and plasma cell diseases (multiple myeloma, MGUS, B-cell lymphoma), amyloid deposition in the tubular basement membrane is more frequent than in previous studies, being observed in one third of patients.
2. Amyloid deposition in the TBM is associated with advanced glomerulosclerosis and interstitial fibrosis in the majority of patients, i.e. most often an advanced stage, a consequence of prolonged/larger light chain burden.
3. TBM amyloid deposition can occur in the early stages of haematological disease and renal involvement, is not clinically significant *per se*, and does not enhance the progression of renal disease.
4. Local factors such as diabetic fibrosis may also play a role in amyloid TBM deposition.

### **Study 2**

5. We first demonstrated acute kidney injury due to tubulointerstitial nephritis with ibrutinib.
6. Regular monitoring of renal function and urine and consideration of renal biopsy in the event of other adverse events (purpura, neuropathy, atrial fibrillation) should be considered when ibrutinib treatment is indicated.
7. Timely recognition, discontinuation of ibrutinib and short courses of steroid administration shall result in rapid and complete improvement in renal function, allowing subsequent chemotherapy regimens to be continued.

## Summary

When assessing renal diseases associated with haematological pathologies, it should always be borne in mind that the acute or chronic kidney damage that develops may be a consequence of the underlying disease itself or of its treatment. In case of AKI, exploring the background is urgently needed, and after ruling out prerenal and postrenal causes, renal biopsy is very often helpful in the differential diagnosis of possible diseases. In cases of suspected chronic kidney disease associated with haematological pathology, renal biopsy can also help to assess whether the renal damage is due to interstitial infiltration, immunoglobulin-related or immune-mediated. In our first study, we investigated the significance of AL deposition into the TBM in chronic kidney disease caused by light chain amyloidosis. Based on our cohort of 12 patients, we found that TBM amyloid deposition was associated with advanced glomerulosclerosis and interstitial fibrosis in the third of the patients, *i.e.* most of them were in an advanced stage caused by prolonged/higher light chain burden. However, TBM amyloid deposition may also occur early in the disease, in which local factors such as diabetic fibrillosis may play a role. It does not have clinical consequences and does not increase the progression of renal disease.

In our second study, acute tubulointerstitial nephritis was confirmed in the background of acute kidney injury in a patient treated with CLL, which was unique in the literature. The ATIN was clearly explained by the ibrutinib treatment started four weeks earlier. After discontinuation of the drug and 3 months of steroid treatment, his renal function normalized.

Our studies aim to emphasise the importance of early detection of CKD and AKI associated with haematological pathologies. For this reason, reliable testing of proteinuria, renal function and urine sediment are required during haematological follow-up. It is reasonable to perform these screening tests in addition to routine tests for renal symptoms (*e.g.* oedema, hypertension) for any new significant symptom that does not appear to be related to renal disease.

Emphasis should be placed on elucidating the etiopathogenesis of both AKI and CKD, in order to reduce the extent of kidney injury and the progression of kidney disease by eliminating the underlying cause and by treating the pathomechanism. For this reason, in the case of progression of haematological disease or haematuria, proteinuria, Scr elevation not explained by other causes in the context of drug treatment, we recommend the discontinuation of the suspected agent and the consideration of renal biopsy. In cases of severe renal impairment, we also consider early administration of steroids to be appropriate, which allows the continuation of chemotherapy by reducing renal impairment and reducing the risk of superimposed AKI. By achieving renal remission, quality of life may be improved, other organ damages and cardiovascular mortality may be reduced.

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### List of publications related to the dissertation

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*Ren. Fail.* 45 (1), 1-6, 2023.  
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2. **Markóth, C.**, Kuskáné File, I., Szász, R., Bidiga, L., Balla, J., Mátyus, J.: Ibrutinib-induced acute kidney injury via interstitial nephritis.  
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### List of other publications

3. Hashmi, A., Bidiga, L., **Markóth, C.**, Kuskáné File, I., Balla, J., P. Szabó, R.: Recurrent IgA Nephropathy in A Kidney Transplant Patient: a Case Report.  
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4. Berczi, C., Mátyus, J., **Markóth, C.**, Somogyi, T., Kerekes, G., Flaskó, T.: Szokatlan, súlyos uroszepszist okozó candidafertőzés.  
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5. **Markóth, C.:** Primer membranosus nephropathia: a foszfolipáz A2 receptor érán innen és túl.  
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