Monoclonal Gammopathy and Kidney Disease

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Abstract

Kidney disease associated with monoclonal gammopathy is increasingly recognized as a complex group of conditions associated with significant morbidity. Advances in treatments to suppress clonal disorders over the last two decades have resulted in significant improvement in survival in patients with multiple myeloma (MM) and subsequent changes to its natural history, including the impact on kidney disease. The work in this thesis evaluates the prevalence of kidney diseases at diagnosis of monoclonal gammopathy and how kidney function changes during the course of the disease. At presentation patients with monoclonal gammopathy may have renal impairment (RI) related to the monoclonal gammopathy, but also this generally elderly population have a high prevalence of RI, caused by unrelated pathogenesis such as hypertension and/or diabetes. I have characterized three main disease groups 1) MM 2) Monoclonal gammopathy of renal significance (MGRS) and 3) Monoclonal gammopathy of undetermined significance (MGUS).

The diagnostic pathway in MM is often delayed but simple measures can be effective in improving the time to diagnosis. An audit of 56 patients with newly diagnosed multiple myeloma (NDMM) and acute kidney injury (AKI) showed delays in diagnosis that indicated a need for direct communication of high free light chain (FLC) results to the haematology team, and this was shown to halve the time to anti-myeloma therapy in the next 19 patients.

Kidney disease in MM is common, 39% of the 4158 patients had an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 at presentation. Novel and intensive therapies have dramatically improved survival in myeloma, however, the impact on renal outcomes is not well defined. At 12 months, 62% (1450/2334) of the NDMM patients have unchanged kidney function and overall renal outcomes are reassuring. In the 38% showing a >25% change in eGFR from baseline; this comprised 22% (502/2334) patients who had an improvement and 16% (382/2334) who had a decline in eGFR at 12 months. Improved renal function was more likely if patients were <70 years old, male, had an average eGFR <60 ml per minute per 1.73m^2, a higher baseline FLC level >1000 mg/L, and/or a FLC response of >90% whilst M-protein response did not correlate with renal improvement. Despite improved survival with new therapies patients with an eGFR ≥30 ml/min still survive longer compared to those with eGFR <30 ml/min.

Albuminuria is a powerful adverse determinant of outcomes in kidney disease but there are limited data on patients with MM. Patients who at 12 months had an improvement in eGFR or a decline in eGFR compared to baseline, were matched with those who had no change in eGFR. The two groups comprised (i) improvement matched with no change (184 pairs) and (ii) decline matched with no change (123 pairs). These groups were matched for age, sex, baseline difference between the involved and the uninvolved FLC (dFLC) and 12-month dFLC response.

At presentation 305 patients (58%) had albuminuria (albumin creatinine ratio (ACR) >3 mg/mmol). In 15 patients (3%) this approximated to ≥1 gram/24 hours (ACR ≥70 mg/mmol). Albuminuria at baseline was not independently associated with a lower eGFR category at 12 months. At 12 months, 162 patients (30%) had albuminuria.

Patients with unexplained RI, atypical clinical course, risk factors for MGRS or those on the renal transplant waiting list with unknown primary renal disease should be evaluated for MGRS. Clone specific chemotherapy is required to target the underlying clone, and this may improve graft survival; however, this can be challenging as many patients are elderly with age related comorbidities and may have complications associated with increasing immunosuppression. Albuminuria in myeloma is not associated with a decline in kidney function at 12 months; however, it is of critical importance in MGUS cohorts to identify undiagnosed MGRS lesions.

Dedication

To kidney patients who live with such grace.

To my husband Vijay and my daughters Nayantara and Ojaswi for their love, patience, understanding and encouragement to pursue my dreams.

Acknowledgments

My research time has been a gratifying and enjoyable experience. This much needed time helped me pause, reflect, and think deeply about this fascinating world of renal medicine. As a result, I have progressed personally and professionally, and I take with me a desire to understand my subject and improve patient care further.

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Abbreviation	
AKI	acute kidney injury
AL	immunoglobulin light chain amyloidosis
ACR	albumin creatinine ratio
ALP	alkaline phosphatase
AUC	area under the curve
APEX	assessment of proteasome inhibition for extending remissions
AF	atrial fibrillation
AI	auto immune
ASCT	autologous stem cell transplantation
β2M	beta 2 microglobulin
VCD	bortezomib, cyclophosphamide and dexamethasone
VRd	bortezomib, lenalidomide and dexamethasone
CNI	calcineurin inhibitor
KCRD	carfilzomib, cyclophosphamide, lenalidomide, and dexamethasone
CKD	chronic kidney disease
CLL	chronic lymphocytic leukemia
COPD	chronic obstructive pulmonary disease
CNS	clinical nurse specialist
C3GN	complement 3 glomerulonephritis
CR	complete response
CR renal	complete response renal
CT	computed tomography
CAPD	continuous ambulatory peritoneal dialysis
CrCl	creatinine clearance
CSH	crystal storing histiocytosis
CRD	cyclophosphamide lenalidomide and dexamethasone
CVD	cyclophosphamide, bortezomib and dexamethasone
CTD	cyclophosphamide, thalidomide and dexamethasone
CTDa	cyclophosphamide, thalidomide and dexamethasone attenuated
DRd	daratumumab, lenalidomide and dexamethasone
dFLC	difference between the involved and the uninvolved free light chain
DNAJB9	DNAJ homologue subfamily B member 9
EM	electron microscopy
ESRF	end stage renal failure
ELISA	enzyme linked immunosorbent assay
eGFR	estimated glomerular filtration rate
FISH	fluorescence in situ hybridization
FDG PET	fluorodeoxyglucose positron emission tomography

FSGS	focal segmental glomerulosclerosis
FFPE	formalin fixed paraffin embedded
FLC	free light chain
GGT	gamma glutamyl transferase.
GBM	glomerular basement membrane.
H&E	haematoxylin and eosin
HR	hazard ratio
HCDD	heavy chain deposition disease
HCs	heavy chains
HD	hemodialysis
HDT	high dose therapy
HLA	human leucocyte antigen
HTN	hypertension
IFE	immunofixation electrophoresis
IF	immunofluorescence
Ig	immunoglobulin
IDDM	insulin dependent diabetes mellitus
IQR	inter quartile range
IMWG	International Myeloma Working Group
ISS	International staging system
κ	kappa
KCR	kappa creatinine ratio
KDIGO	Kidney Disease Improving Global Outcomes
λ	lambda
LCR	lambda creatinine ratio
LVH	left ventricular hypertrophy
LHCDD	light and heavy chain deposition disease
LC	light chain
LCDD	light chain deposition disease
LCO	light chain only
LCPT	light chain proximal tubulopathy
LC-MS	liquid chromatography and mass spectrometry
MRI	magnetic resonance imaging
MS	mass spectrometry
MALDI	matrix assisted laser desorption/ionization
MP	melphalan plus prednisolone
MPGN	membranoproliferative glomerulonephritis
MRD	minimal residual disease
MR	minimal response
MR renal	minor response renal

MDRD	modification of diet in renal disease
MW	molecular weight
MBL	monoclonal B cell lymphocytosis
MGUS	monoclonal gammopathy of undetermined significance
MGFD	monoclonal gammopathy with fibrillary deposits
mi-RAMM	monoclonal Ig rapid accurate mass measurement
MIg	monoclonal immunoglobulin
MIDD	monoclonal immunoglobulin deposition disease
M-protein	monoclonal protein
MM	multiple myeloma
MMF	mycophenolate mofetil
MCN	myeloma cast nephropathy
NT pro BNP	N-terminal pro B-type natriuretic peptide
NAC	National Amyloidosis Centre
NICE	National Institute of Clinical Excellence
RaDaR	National Registry of Rare Kidney Diseases
NDMM	newly diagnosed multiple myeloma
NC	no change
NIDDM	non-insulin dependent diabetes
NSMM	non-secretory multiple myeloma
NSAIDS	non-steroidal anti-inflammatory drugs
NF-κB	nuclear factor κB
OSA	obstructive sleep apnoea
OS	overall survival
OUH	Oxford University Hospitals
PR	partial response
PR renal	partial response renal
PUJ	pelvi ureteric junction
POEMS	polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes
PET	positron emission tomography
PI	post induction
PFS	progression-free survival
PD	progressive disease
PGNMID	proliferative glomerulonephritis with monoclonal immunoglobulin deposits
PCR	protein creatinine ratio
RI	renal impairment
RRT	renal replacement therapy
RA	rheumatoid arthritis
SAP	serum amyloid P

sFLC	serum free light chain
SPEP	serum protein electrophoresis
SLAMF7	signalling lymphocytic activation molecule F7
SMM	smouldering multiple myeloma
SCT	stem cell transplant
TMA	thrombotic microangiopathy
TOF	time of flight
USS	ultrasonography
UHB	University Hospitals Birmingham
UPEP	urinary protein electrophoresis
VTE	venous thromboembolism
VGPR	very good partial response
WM	Waldenström macroglobulinemia

Chapter I.

Introduction

Monoclonal gammopathy related kidney disease represents a group of rare, complex renal diseases that are the result of MM, Waldenström macroglobulinemia (WM), chronic lymphocytic leukemia (CLL) and MGRS.

Patients with monoclonal gammopathy related kidney disease are an understudied cohort. There is little UK and international data regarding the burden of renal disease in patients with a monoclonal gammopathy and its impact on outcomes. Understanding the characteristics and factors associated with organ dysfunction, outcomes and patient survival in this cohort is crucial for risk stratification, counselling and targeting treatment strategies.

To provide the context for the chapters reported in this thesis, this introductory chapter will provide an overview of monoclonal gammopathy related kidney disease including MGRS and myeloma.

1.1 Extent of personal contribution

This chapter is based on the following published first author articles [1, 2]. The first drafts of the manuscript were written by myself, and I was responsible for all the edits and revisions.

Rana, R., Cockwell, P. and Pinney, J., 2022. Multiple Myeloma and the Kidney. In *Primer on Nephrology* (pp. 843-859). Springer, Cham [1].

Rana, R., Pinney, J.H. and Lachmann, H.J., 2019. Paraprotein-related renal disease. *Medicine*, *47*(10), pp.666-671 [2]

1.2 Monoclonal gammopathy

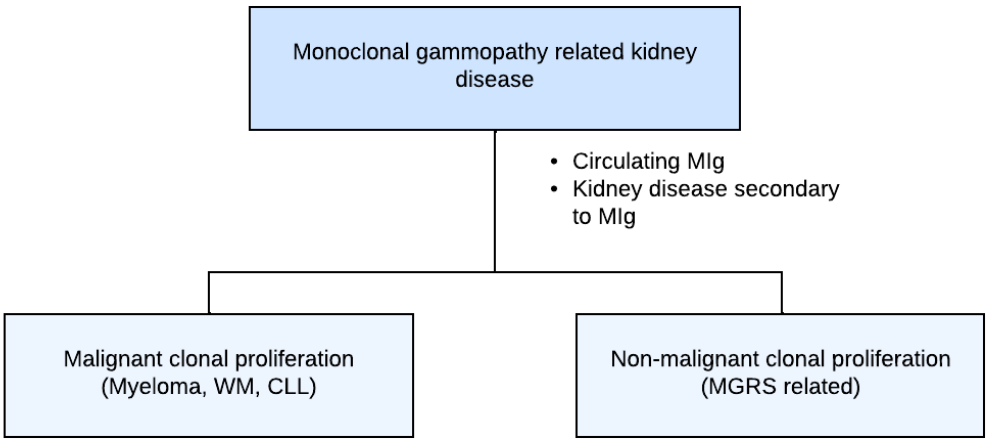
A monoclonal immunoglobulin (MIg) or paraprotein refers to an abnormal immunoglobulin (Ig) or its components (heavy chain (HC) or light chain (LC)) present in the serum or urine; produced by an abnormal monoclonal proliferation of a plasma cell or another cell of B-cell lineage. Monoclonal gammopathy refers to a disturbance in the Ig production because of clonal proliferation of cells in the B lymphocyte lineage.

The spectrum of disease associated with a monoclonal gammopathy is wide, from overt malignancy such as MM to the pre-malignant condition MGUS. This latter term is used when a circulating MIg causes no detectable tissue damage. MGUS is a relatively common finding in the elderly; with an estimated prevalence of 3% in those older than 50 years [3] and carries a 1% per year risk of progression [4] [5]. In the majority of patients with kidney disease and a monoclonal gammopathy, the paraprotein is an incidental finding [6].

1.3 Monoclonal gammopathy related kidney disease

Monoclonal gammopathy related renal disease represents a diverse group of rare diseases characterized by distinct renal injury due to direct or indirect effects of a nephrotoxic paraprotein, secreted by a clone of B cells. The clonal proliferation of plasma cells or other cells of B-cell lineage can be malignant or non-malignant (Figure 1.1)

Figure 1.1: A broad classification of monoclonal gammopathy related renal disease.

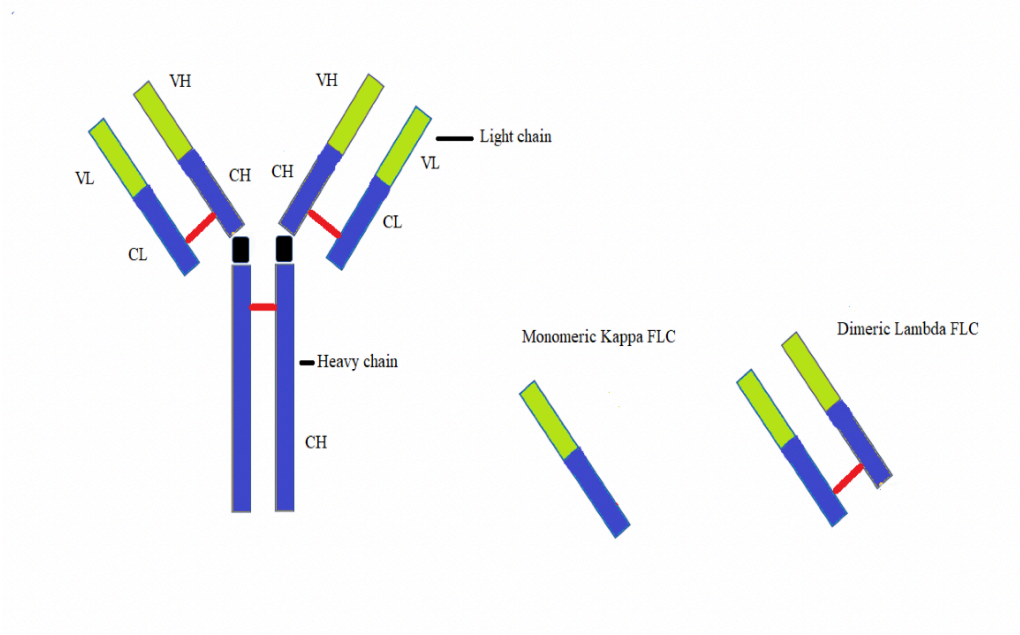


Here the renal disease is often due to the direct effects of the monoclonal LCs, however it can also be due to intact Ig or HC Ig. Occasionally (e.g., C3 glomerulopathy) the renal disease is due to a distal effect of a MIg. Monoclonal gammopathy related kidney diseases although diverse in their presentation and histopathology, are commonly progressive in nature and have a tendency to recur after transplantation [7]. These are poorly responsive to conventional immunosuppression and require clone-directed chemotherapy [8-11]. A kidney biopsy with detailed hematologic work up is required to link the paraprotein to the kidney disease. Treatment targeting the underlying clone may improve outcomes for many of these disorders.

1.4 The biology of immunoglobulin light chains

The renal lesions associated with MM and MGRS are caused by clonal Ig, usually the LC component; direct infiltration by clonal cells is rare. Igs are symmetrical molecules composed of two identical HCs and identical two LCs, each containing variable and constant domains (Figure 1.2).

Figure 1.2: Diagrammatic representation of intact immunoglobulin.
Adapted from Rana et al., 2022 [1].



Each light chain consists of variable domain, VL and one constant domain, CL. The heavy chain consists of a variable domain, VH and three constant domains, CH.

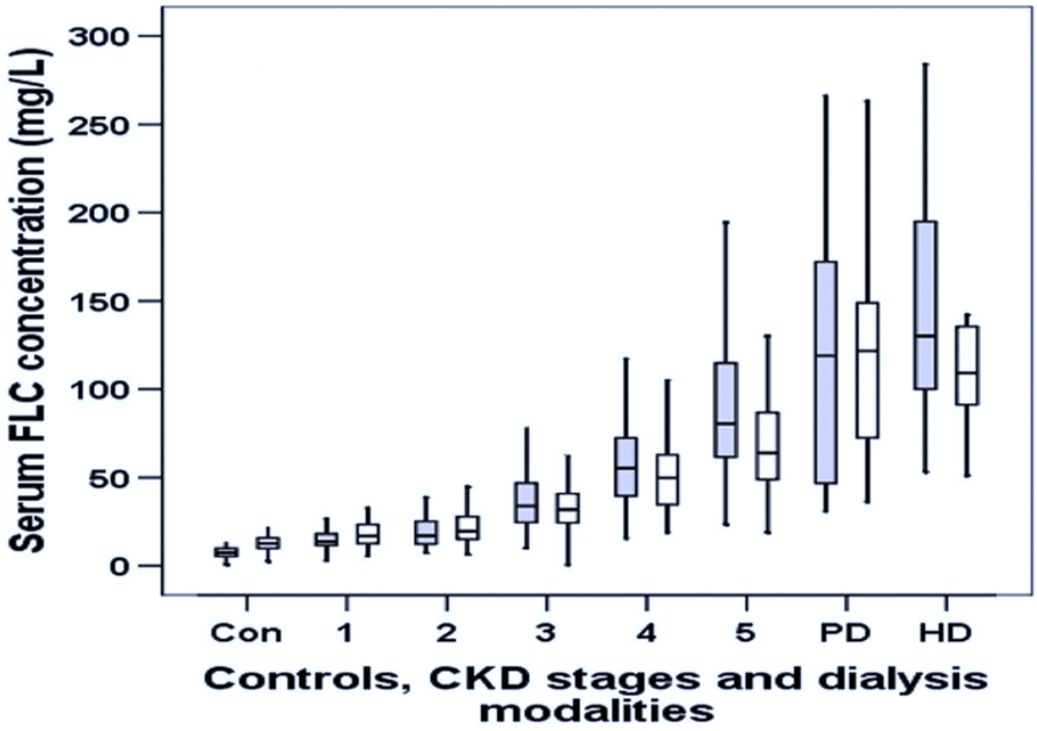
Ig can be produced by all cells of B-cell lineage; however, the predominant source are plasma cells: each cell produces an Ig of unique specificity consisting of HCs and LCs of a single isotype.

When a clone develops from sustained proliferation of a single aberrant plasma cell, there is usually excess production of clonal Ig; this molecule has no useful biological role. There are two isotypes of LC, kappa (κ) and lambda (λ). In health there is a 2:1 ratio of κ:λ producing plasma cells in humans. Whilst the majority of produced LC is incorporated into the intact Ig molecule, around 40% is freely released k LC (a monomeric protein with a molecular weight (MW) of 22.5 Kd) or λ LC (as a dimeric protein, MW 45 Kd), although oligomers and polymers of the clonal isotype are present in some patients (Figure 1.2).

Renal involvement usually occurs because of interactions between LCs and resident renal cells. Different LCs exert differential pathogenicity due to their differing physico-chemical properties [12]. Serum free light chains (sFLC) are cleared by glomerular filtration at 40% for k FLC and 20% for λ FLC, equating to half-lives of 2-3 hours and 4-6 hours respectively in patients with normal renal function. In contrast, IgG has a MW of 160 kDa and has minimal renal clearance, with a half-life of 21 days. In end stage renal failure (ESRF), the half-life of serum FLC increases to 18 hours or more with slow clearance by the reticulo-endothelial system.

Serum FLC increase progressively with each chronic kidney disease (CKD) stage and those on renal replacement therapy (RRT) have significantly higher concentration than patients with CKD stage 5 (Figure 1.3).

Figure 1.3: Serum FLC concentrations in controls, CKD stages (1-5) and in patients on renal replacement therapy.
Adapted from Hutchison et al., 2008 [13].



Blue boxes represent kappa and white boxes lambda light chain.
Abbreviations: Con, controls; PD, peritoneal dialysis; HD, hemodialysis.

The relationship between the MW of LCs, renal clearance and serum half-life is crucial in the development of AKI. Furthermore, because of the big differences in half-life between the serum FLC and intact Ig, changes in involved serum FLC levels are a better marker of early disease response than intact (clonal) Ig.

Given the important role of kidneys in the clearance of FLCs, in patients with a low eGFR the normal serum FLC ratio shifts up because κ FLC is differentially retained as it has a lower MW: this means that the upper limit of the normal range for the serum FLC ratio in a patient receiving dialysis treatment is 3.17. The normal range ratio in normal kidney function is 0.26-1.65 [14].

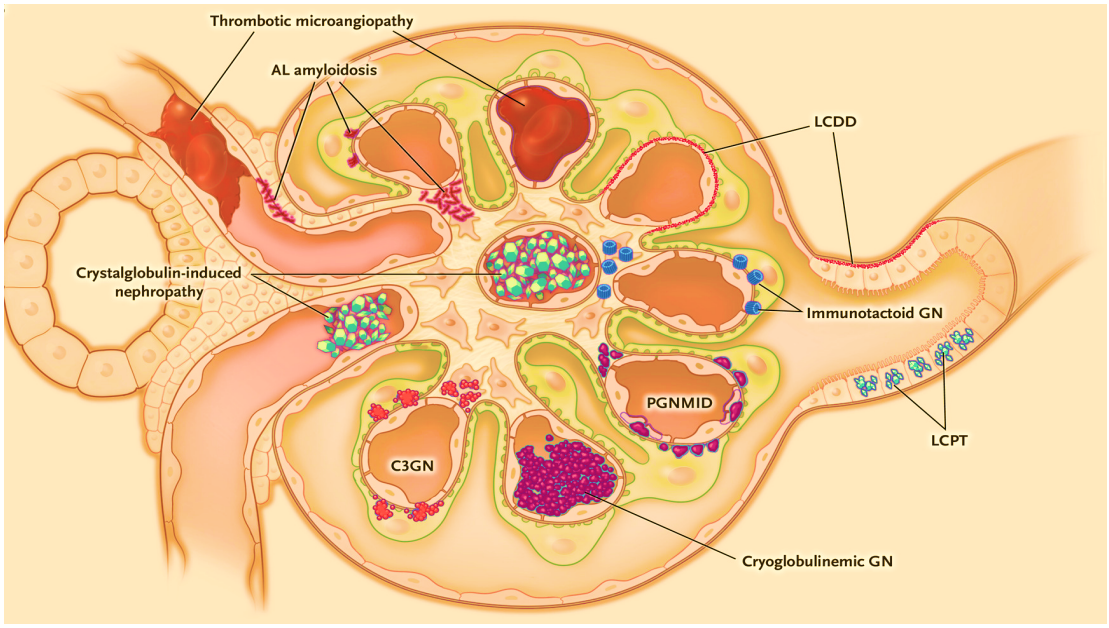
1.5 MGRS

The term MGRS is used to describe a B-cell lineage clonal proliferation with 1) one or more renal lesions caused by the produced MIg and 2) a clone that does not cause tumour complications or meet current haematological criteria for immediate specific therapy. Most of these patients have a low-grade clone consistent with that seen in MGUS; however, unlike in MGUS the MIg causes end-organ damage. The renal lesion is a consequence of the physico-chemical properties of the clone rather than the nature of the underlying clonal lymphoproliferation. Renal disease secondary to MGRS is termed an MGRS-associated renal lesion.

MGRS associated diseases have different clinical characteristics, treatment requirements and need to be distinguished from polyclonal Ig related kidney disease and/or another kidney disease where the Ig is not nephrotoxic to ensure appropriate treatment is given. The range of hematological conditions that can produce a nephrotoxic MIg and result in MGRS includes smouldering MM (SMM), smouldering WM, monoclonal B cell lymphocytosis (MBL), low grade CLL and other low-grade lymphomas. Once the hematological condition progresses to requiring treatment i.e., overt MM, WM, advanced CLL or malignant lymphoma these are no longer considered MGRS and managed according to disease specific protocols [15].

The deposition of the MIg can occur in the glomeruli, tubulointerstitium, and or the vasculature, with glomerular capillaries and mesangium being a preferred site for deposition [16]. The site of the various MGRS lesions is shown in Figure 1.4. LC deposition in the kidney is most seen, deposition of the whole Ig is less frequent, and monoclonal heavy chain deposition is rare.

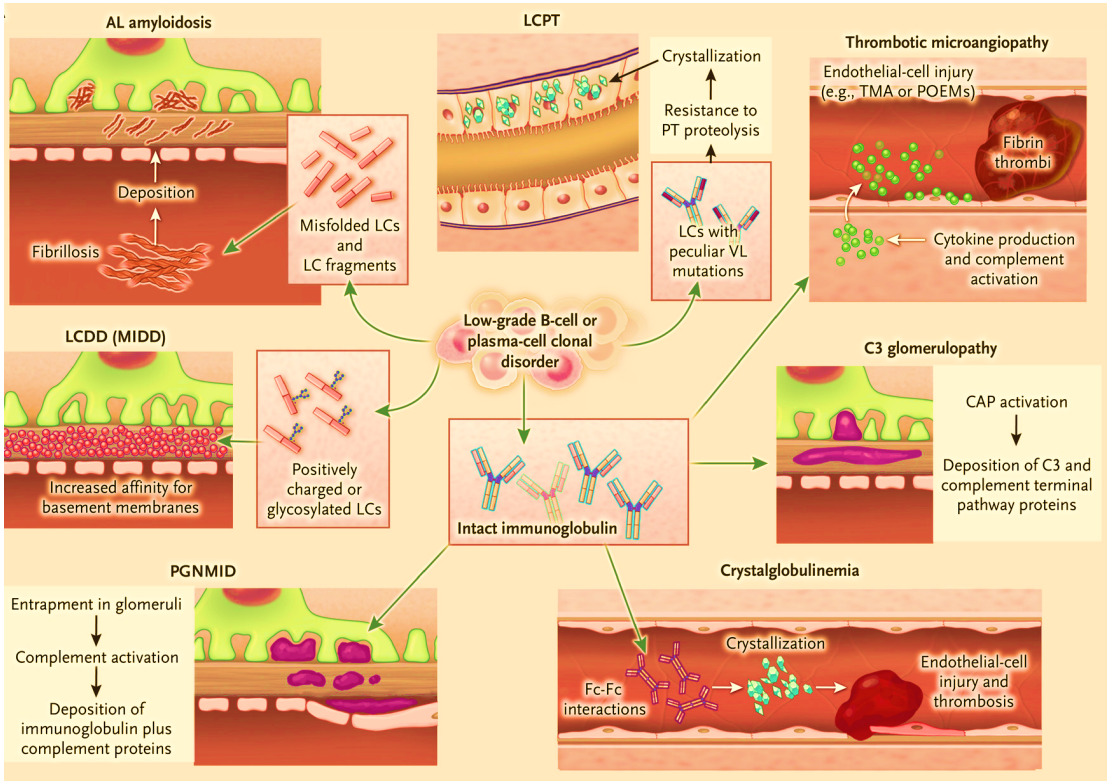
Figure 1.4: The site of various MGRS associated lesions in a nephron.
Reproduced with permission from Leung et al [17], Copyright Massachusetts Medical Society.



Abbreviations: AL, Ig light chain amyloidosis; LCDD, light chain deposition disease; LCPT, light chain proximal tubulopathy; PGNMID, proliferative glomerulonephritis with monoclonal immunoglobulin deposits; C3GN, C3 glomerulonephritis.

The MIg can cause renal injury by intratubular cast formation as in cast nephropathy (this will be discussed separately later in the chapter), direct tubular toxicity, or deposition in various compartments (Figure 1.5). Although the underlying clone does not cause tumour complications nor meet current haematological criteria for treatment, specific therapy aimed at the clone may be required to prevent further renal damage or slow progression to ESRF [15].

Figure 1.5: Mechanisms of renal injury in MGRS.
Reproduced with permission from Leung et al [17], Copyright Massachusetts Medical Society.

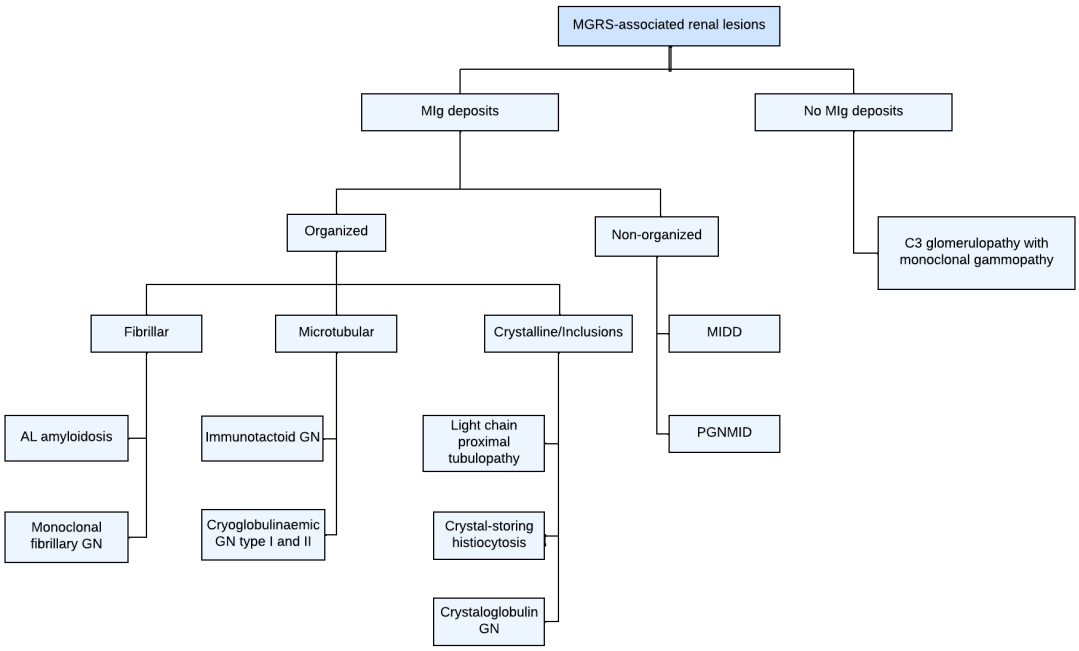


1) AL amyloidosis; deposition of misfolded monoclonal Ig LCs in the glomerular basement membrane and subepithelial space 2) LCPT; LCs with mutations in the variable domain are resistant to proteolysis and crystallize in the proximal tubular cells 3) Thrombotic microangiopathy; Igs can activate complement or act as autoantibodies against regulators of alternative pathway leading to endothelial cell injury 4) C3 glomerulopathy; MIg activates complement through the alternative pathway leading to deposition of C3 and glomerular inflammation 5) Crystaloglobulinemia; MIg crystallizes in arterioles, capillaries and glomeruli 6) PGNMID; MIg get trapped in glomeruli causing complement activation and glomerular inflammation 7) LCDD; abnormal light chains get deposited in the vascular, glomerular and tubular basement membranes.

1.5.1 Classification of MGRS

The International Kidney and Monoclonal Gammopathy (IKMG) research group has proposed a classification system for MGRS associated lesions based on immunofluorescence (IF) and the ultrastructural appearance of in situ deposits, categorized as organized and non-organized (Figure 1.6).

Figure 1.6: Classification of MGRS associated renal lesions.
Adapted from Leung et al., 2019 [15].



Abbreviations: MIg, monoclonal immunoglobulin; AL, immunoglobulin light chain amyloidosis; GN, glomerulonephritis; MIDD, monoclonal immunoglobulin deposition disease; PGNMID, proliferative glomerulonephritis with monoclonal immunoglobulin deposits.

MGRS can only be fully assessed by pathology departments that have available IF for a full panel of antibodies and electron microscopy (EM) and access to this technology is not universal across hospital trusts in the UK. The renal deposits are initially categorized as organized, non-organized and non-Ig [18].

1.5.1.1 Lesions with organized deposits

Organized deposits of MIg can be further categorized into fibrillar, microtubular or crystalline and/or inclusionary. Amyloid fibrils stain with Congo red and are solid, non-branching and randomly arranged with diameter of 7-12 nm. In fibrillary glomerulonephritis the fibrils are twice as thick (10-30 nm) compared to amyloid fibrils and generally do not stain with Congo red [19]. Microtubules are distinguished from fibrils by hollow centres and large diameters (17-52 nm) [20].

Fibrillar

The fibrillar category can be further split into Ig light chain (AL) amyloidosis and fibrillary glomerulonephritis. Previously AL amyloidosis was the only recognised entity in the fibrillar category [21]. In 1987 Alpers et al reported a case series of fibrillary glomerulonephritis distinct from amyloid [22] and these findings were confirmed in a study by Iskandar et al in 1992 to be distinctive enough to label fibrillary glomerulonephritis as a separate entity [23].

1. AL Amyloidosis

AL amyloidosis is the most common MGRS lesion. In the UK patients are referred to the UK National Amyloidosis Centre (NAC), and the development of a national cohort has enabled significant research into the natural history and biology of this disease. Amyloidosis is a disorder of protein misfolding and refers to extracellular deposition of low molecular weight proteins as fibrils. In AL amyloidosis there is a clonal expansion of CD38+ plasma cells that produce abnormal LCs, commonly of λ isotype [24]. A conformational change in the structure of LC to a beta pleated sheet, results in the formation of insoluble amyloid fibrils, that get deposited in tissues and interferes with organ function (Figure 1.4 and Figure 1.5). The most common organs involved are heart and kidneys [25].

AL amyloidosis can occur in patients with other malignant plasma cell dyscrasias like MM and WM and less frequently may be associated with CLL or non-lympho-plasmacytic lymphoma [26]. AL amyloidosis is usually a systemic disease with multiple organ and tissue involvement and less frequently presents as a localized disorder restricted to single organ/tissue [27]. An increase in the free LCs pre-dates all patients who eventually become symptomatic with the disease [28]. An estimated 500-600 people are diagnosed in the UK every year [29]. The median age at diagnosis is 64 years and it is more common in males [30-32]. AL amyloidosis is 5 to 10 times less frequent than MM [30]. In a patient with visceral amyloidosis and an Ig abnormality, amyloidosis may be non-AL with an incidental un related MGUS [33].

Differentiating AL amyloidosis from other forms of amyloidosis with tissue typing is mandatory as the latter will not benefit from chemotherapy directed to clone. The clinical presentation in AL amyloidosis depends on number and nature of organs affected (Table 1.1). Many patients present with non-specific symptoms such as malaise and weight loss. Renal manifestations are the most common and characterised by heavy proteinuria, nephrotic syndrome, and impaired renal function. Nearly 50% patients present with diastolic heart failure [34].

Amyloidosis should be suspected in any patient with a MGUS or SMM who presents unwell with oedema, fatigue or weight loss [33].

Table 1.1: Clinical manifestations of AL amyloidosis.
Adapted from Rana et al., 2019 [2].

Clinical manifestations of AL amyloidosis
Visible tissue infiltration (10% of cases)
Easy bruising (often periorbital)
Macroglossia with altered taste and dry mouth
Thyroid and salivary gland enlargement
Muscle and joint pseudo-hypertrophy
Autonomic neuropathy (15% of cases)
Orthostatic hypotension
Cardiac arrhythmias
Gastroparesis
Diarrhoea or constipation
Impaired bladder emptying
Erectile dysfunction
Peripheral neuropathy (20% of cases)
Carpal tunnel syndrome
Symmetrical distal axonal sensorimotor neuropathy
Hepatic (30% of cases)
Hepatomegaly
Increased ALP and GGT
Hepatic failure (rare)
Cardiac (60% of cases)
Diastolic heart failure/heart failure with preserved ejection fraction
Infiltrative cardiomyopathy
Symptoms of coronary heart disease
Renal (70% of cases)
Progressive chronic kidney disease
Proteinuria
Gastrointestinal
Weight loss, anorexia, diarrhoea, bloating
Blood loss
Adrenal axis
Biochemical hypoadrenalism (rare)
Pulmonary
Recurrent pleural effusions
Interstitial lung involvement is usually asymptomatic
Lymphoreticular system
Splenomegaly, features of hyposplenism
Lymphadenopathy
Haematology
Haemorrhagic complications

Abbreviations: ALP, alkaline phosphatase; GGT, γ-glutamyl transferase.

Screening for AL amyloidosis includes serum immunofixation (IFE), 24 urine IFE and sFLC assay. The diagnosis of AL amyloidosis requires evidence of a monoclonal plasma cell disorder and direct demonstration of amyloid fibrils on biopsy which on typing reveal LC protein subunits.

The amyloid fibrils have a characteristic appearance on EM i.e. randomly arranged fibrils 7-10 nm in external diameter [35-37] and bind to Congo Red with apple green birefringence under polarized light. Routine Congo Red staining of the marrow in MGUS or myeloma is not usually performed as only a small proportion of patients with positive bone marrow Congo Red will develop symptomatic amyloidosis [38]. The type of protein within the amyloid fibrils can be determined by immunohistochemical staining, IF or laser microdissection with mass spectrometry (MS). MS based proteomic analysis is the gold standard for typing protein subunits in amyloid deposits, is superior to immunohistochemistry in identifying protein subunit [39] and has led to discovery of new types of amyloidosis. The main limitation of MS is its availability in only selected centres. If the nature of amyloid fibrils cannot be determined genetic studies are required to diagnose hereditary amyloidosis.

Whole body ^{123}I-labelled serum amyloid P (SAP) scintigraphy can identify amyloid in solid organs and can be used in serial follow-up assessments to track the total body amyloid load (Figure 1.7).

Figure 1.7: Anterior whole body scintigraph image following injection of ^{123}I-human serum amyloid P in a patient with AL amyloidosis.



Uptake is seen in the bones, liver, and spleen.

Cardiac involvement is an adverse prognostic marker in AL amyloidosis [40]. In patients with renal AL amyloidosis who have received chemotherapy, baseline creatinine and proteinuria have been shown to predict progression to dialysis [41]. Low baseline proteinuria and cardiac troponin are independently associated with a renal response after autologous stem cell transplantation (ASCT) [42]. Patients who require dialysis are doing better than previously reported; however, mortality remains high in those with cardiac involvement [43].

Haematologic response is the strongest predictor of outcome and the best predictor of organ response [44]. The goal of treatment is a complete response (CR) as elimination of plasma cell clone may lead to reversal of organ damage [42, 45] and improved survival [46, 47]. Renal response follows a haematologic response [42] and those who achieve a renal response have better survival [42].

First line therapy with best outcome is daratumumab, bortezomib, cyclophosphamide and dexamethasone [33]. Bortezomib is the backbone of therapy and has been shown to improve cardiac function [48]. Daratumumab produces deep clonal responses making it ideal for AL amyloidosis [49] and in those who fail to achieve this response options include pomalidomide, stem cell transplantation (SCT), venetoclax and bendamustine [33].

Patients with AL amyloidosis now have a much better outlook with a 5-year overall survival of nearly 50% [50]. Renal transplantation in selected patients with sustained clonal remissions can achieve an excellent outcome and recurrence in the graft is not common [51].

2. Fibrillary glomerulonephritis

In 1977 Rosenmann and Eliakim reported this entity, characterized by infiltration of glomeruli by a fibrillar material like amyloid but not staining with Congo Red [52]. Duff et al used the term fibrillary to describe the ultrastructure of these deposits [53] and Alpers et al adopted the term fibrillary glomerulonephritis for this disease [22].

Fibrillary glomerulonephritis is a rare renal limited glomerular disease and accounts for approximately up to 1% of all kidney biopsy diagnosis [23]. It is characterized histologically by randomly arranged Congo Red negative fibrils, with an average diameter of 20 nm (larger than amyloid), in the mesangium and/or glomerular basement membrane [54, 55]. On IF polyclonal IgG is dominant and often the only Ig class with IgG4 subclass [23]. DnaJ homologue subfamily B member 9 (DNAJB9) is a highly specific and reliable proteomic biomarker for fibrillary glomerulonephritis [56] and is not seen in patients with amyloidosis or other glomerular diseases. Most cases are idiopathic although there is some evidence that fibrillary glomerulonephritis is more common in the context of malignancy, dysproteinaemias, hepatitis C and autoimmune disease.

In a small proportion (7-17 %) of patients with fibrillary glomerulonephritis there may be evidence of a monoclonal gammopathy. In 3-15% of these patients the IgG deposits exhibit LC restriction [54, 57], this is termed as monoclonal fibrillary glomerulonephritis and classified as MGRS lesion. Parafffin IF is required to make the diagnosis of monotypic fibrillary glomerulonephritis to unmask the polytypic deposits not apparent on standard IF on frozen tissue [58]. Occasionally monoclonal fibrillary glomerulonephritis can show Congo red positivity and staining for DNAJB9 and MS can help distinguish this from amyloidosis [59].

Nearly half of the patients present with nephrotic range proteinuria. Haematuria and hypertension are also common features. Renal prognosis is poor with nearly half of all patients progressing to ESRF within 2 to 4 years [23, 57]. Kidney transplantation can be associated with disease recurrence [54, 60].

There is heterogenous data regarding response to treatment and the optimal management of this condition has yet to be established. Patients with a possible cause i.e., monoclonal gammopathy may benefit from treatment of the underlying disorder.

Microtubular

Microtubules have hollow centres and large diameters (17-52 nm) compared to fibrils [20].

1. Immunotactoid glomerulonephritis

Immunotactoid glomerulonephritis is a very rare renal limited disease, seen in 0.06% of native kidney biopsies [57]. Deposits here are larger composed of parallel microtubules with predominant subepithelial and subendothelial localization (Figure 1.4) [61]. The microtubular structure of deposits is a function of abnormal Ig structure, its physicochemical properties and tissue affinity. In majority deposits contain IgG and C3 and on show LC restriction on IF [57].

Patients can present with nephrotic proteinuria, haematuria, renal insufficiency, and hypertension. It is often associated with low complement levels, monoclonal gammopathy and lymphoproliferative disorders and can recur in transplant kidney.

Fibrillary glomerulonephritis is distinct from immunotactoid glomerulonephritis: patients with former are less likely to have hypocomplementemia or dysproteinaemia and have deposits that are polyclonal [54, 57, 61, 62].

2. Type I and II Cryoglobulinaemic glomerulonephritis

Cryoglobulins are Igs that precipitate at cold temperature (below 37°C) and redissolve on warming. Cryoglobulinemia refers to the presence of cryoglobulin in a patient's serum. Brouet el al classified cryoglobulins into three distinct groups based on their Ig composition: Type I are monoclonal Igs, commonly IgG or IgM; Type II are mixture of monoclonal Ig with rheumatoid factor activity and polyclonal Ig and Type III are a mixture of polyclonal Igs [63]

Type I and II cryoglobulinaemias are considered MGRS associated disorders (Figure 1.4) [18]. Patients often present with vasculitic rashes, skin ulcers, glomerulonephritis, vasomotor symptoms like Raynaud's phenomenon, peripheral neuropathy and arthralgias.

Cryoglobulinemia should be suspected if these symptoms occur in the setting of a clonal hematologic disease (MGUS, MM or WM), viral infection (hepatitis B or hepatitis C virus), or connective tissue disease (systemic lupus erythematosus, Sjogren's or rheumatoid arthritis (RA)).

The presence of a cryoglobulin with a low C4 complement level is the hallmark of cryoglobulinemia syndrome [64]. The typical characteristics of cryoglobulinaemic glomerulonephritis are glomerular protein thrombi and arteriolar or arterial vasculitic lesions (Figure 1.4). The deposits appear as short, curved, or straight microtubules with predominant intraluminal and subendothelial localization (Figure 1.4) [65-67].

As is the case for all MGRS lesions treatment should focus on the underlying haematologic malignancy that is producing the cryoglobulin and the presence of complications like hyperviscosity syndrome [68]. For patients with underlying non-IgM MGUS and myeloma, novel myeloma therapy should be considered while rituximab-alkylator based therapy may be more appropriate for IgM MGUS and WM [69]. Plasmapheresis may be effective treatment for patients with severe skin involvement or renal disease if initiated earlier [70, 71].

Inclusions or crystalline deposits

1. Light chain proximal tubulopathy

Light chain proximal tubulopathy (LCPT) is a very rare renal disease, characterised by abnormal intracellular deposits of monoclonal LC in the form of crystals or inclusions within the cytoplasm of the epithelium of proximal tubule (Figure 1.4 and 1.5) [72, 73]. Here the light chains are of VK1 subgroup and have an intrinsic ability to resist proteolysis and undergo aggregation and crystal formation (Figure 1.4 and 1.5) [74-76]. Diagnosis can be a challenging due to rarity of this condition, indolent presentation, and often subtle non-specific findings on light microscopy. Antigen retrieval on formalin fixed, paraffin embedded tissue is required prior to IF [77] and careful EM study is required

Patients present with Fanconi syndrome (e.g., aminoaciduria, normoglycemic glycosuria, hypophosphatemia, hypouricemia, sub nephrotic-range proteinuria). The IKMG recommends chemotherapy or SCT to delay renal progression and minimize post-transplant recurrence [68]. A few case reports and series have shown benefit from these therapies [73, 78, 79].

2. Crystal storing histiocytosis

Crystal storing histiocytosis (CSH) is rare complication of monoclonal gammopathy, characterized by accumulation of monoclonal Ig fragments as crystals in histiocytes [80]. In the kidney these crystal laden histiocytes are seen commonly in the interstitium or in the glomerular mesangium [81-83] or very rarely within glomerular capillary loops [84, 85].

Although the exact mechanism is not known, it is hypothesized that there is either production of excess and/or structurally aberrant LC by the plasma cell tumor that is resistant to proteolysis and gets deposited as crystals [81]. These via systemic circulation can involve any anatomical site [80, 86-88].

Diagnosis requires careful ultrastructural evaluation by EM and proteome studies.

3. (Cryo)crystalglobulin glomerulonephritis

Crystalglobulinaemic glomerulonephritis is rare and characterized by crystallization of MIgs in the systemic vasculature leading to vascular injury (Figure 1.4 and 1.5) [89, 90]. When the crystallization process is precipitated by cold exposure it is termed as cryocrystalglobulinaemia [91]. In the literature, crystalglobulinemia has mainly been described in patients with MM but rarely can be seen in patients with MGRS [91].

Patients can present with wide range of manifestation like purpura, skin ulcers, arthropathy and renal involvement. Early recognition and prompt initiation of chemotherapy is key to better outcomes [92].

1.5.1.2 Lesions with non-organised deposits

1. Monoclonal immunoglobulin deposition disease

Monoclonal immunoglobulin deposition disease (MIDD) is characterized by deposition of MIgs or its components in various organs; the kidney is most affected, other organs reported to be affected are the heart and liver. There are three subtypes are: light chain deposition disease (LCDD), heavy chain deposition disease (HCDD), and light and heavy chain deposition disease (LHCDD). Of these LCDD is the most common accounting for 80% of cases.

LCDD is characterized by deposition of linear non-organized granular deposits composed of MIg LCs along the glomerular and tubular basement membranes (Figure 1.4 and 1.5). The diagnosis is usually made on EM.

The kidney is the principal target organ as LCs are filtered by the glomeruli, reabsorbed in proximal tubules, and degraded in tubular cells by lysosomal enzymes. Deposits in heavy-chain MIDD or light-and-heavy-chain MIDD lack the first constant domain of the Ig [93, 94].

LCDD usually presents in the sixth decade and is more common in men. Presentation is usually with hypertension and microscopic haematuria, and 50% of cases have associated nephrotic syndrome. Most cases have advanced CKD, ESRF occurs in up to 30%.

LCDD is associated with κ light chains in 80% of cases and more than 60% have underlying myeloma. Extra-renal manifestations of LCDD are seen in approximately 30% of cases, with cardiac infiltration occurring in 20% and clinically overt liver involvement in 10–20%. There have also been reports of LCDD in gastrointestinal tract and skin biopsies but this is extremely rare.

LCDD has a better outcome than other paraprotein-related renal diseases with a median patient survival of 7 years. LCDD should be aggressively treated with chemotherapy as achieving a deep clonal response prolongs renal survival and prevents allograft failure from recurrent LCDD in those who receive renal transplants [10]. Extra-renal involvement and lack of clonal response to chemotherapy are both features of a poor prognosis.

2. Proliferative glomerulonephritis with monoclonal immunoglobulin deposits.

Proliferative glomerulonephritis with monoclonal immunoglobulin deposits (PGNMID) mimics an immune complex glomerulonephritis. The most common pattern is a membranoproliferative glomerulonephritis (MPGN).

IF reveals monotypic deposits, and 20% patients have a detectable paraprotein. The deposits contain intact Igs and are confined to the glomeruli i.e., present in the mesangium, subendothelial space and occasionally in the subepithelial space (Figure 1.4 and 1.5) [94, 95]. Generally, PGNMID is associated with IgG3 but can be IgA or (rarely) IgM related [96, 97].

Various chemotherapy regimens have been used to treat PGNMID with varying results [95]. The renal prognosis of these patients remains poor [95] and PGNMID recurs frequently post renal transplant [98]. Patients with MPGN on light microscopy should be evaluated for an underlying clone.

1.5.1.3 Lesions without deposits

1. C3 glomerulopathy with monoclonal gammopathy

C3 glomerulopathy is characterized by glomerular lesions with predominant complement C3 fragment deposition without MIg deposits (Figure 1.4 and 1.5), even though 60-80% patients over 50 years have a clone at the time of diagnosis [99-102].

Here the MIg acts as a C3 nephritic factor or anti factor H antibody and results in uncontrolled activation of alternative complement pathway. Patients generally present in the fifth to sixth decade of life [100, 103] with hypertension, nephrotic range proteinuria and/or chronic kidney disease (CKD).

Light microscopy can exhibit a variety of proliferative patterns including membrano proliferative, mesangial proliferative, endocapillary proliferative and crescentic glomerulonephritis [100, 103]. EM shows ill-defined, moderately electron-dense mesangial, subepithelial and subendothelial deposits are seen [8].

IF reveals diffuse, bright, granular glomerular staining for C3 without staining for Ig heavy or light chains, C4 or C1q [100, 103]. Masked monotypic Igs can be present in one-third of patients and additional IF on protease digested, paraffin embedded tissue should be considered for patients with an MPGN pattern on light microscopy and negative IF [104, 105]. The IKMG recommendations are to adapt treatment strategy to the nature of clone (plasmacytic or lymphocytic) and severity of renal disease [68] and achievement of clonal response may influence renal outcomes [8].

1.5.1.4 Lesions with provisional status

The following lesions are not currently defined as MGRS but have a provisional status.

1.Thrombotic microangiopathy associated with monoclonal gammopathy

Here the thrombotic microangiopathy (TMA) occurs in the setting of a monoclonal gammopathy. The exact mechanism of how the monoclonal gammopathy induces TMA is not clear and the MIg may act as an autoantibody against a complement regulatory protein (Figure 1.4 and 1.5). Targeting the monoclonal gammopathy may be a novel approach in treatment of TMA [106].

2. Glomerular microangiopathy associated with polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes

Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes (POEMS) syndrome is a rare multisystem disorder due to an underlying plasma cell dyscrasia. RI is common and is thought to be due to cytokine mediated endothelial injury. Novel chemotherapy agents are effective, and this may improve outcomes [107] .

1.5.2 Evaluation of MGRS

Patients with unexplained urinary abnormalities (proteinuria and/or hematuria), or RI should have a MIg screen as a part of diagnostic work up. This includes serum protein electrophoresis (SPEP) with IFE, urine protein electrophoresis (UPEP) with IFE and sFLC analysis.

Measurement of LCs is important as can help identify 20% of those with AL amyloidosis [24] and a similar proportion of myeloma (i.e. light chain only (LCO) myeloma) patients that have no detectable MIg by serum IFE and UPEP, but detectable FLCs by sFLC analysis [108]. Detection of MIg helps establish the diagnosis and is important for response assessment.

A renal biopsy is required to diagnose the underlying MGRS associated renal lesion, which can help to guide treatment and is prognostically important. A kidney biopsy should include IF and EM to establish monoclonality and characterize the immunoglobulin Ig deposit ultra-structurally. In some patients' ancillary techniques may be needed to establish the diagnosis like protease IF, ultrastructural immunogold labelling and laser microdissection followed by liquid chromatography and mass spectrometry (LC-MS) [109]. The current gold standard for amyloid typing is laser microdissection followed by LC-MS, but this is available only in a few specialized centres [18]. These techniques are discussed in detail in Chapter II of this thesis.

A detailed hematologic work up (bone marrow aspirate and biopsy, flow cytometry, immunohistochemistry, fluorescence in situ hybridization testing +/- lymph node biopsy) including imaging studies is required to correlate the specific Ig found on renal biopsy with that found on hematologic work up to establish a link between paraprotein and kidney disease.

1.5.3 Treatment of MGRS

Treatment of MGRS associated kidney disease is often indicated to preserve or restore kidney function and prevent recurrence after kidney transplantation. The choice of chemotherapy regimen depends on the nature of underlying B-cell clone i.e. lymphocytic or plasmacytic in origin [68]. The renal response strongly correlates with the nature of the hematologic response and rapid suppression of Ig secretion with chemotherapy is required to improve outcomes [68]. For patients who have progressed to ESRF, kidney transplantation may be an option for those with a sustained hematologic response [110].

1.6 Multiple myeloma and the kidney

Myeloma is a cancer of the bone marrow, caused by clonal expansion of plasma cells. The clonal plasma cells usually produce a MIg. MM is classified by the type of abnormal Ig produced and diagnostic criteria are based on organ involvement (a myeloma defining event) or a high tumor burden. Diagnosis in myeloma is often delayed; a recent study showed that patients had multiple visits (average of 3) to primary care before referral to a hospital specialist [111].

RI is common in MM and up to half of the patients with MM on presentation have an eGFR <60 ml/min/1.73m2, and in 20% renal impairment is severe to classify this as a myeloma defining event (a serum creatinine > 173 μmol/l) [112, 113].

Up to 90% of severe AKI in myeloma is caused by cast nephropathy. Around 3.5% of patients with new presentation MM require dialysis treatment for AKI [38]. Severe RI adversely affects patient survival and in patients who develop ESRF median survival is only 18.3 months [114]. Recovery of renal function translates into improved survival [115]. There is limited but emerging data relating to renal transplantation for certain MM patients with ESRD [116].

Patients who present with AKI and a co-incident paraprotein require prompt workup. Delays in diagnosis and treatment can be the difference between life-long dialysis or recovery of independent renal function, with a profound impact on patient survival.

1.6.1 Clinical features

The symptoms and signs of MM can be both local and systemic.

Bone marrow infiltration with clonal plasma cells may result in anemia, leucopenia, thrombocytopenia, and immune paresis, with a high risk of subsequent infections. Bone involvement leads to hypercalcemia and lytic lesions, and tumor masses arising from the bones may result in spinal cord compression or nerve root compression [117]. If MM involves extramedullary organs, which is rare, the symptoms are similar to those of a solid tumor arising from the involved organ [117].

Systemic clinical features include malaise, weight loss, recurrent infections, consequences of hypercalcemia, and bone pain. The nonspecific nature of the clinical symptoms can often contribute to delays in diagnosis. Patients occasionally may present primarily with AKI without other obvious features of myeloma, although a disease related trigger (e.g., hypercalcemia, dehydration, sepsis) is common. Rarely a kidney biopsy performed for unexplained AKI may lead to MM diagnosis.

1.6.2 Epidemiology

There are around 5,500 new cases/year of MM in the UK [118]. It is the seventeenth most common cancer in the UK [119] and the second most common hematological malignancy after non-Hodgkin's lymphoma [120]. The reported international incidence for MM varies from 4 to almost 50 per million population/year, with the highest rates reported in high income countries [121]. The median age at onset is 70 years and it occurs more frequently in men, and in African Americans [119].

The median survival in MM with novel therapies is approximately 6 years [122]. In those eligible for intensive treatment, median survival is > 8 years [123, 124] and in the elderly (>75 years) median survival is 5 years [122, 125]. New therapy regimens are increasing survival for even the ultra-high-risk patients and emerging therapies (chimeric antigen receptor T cell) may improve survival further.

1.6.3 Pathogenesis of myeloma

Myeloma is a genetically complex disease and intra-clonal heterogeneity is a common feature [126]. A plasma cell becomes malignant through an accumulation of genetic hits across different cellular pathways, leading to dysregulation of the intrinsic biology of the cell over time [127].

There appears to be interplay between tumor cell genetics and the local microenvironment. The first oncogenic events may occur in the germinal centre during somatic hypermutation and isotype switching, and the plasma cell differentiates into a long lived cell [128]. These initial mutations also occur in premalignant plasma cell dyscrasias (MGUS and SMM), suggesting they are necessary but not especially causative in the pathogenesis of MM.

Later oncogenic events include mutations or expression mediated activation of oncogenes, loss of function of onco-suppressor genes, overexpression of anti-apoptotic proteins, secretion of various cytokines, growth factors and chemokines.

Each of these processes may have a role in supporting cancer cell growth, survival and resistance to therapies and occur in the bone marrow [127]. Genetic, epigenetic, and biological events occurring in the cancer clones and BM microenvironment all play a role in pathogenesis [127].

1.6.4 Evolution and development of myeloma

MM almost always evolves from an asymptomatic premalignant stage termed MGUS. Patients with MGUS have a 1% per year lifelong rate of malignant transformation, therefore most individuals with MGUS do not develop MM. The risk of progression is related to the concentration of the monoclonal protein, sFLC ratio, bone marrow plasmacytosis, proportion of phenotypically clonal plasma cells, and presence of immune paresis [129, 130]. Patients with certain cytogenetics: t (4;14) translocation, del (17p) and gain (1q) are at a higher risk of progression to MM and the monitoring strategy for a patient will be determined by risk of progression [131, 132].

The incidence of MGUS increases with age. MGUS is present in 3% of white individuals >=50 years old [3] and the prevalence is twofold higher in Blacks.. About 80% of MM originates from non-IgM intact Ig MGUS, and 20% from LCO Ig MGUS [133]. LCO MGUS has a lower rate of evolution to MM compared to intact Ig producing MGUS [134]. When IgM MGUS evolves into a disease this is usually to WM; rarely IgM MGUS can progress to IgM Myeloma [135, 136].

The diagnosis of MGUS requires the absence of end organ damage attributed to the clone of B-cell lineage or the monoclonal gammopathy produced by the clone.

SMM is an intermediate asymptomatic clinical stage between MGUS and MM, and patients have a 10%/year risk of progression to MM within the first 5 years of diagnosis, which then progressively reduces but never disappears; therefore SMM requires more frequent monitoring than MGUS [137].

It is important to note that not all patients with myeloma precursor diseases will develop overt MM. Refer to Table 1.2 for International Myeloma Working Group (IMWG) definition and diagnostic criteria for MGUS and SMM. For MM, the diagnosis requires either a myeloma defining event (Table 1.3) or a heavy tumor burden.

Table 1.2: Diagnostic criteria and classification for MGUS and SMM.
Adapted from Rajkumar et al., 2014 [133].

	Definition	**Progression rate**	**Primary progressing events**
Non-IgM MGUS	Serum monoclonal protein (non-IgM type) <30 g/L Clonal bone marrow plasma cells <10% Absence of end-organ damage i.e. CRAB lesions or amyloidosis that can be attributed to the plasma cell proliferative disorder.	1% per year	MM, solitary plasmacytoma, AL amyloidosis.
IgM MGUS	Serum IgM monoclonal protein <30 g/L Bone marrow lymphoplasmacytic infiltration <10% No evidence of anaemia, constitutional symptoms, hyperviscosity, lymphadenopathy, hepatosplenomegaly, or other end-organ damage that can be attributed to the underlying lymphoproliferative disorder.	1·5% per year	Waldenström macroglobulinaemia, AL amyloidosis.
Light-chain MGUS	Abnormal FLC ratio (<0·26 or >1·65) Increased level of the appropriate involved light chain (increased κ FLC in patients with ratio >1·65 and increased λ FLC in patients with ratio <0·26) No immunoglobulin heavy chain expression on immunofixation. Absence of end-organ damage such as CRAB lesions or amyloidosis that can be attributed to the plasma cell proliferative disorder. Clonal bone marrow plasma cells <10% Urinary monoclonal protein <500 mg/24 h	0·3% per year	Light chain MM, AL amyloidosis
Smouldering MM	Both criteria must be met: Serum monoclonal protein (IgG or IgA) ≥30 g/L or urinary monoclonal protein ≥500 mg per 24 h and/or clonal bone marrow plasma cells 10–60% Absence of myeloma defining events or amyloidosis.	10% per year in the first 5 years	MM

1.6.5 Diagnosis

The IMWG updated the diagnostic criteria for MM in 2015 (Table 1.3). These criteria allow for early diagnosis and treatment initiation before end organ damage has occurred. The revised criteria comprise the presence of: ≥10% clonal plasma cells on bone marrow examination or a biopsy-proven plasmacytoma and one or more myeloma defining event of the classic 'CRAB' features of hyper**c**alcemia, **r**enal failure, **a**nemia and/or destructive **b**one lesions, or evidence of a heavy tumor burden defined by ≥ 60% plasma cells on bone marrow biopsy, sFLC ratio of ≥ 100, or >1 focal bone lesion on MRI scan [133].

The requirement for MIg as a part of diagnostic test criteria is not mandatory as 3% of MM patients have non-secretory MM (NSMM) characterized by no MIg in the serum or urine on immunofixation at the time of diagnosis; 30% of these patients with NSMM have a normal FLC assay, despite clearly having MM as defined by other criteria.

Table 1.3: Revised IMWG diagnostic criteria for MM.

Adapted from Rajkumar et al., 2014 [133].

Definition of MM
Clonal bone marrow plasma cells ≥10% or Biopsy-proven bony or extramedullary plasmacytoma* and Any one or more of the following myeloma defining events: Evidence of end organ damage that can be attributed to the underlying plasma cell proliferative disorder, specifically: Hypercalcaemia: >2·75 mmol/L Renal insufficiency: creatinine clearance <40 mL per min † or serum creatinine >177 micromol/L Anaemia: haemoglobin value <100 g/L Bone lesions: one or more osteolytic lesions on skeletal radiography, CT, or PET-CT ‡ Any one or more of the following biomarkers of malignancy: Clonal bone marrow plasma cell percentage* ≥60% Involved: uninvolved serum free light chain ratio ≥100 § >1 focal lesions (size 5 mm or more) on MRI studies * Clonality should be established by showing κ/λ-light-chain restriction on flow cytometry, immunohistochemistry, or immunofluorescence †Measured or estimated by validated equations •‡ If bone marrow has less than 10% clonal plasma cells, more than one bone lesion is required to distinguish from solitary plasmacytoma with minimal marrow involvement. § These values are based on the serum Freelite assay (The Binding Site Group, Birmingham, UK). The involved free light chain must be ≥100 mg/L.

1.6.6 Prognostic factors

Prognosis in MM can be stratified by the International Staging System (ISS) and chromosomal abnormalities [138]. The ISS incorporates Beta 2 microglobulin (β2M), serum albumin, and reflects tumor burden, renal function (as a function of β2M renal clearance) and patient fitness. Bone marrow karyotype, translocations, chromosome number and gene expression profiling also have prognostic value [139].

Although detection of any cytogenetic abnormality is considered to suggest higher-risk disease, the specific abnormalities considered poor risk are cytogenetically detected chromosomal 13 or 13q deletion, t(4;14) and del17p, and detection by fluorescence in situ hybridization of t(4;14), t(14;16), and del17p [140]. Detection of 13q deletion by fluorescence in situ hybridization only, in the absence of other abnormalities, is not considered a high-risk feature.

1.6.7 Definition of renal failure in MM

The IMWG recommends using eGFR for the evaluation of renal function in patients with MM with a stable creatinine. An eGFR threshold of less than 40 ml/min/1.73m^2 has now been adopted to define RI in MM consistent with evidence of renal end-organ damage that fulfils the diagnostic criteria.

The IMWG criteria has been recently updated to clarify that only renal failure caused by LC cast nephropathy (based on typical histological changes or presumptive diagnosis based on the presence of high involved FLC levels, >1500 mg/l) is regarded as a myeloma defining event. The IMWG threshold for cast nephropathy is higher than that recommended by IKMG research group (>500 mg/L). The published literature indicates that the threshold sFLC at which there is a risk of MCN by the involved LC is 500 mg/L.

A renal biopsy should be reserved for diagnostic uncertainty, such as patients with an involved FLC level <500 mg/L and or heavy albuminuria, which may indicate the presence of a different renal pathology (e.g., AL amyloidosis).

1.6.8 Impact of kidney disease on prognosis

The survival of patients who present with impaired kidney function has been improved by the use of novel agents, in particular regimens that included the proteasome inhibitor Bortezomib [141]. Dialysis has a major impact on the survival of patients with MM, however survival is improving in patients with MM who require dialysis with a median overall survival of 14 months [38]. Over 50% of patients who present with AKI and require dialysis now recover independent renal function with an associated improvement in survival [142].

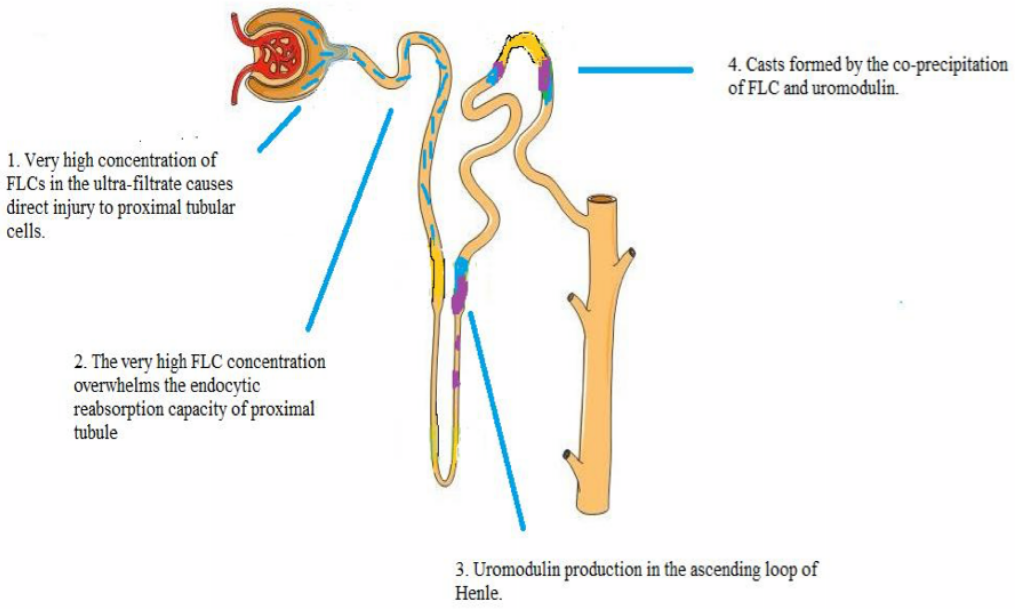
1.6.9 Pathogenesis of MM and kidney disease

Many patients with NDMM have mild to moderate RI which is usually transient and reversible. This is generally a consequence of dehydration, infection, hypercalcemia, use of contrast agents, and /or prescription of non-steroidal anti-inflammatory drugs (NSAIDs). With more severe AKI the dominant lesion is MCN; for patients who have dialysis dependent AKI, MCN is present in 90%.

Serum FLC are normally freely filtered by the glomerulus and reabsorbed in the proximal tubule via receptor mediated endocytosis. MCN develops when an excessive load of the involved FLC overwhelms the capacity of the tubular cells to catabolize them, with subsequent significant level in the tubular fluid of distal nephron segments.

Here FLCs bind to Tamm-Horsfall protein (uromodulin), a glycoprotein synthesized by the cells in the medullary thick ascending limb of the loop of Henle to form tubular casts (figure 1.8).

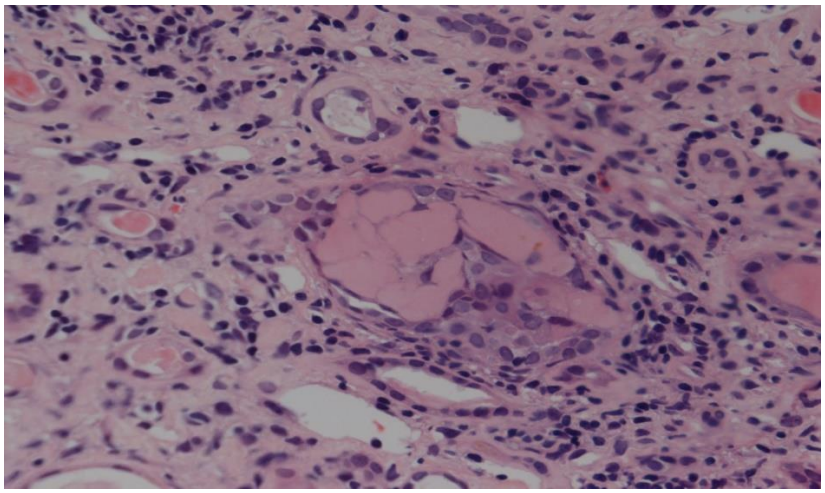
Figure 1.8: Mechanism of FLC induced AKI
Adapted from Rana et al., 2022 [1].



LCs interact through their complementary determining region with a specific binding site on Tamm-Horsfall protein to form aggregates and casts that subsequently lead to the tubular obstruction of the distal tubule and the thick ascending loop of Henle.

Tubular obstruction increases intraluminal pressure, reduces GFR and reduces interstitial blood flow, thus further compromising renal function [143]. The reduced tubular clearance of FLC further increases their concentration in the tubules and contributes to the vicious cycle that results in MCN (Figure 1.9). This lesion can quickly progress to ESRF[143].

Figure 1.9: The histological appearances of cast nephropathy
Adapted from Rana et al., 2022 [1].



Typical appearance of myeloma cast nephropathy: tubules contain hard, often fractured, distal tubular protein precipitates (casts) consisting of uromodulin and free light chain and surrounding cellular reaction.

An involved FLC level in excess of 500 mg/l can cause MCN [144]. However, most patients with levels >500 mg/L will not develop the lesion and require serum levels of several g/L of the involved LC before MCN develops.

The threshold for cast formation is dependent on the structural features of the involved LC and co-factors that increase the likelihood of cast formation including low pH, dehydration, hypercalcemia, NSAIDs, diuretics, and radiological contrast media. LC endocytosis may also cause acute tubular necrosis; aggregation of LCs after endocytosis may initiate a cascade resulting in tubular cell death [143]. LCs may lead to functional impairment of tubular cells, in which case Fanconi syndrome may present. Focal loss of microvilli and inhibition of Na-K-ATPase may lead to reabsorption defects [145].

Some myeloma patients also have a urine concentration defect, probably due to tubulo-interstitial changes, and nephrogenic diabetes insipidus due to unresponsiveness to anti diuretic hormone, thus further promoting dehydration [146]. Hypercalcemia is an important common cause of RI in MM. Hypercalcemia impairs renal concentrating ability. It causes vasoconstriction of renal vasculature and enhances diuresis, which may result in hypovolemia and pre-renal azotemia [143]. Concentrated urine and reduced urine flow enhance cast formation, thus leading to further renal damage [143].

1.6.10 Diagnostic workup in patients with suspected myeloma.

An approach to the diagnostic work up in those with suspected myeloma is shown in Table 1.4.

Table 1.4: Diagnostic workup in suspected myeloma.

Diagnostic workup in patients with suspected MM
1. History and physical examination 2. Routine testing Full blood count with differential and peripheral blood film Chemistry panel including calcium, creatinine and eGFR SPEP, IFE and serum FLC Nephelometric quantification of serum Igs. Routine urinalysis; 24-hour urine collection for proteinuria or quantification of proteinuria with ACR or PCR, urine electrophoresis and immunofixation. 3. BM aspirate plus trephine biopsy with testing for cytogenetics, fluorescent in situ hybridisation and immunophenotyping. 4. Imaging: Bones; including spine, pelvis, skull, humeri, and femurs. The IMWG recommends either a PET-CT or low dose whole body CT or MRI of whole body or spine in all patients with suspected myeloma.

SPEP tests for an intact Ig. If SPEP is positive, IFE will provide isotype characterization. All patients should be tested for sFLC. If this test is not available UPEP or urinary immunofixation electrophoresis (UIFE) for urinary LC (Bence Jones proteinuria) can be used.

The Freelite™ assay has undergone the most extensive clinical evaluation and is the most widely used FLC assay [147]; the test measures sFLC to below the normal range on a single blood sample. As previously discussed, elevation of both isotypes of serum free LC occurs in patients with RI. Hence an abnormal ratio and elevation of the involved free LC are required for the diagnosis of a LC clone.

The hematological response to treatment is classified based on serial monitoring of involved LC. Occasionally the sFLC assay can produce a false negative result because of antigen excess. If the test is negative and there is significant suspicion, then the laboratory should be informed and provided with a second sample that can be appropriately diluted.

Once an M-protein is identified in a patient with AKI, an immediate referral should be made to a hematologist.

1.6.11 Management of patients

1.6.11.1 Historical perspective

The treatment for myeloma has developed markedly over the last two centuries, from early remedies like rhubarb, leeches, orange peel and quinine [148] in 1840 to the current novel therapies.

A brief overview of the historical milestones in myeloma research are discussed below.

In 1947, Alwall et al reported 2 cases of myeloma treated with urethane, of which one responded with improvement in hemoglobin, disappearance of proteinuria and decrease in bone marrow plasma cells [149]; and urethane remained the standard of therapy in myeloma for nearly two decades [149, 150].

In 1966 Holland et al conducted a randomized controlled trial of urethane vs placebo in 83 patients with symptomatic myeloma, and reported that urethane administration was characterized by a shortened survival, and its use in myeloma was not advised [151].

The first breakthrough in treatment of myeloma came with the introduction of melphalan. In 1958, Blokhin from Moscow reported the application of melphalan or sarcolysin in myeloma [152]. It was the first drug to show consistent response in myeloma patients and for those who responded to melphalan, survival was 30 months longer compared to the unresponsive group [153].

In the late 1960s, prednisolone was added to melphalan. Prednisolone enhanced the activity of melphalan. The combination resulted in additional survival of 6 months when compared with melphalan as single treatment [154, 155].

In 1972 Harley et al introduced the combination of alkylating agents in the treatment of myeloma and for the next several decades various combinations of alkylating agents were used [156]. However, melphalan plus prednisolone (MP) remained the mainstay of myeloma treatment for decades, as trials failed to show a significant advantage in survival for other combinations [157].

The next major breakthrough in myeloma treatment came in 1983, when Tim McElwain and Powles reported that a high dose of melphalan could induce complete remission in patients with myeloma including those resistant to standard melphalan [158] and this was later confirmed in a larger study [153].

Barlogie et al played a major role in establishing high dose therapy (HDT) and stem cell rescue as standard therapy for myeloma [159, 160].

The past two decades have seen major therapeutic advances in myeloma with the emergence of proteosome inhibitors and immunomodulatory therapy, forming the backbone of contemporary anti-myeloma therapy.

1.6.11.2 Antimyeloma therapy

Prompt commencement of disease specific therapy is the single most important factor in the management of patients with MM. The introduction of novel agents such as bortezomib, thalidomide, and lenalidomide in the treatment of both transplant and non-transplant candidates has revolutionized the treatment. The various drugs used in myeloma treatment are discussed below.

Dexamethasone

Steroids are cytotoxic for myeloma cells and have the advantage of oral administration without being myelotoxic. Dexamethasone is the most effective steroid for rapid tumor kill, particularly when used in combination with other therapies. The most common dosing strategy is four-day pulses of 40mgs a day of dexamethasone, repeated weekly for three cycles, followed by less frequent pulses depending on disease response and the combination regimen that is being followed.

Adverse effects include hypertension, hyperglycemia, gastritis, weight gain, fluid retention, mood swings, opportunistic infections, insomnia, osteopenia, and Cushing's syndrome. Smaller doses of dexamethasone (10-30 mgs) are generally used in older patients to minimize side effects.

Proteasome inhibitors

Bortezomib

Bortezomib (formerly known as PS-341) is a boron containing molecule that specifically and reversibly inhibits the threonine residue of the 26S proteasome, an intracellular organelle that regulates protein homeostasis within the cell. Inhibition leads to cell cycle arrest, cellular apoptosis with malignant transformed and proliferating cells being more susceptible [161, 162].

In myeloma bortezomib causes inhibition of nuclear factor κB (NF-κB) activation and thereby inactivation of multiple downstream pathways known to be important in myeloma cell signaling [163].

It decreases the adhesion of the myeloma plasma cell to stromal cells which increases sensitivity to apoptosis, as well as interrupting pro-survival cytokine loops in the bone marrow microenvironment mediated by interleukin-6, insulin-like growth factor 1, vascular endothelial growth factor and tumor necrosis factor-α [164]. Other effects in myeloma include inhibition of angiogenesis, inhibition of DNA repair and impairment of osteoclast activity [165].

Bortezomib is metabolized in the liver via cytochrome P450 enzymes and no dose adjustment is necessary for patients with RI, including those on dialysis [113]. For hemodialysis patients, bortezomib should be administered after dialysis since dialysis may reduce concentrations [166].

In the initial studies with bortezomib peripheral neuropathy was a major concern. This can be diminished by administering bortezomib once a week instead of twice weekly [167, 168] and using the subcutaneous route instead of intravenous [169]. The common side effects are peripheral neuropathy and hematological toxicity, other are electrolyte abnormality, mood disturbance, hypotension, and gastrointestinal disturbances.

Bortezomib was approved in 2003 for the treatment of advanced MM, with approximately one third of patients with relapsed and refractory MM showing an overall response and 10% CR or near CR [170-172]. The assessment of proteasome inhibition for extending remissions (APEX) study comparing bortezomib to dexamethasone in relapsed/refractory myeloma patients demonstrated superiority of bortezomib for all endpoints, with an overall response rate of 38% for bortezomib vs 18% for high-dose dexamethasone, including CR/near-CR of 13% versus 2% respectively [173, 174].

A subgroup analysis of this study reported that this advantage of efficacy is maintained regardless of RI. Bortezomib treatment resulted in higher response rates than dexamethasone in all four renal subgroups (CrCl of <30, 30–50, 51–80 and >80 ml/ min) [175]. Proteasome inhibition may sensitize tumor cells to other chemotherapeutic agents and pre-clinical studies using chemo resistant MM cell lines showed that drug resistance may be overcome by combining bortezomib with other agents [176, 177].

Studies looking at use of bortezomib upfront in combination with; dexamethasone [178-180], cyclophosphamide and dexamethasone [181], thalidomide [182], melphalan and prednisolone [183] or adriamycin and dexamethasone [184] have shown encouraging response rates with no impairment in the capacity to harvest stem cells [185].

Bortezomib based maintenance is preferred for high risk patients [186].These drugs can maximize the likelihood of renal recovery with a major improvement in overall survival [141].

Carfilzomib

Carfilzomib is a second-generation proteasome inhibitor and is currently being examined in relapsed refractory myeloma and in newly diagnosed myeloma [187].

Ixazomib

Is an oral proteasome inhibitor and has the advantage of once weekly administration. Compared to bortezomib it has more gastrointestinal adverse events but lower risk of neurotoxicity.

It is active in patients with relapsed refractory myeloma and in the newly diagnosed patients [188, 189] and is approved for combination with lenalidomide in myeloma patients who have received at least one prior therapy [189].

Immunomodulatory therapy

Thalidomide

In 1999 Singhal et al reported on the antitumor activity of thalidomide in 84 patients with advanced myeloma, of which 32% patients responded; making it the first new drug with single agent activity for myeloma in more than 3 decades [148, 190].

Thalidomide has an anti-angiogenic effect, causes apoptosis of myeloma cells, and enhances tumor cell immuno-surveillance [190]. Specific effects include alteration of expression of adhesion molecules [191], suppression of tumor necrosis factor α [192], increased production of interleukin 10 [193], enhanced cell mediated immunity by directly stimulating cytotoxic T cells [194], increase in the total number of lymphocytes as well as CD8+ and CD4+T cell counts, and increase in the levels of interleukin-2 receptor [195].

Thalidomide undergoes biotransformation via nonenzymatic hydrolysis in the liver, and <1 % of the unchanged drug is excreted in the urine [196]. Thromboembolism is the single most important complication of thalidomide therapy. The risk of thromboembolism events increases substantially when thalidomide is given in conjunction with glucocorticoids or other agents [197] and whilst the majority of thrombotic events are venous, arterial thrombotic events are also reported [198].

Other side effects of thalidomide include peripheral neuropathy (can be irreversible if drug not promptly withdrawn), sedation, constipation, hypothyroidism, and skin rashes [199].

The use of thalidomide in patients with myeloma and renal failure (including those on dialysis) may be associated with hyperkalemia and close monitoring of potassium levels is prudent for such individuals [200, 201]. Dose adjustment is not necessary for patients with RI, including those on dialysis.

The combination of dexamethasone with thalidomide potentiates the anti-myeloma effect of thalidomide and induces a higher response rate [199].

The Myeloma IX study investigated thalidomide containing induction combination cyclophosphamide, thalidomide and dexamethasone (CTD) with cyclophosphamide, vincristine, doxorubicin hydrochloride (adriamycin), and dexamethasone (CVAD); and reported that the CTD combination was associated with improved response rates, with responses occurring more rapidly and with fewer infections [202].

When thalidomide was compared as maintenance therapy with observation in patients with newly diagnosed MM, treatment with thalidomide improved progression free survival (PFS) but there was no improvement in the OS. In patients with cytogenetic high-risk disease, thalidomide did not improve PFS or OS. [203].

Thalidomide is useful both as single agent and in combination with other therapies in patients with de novo and advanced myeloma and is effective in patients with renal failure [204, 205].

Lenalidomide

Lenalidomide is a second-generation immunomodulatory drug. It is structurally related to thalidomide however has an improved toxicity profile and more potent immunomodulatory activity. Lenalidomide has anti angiogenic, indirect immunomodulatory (resulting in activation of natural killer and T cells) and direct tumoricidal effects on myeloma cells [206-209].

In the myeloma cells, lenalidomide targets cereblon, a component of the E3 ubiquitin ligase complex, [206, 210, 211] and this interaction triggers proteasome degradation of transcription factors Ikaros and Aiolos, resulting in down regulation of myeloma survival signals IRF4 and MYC and up regulation of immunoregulatory molecule interleukin 2 [206, 207, 210].

Lenalidomide therapy is associated with an increased risk of thromboembolic events and myelosuppression (20%) mainly neutropenia and thrombocytopenia. Lenalidomide in combination with dexamethasone has synergistic effects, making this regimen one of the standards of care in patients with newly diagnosed myeloma and relapsed/refractory MM [212-216].

The Myeloma XI trial showed a significant benefit of lenalidomide maintenance therapy in terms of PFS, for patients in the intensive and non-intensive pathway, as well as across all cytogenetic risk groups, even those with high-risk disease [217]. In a meta-analysis of randomized trials, a significant benefit was seen in PFS and OS with lenalidomide maintenance compared to placebo or no therapy [218]. Lenalidomide is the standard of care for maintenance therapy for most patients [214, 219-223].

Lenalidomide is associated with a 2-3 fold increase in the risk of secondary cancers and patients should be counselled and monitored [224]. Lenalidomide is excreted through the kidneys [225] and requires dose adjustments according to the degree of renal impairment (RI) [226]. A single session of 4-hour hemodialysis only removes 30% of the administered dose [227].

Patients with creatinine clearance (CrCl) <40 ml/min are 8.4-fold more likely to require lenalidomide dose reduction due to grade 3 or grade 4 myelosuppression [228, 229]. Patients with pre-existing RI are at risk for increased drug toxicity and those with severe RI or on dialysis should be monitored for hematologic toxicity [113, 228, 230-232].

Retrospective analysis of data from two large phase 3 trials, assessing the impact of varying degree of RI on outcomes in patients with relapsed and/or refractory myeloma who received treatment with lenalidomide plus dexamethasone, showed that RI subgroups did not differ in terms of overall response rate, response quality, time to progression and PFS. However, patients with moderate RI (CrCl >= 30 ml/min to < 60 ml/min) and severe RI (CrCl < 30 ml/min) experienced increased incidence of thrombocytopenia, required more frequent dose reduction or interruption and had shorter survival than patients with mild or no RI [230].

Overall studies, evaluating lenalidomide and dexamethasone in patients with RI indicate that with careful monitoring of CrCl, adverse events and appropriate dose adjustments, this treatment is an effective and well tolerated option in MM patients with all degrees of RI [230, 233-237].

Pomalidomide

Pomalidomide is a second-generation immunomodulatory drug and is predominantly metabolized by the liver. Pomalidomide does not require dose adjustments in patients with CrCl ≥45 mL/min and it is being further studied in patients with CrCl <45 mL/min [113].

Monoclonal Antibodies

Daratumumab

Daratumumab is a monoclonal antibody that binds to CD38, causing myeloma cell apoptosis via antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity, or antibody-dependent cellular phagocytosis. It is approved for treatment of myeloma patients who have received at least three prior lines of therapy including a proteasome inhibitor and an immunomodulatory agent or who are double refractory to a proteasome inhibitor and an immunomodulatory agent.

Three-drug regimens containing the anti-CD38 monoclonal antibody daratumumab are some of the preferred regimens for the treatment of initial relapse and have the greatest reduction in risk of progression [238-240]. Daratumumab, lenalidomide, dexamethasone (DRd) is an alternative to bortezomib, lenalidomide, dexamethasone (VRd) as an initial treatment for patients with standard-risk MM who are not transplant candidates, especially for patients who cannot receive or tolerate bortezomib [241].

Elotuzumab

Elotuzumab is a humanized immunostimulatory monoclonal antibody that binds signaling lymphocytic activation molecule F7 (SLAMF7), a glycoprotein that is highly expressed on the surface of myeloma cells, natural killer cells, and some immune cells, but not on other normal tissues [242, 243].

The mechanism of action of elotuzumab includes natural killer cell–mediated antibody-dependent cellular cytotoxicity on SLAMF7-expressing myeloma cells and direct activation of natural killer cells [242-246]. Elotuzumab may facilitate macrophage-mediated killing of myeloma cells [247]. It has synergistic activity when combined with Rd [248]. Elotuzumab may be more active in combination with pomalidomide than with lenalidomide [224]. Elotuzumab, pomalidomide and dexamethasone combination has been approved for myeloma patients who have received at least 2 previous therapies including lenalidomide and a proteasome inhibitor [249].

Isatuximab

Is a monoclonal antibody targeting CD38 and is active in relapsed refractory myeloma. Isatuximab has been approved for the treatment of relapsed refractory myeloma in patients who have received two previous lines of treatment including lenalidomide and a proteasome inhibitor [250].

Other options

Selinexor

Selinexor blocks exportin 1 and leads to accumulation of various tumor suppressor proteins and inhibition of nuclear factor kappa B. Selinexor is approved for relapsed refractory myeloma who have received at least four prior therapies and whose disease is resistant to at least two proteasome inhibitors at least two immunomodulatory agents and an anti CD38 monoclonal antibody [224].

Panobinostat

Is a pan-deacetylase inhibitor approved for treatment of myeloma who have received at least two prior therapies including bortezomib and an immunomodulatory agent.

1.6.11.3 Treatment recommendations for newly diagnosed myeloma patients

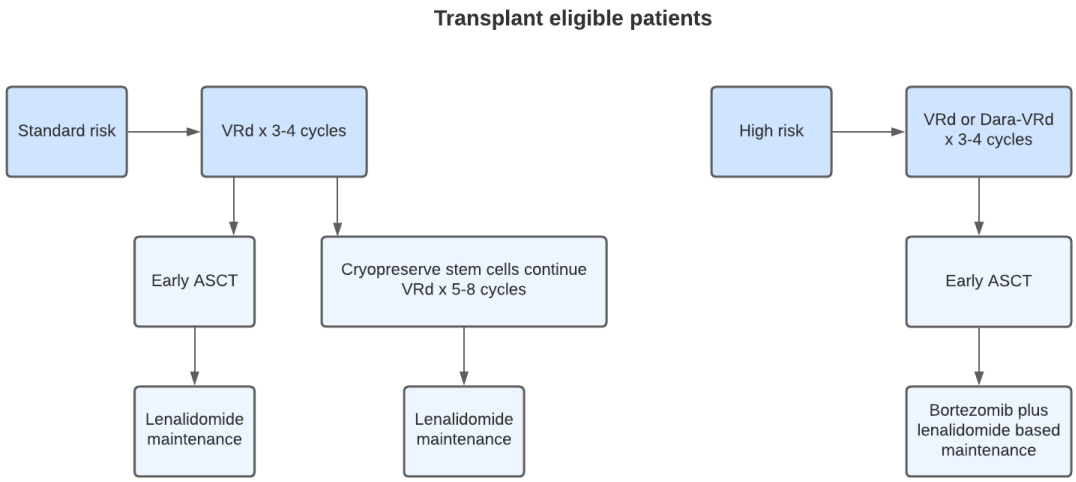
The initial evaluation of patients includes an assessment of eligibility for HDT and ASCT based on age, frailty, performance status, and comorbidities to guide choice of chemotherapy.

Patients eligible for ASCT

The standard of care for NDMM patients is VRd [122]. In standard risk patients VRd therapy is recommended for 3-4 cycles followed by ASCT, and lenalidomide maintenance [251]. An alternative is VRd for 8-12 cycles followed by lenalidomide maintenance therapy. Here stem cells should be collected after first 3-4 cycles of VRd and ASCT must be considered at first relapse [251].

In high-risk patients daratumumab, bortezomib, lenalidomide and dexamethasone (Dara-VRd) is recommended as initial therapy for 3-4 cycles followed by ASCT and then maintenance with bortezomib and lenalidomide. In patients presenting with acute renal failure secondary to cast nephropathy bortezomib-cyclophosphamide-dexamethasone (VCD) or daratumumab plus VCD is recommended [251]. An approach to treatment in this group is shown in Figure 1.10.

Figure 1.10: Approach to treatment of NDMM patients eligible for ASCT. Adapted from Rajkumar et al., 2022 [251].

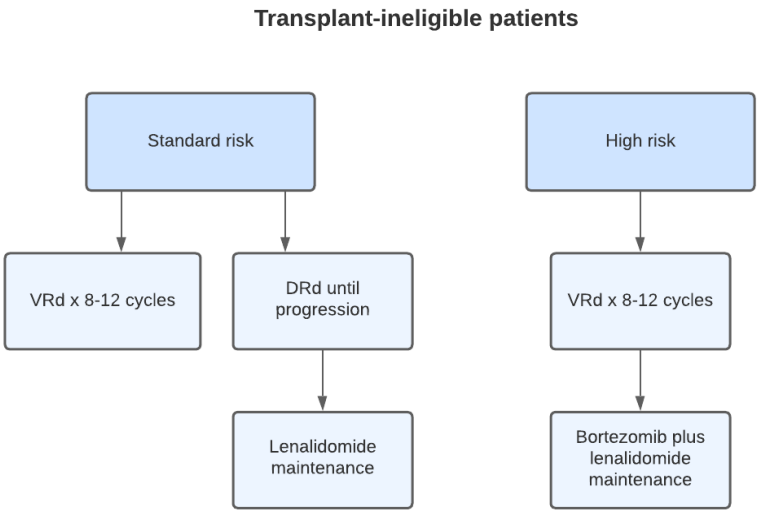


VRd, bortezomib lenalidomide, dexamethasone; Dara-VRd, daratumumab, bortezomib, lenalidomide and dexamethasone.

Patients not eligible for ASCT

In standard risk patients VRd [122] is recommended for 8-12 cycles followed by lenalidomide maintenance. DRd is an alternative [241] but requires treatment until progression making it expensive in the long run [252]. In the high risk patients VRd for 8-12 cycles is recommended followed by bortezomib plus lenalidomide maintenance [251]. An approach to treatment in this group is shown in Figure 1.11.

Figure 1.11: Approach to treatment of NDMM patients' ineligible for ASCT. Adapted from Rajkumar et al., 2022 [251].



VRd, bortezomib lenalidomide, dexamethasone; DRd, daratumumab, bortezomib, lenalidomide and dexamethasone.

1.6.11.4 Treatment of relapsed myeloma

All patients with myeloma will eventually relapse. For relapsed myeloma the remission duration decreases with each regimen [253]. The survival of patients with relapsed myeloma refractory to lenalidomide and bortezomib is poor with survival less than 10 months [254]. In 2015 daratumumab was approved by the United States Food and Drug Administration in relapsed refractory myeloma based on a phase II trial showing a response rate of 30% in heavily pre-treated patients [255]. Patients who are eligible for ASCT should consider ASCT as salvage therapy at first relapse, if they have never had a transplant before or if they have had a prolonged remission with first ASCT [224].

If relapse occurs more than 6 months after stopping therapy the initial treatment that successfully controlled the disease can be re instituted when possible [224]. A triplet regimen containing at least 2 new drugs that the patient is not refractory should be considered.

At first relapse for patients who are not refractory to lenalidomide recommendation is DRd; [224]. At first relapse for patients who are refractory to lenalidomide recommendation is for daratumumab, carfilzomib, dexamethasone; alternatives include pomalidomide based combinations [224].

Additional options for patients with multiple relapses and refractory disease are VCD, bendamustine based regimens, the addition of panobinostat to a proteasome inhibitor containing regimen, quadruplet daratumumab containing regimens, allogenic transplantation in young high-risk patients with a suitable donor and venetoclax in patients with t (11;14) myeloma.

1.6.12 MM and AKI

As previously discussed, early intervention with effective chemotherapy improves outcome and as such AKI in the context of MM management should be instituted as an emergency. The optimal management comprises early institution of novel antimyeloma therapy with good supportive care, to maximize restoration of kidney function. For patients who require dialysis the primary goal should be independence from dialysis.

Myeloma is usually regarded as a contra-indication to kidney transplantation. However, there are reports of successful kidney transplantation and this may be a treatment option for patients who sustain a complete remission and where the long-term hematological prognosis is good. At some stage most patients will require support for end-of-life care; some of these patients will have ESRF and careful MDT involvement to include renal service staff will be required at these times.

1.6.13 Extracorporeal removal of sFLC

Plasma exchange should not be performed for myeloma induced renal disease as previous studies have failed to show a benefit of plasma exchange on patient and renal outcomes [256]. The pathological proteins in myeloma kidney are FLCs. These are distributed throughout the extracellular compartment in patients with MM. Consequently, a short treatment such as plasma exchange will only remove a small amount of total FLC, with rapid re-equilibration of extravascular FLC from the extravascular to the intravascular compartment. This makes plasma exchange ineffective.

A high cut-off dialyzer can remove far more sFLC than plasma exchange. The membrane in this dialyzer removes both FLC isotypes [257, 258]. A few retrospective studies reported an increased proportion of patients with MM and dialysis dependent AKI recovering independent kidney function compared to those historically reported. However, patients in these studies received novel chemotherapy (usually bortezomib based) and assessed the relationship between FLC level and renal function in dialysis dependent patients treated with chemotherapy and high cut off dialysis.

Two randomized controlled trials have reported outcomes: the MYRE and EuLITE studies both reported no difference in renal recovery at 3 months in patients treated with high cut off hemodialysis compared to high flux hemodialysis. There was an increase in overall renal recovery in the MYRE study, but not in the EuLITE study, where there was an increased mortality reported by 2-years in patients who received high cut off hemodialysis [259, 260]. There is not sufficient evidence of benefit to support the use of high cut off hemodialysis in routine clinical practice.

1.6.14 Supportive care

Careful attention to fluid and electrolyte, infection, drug use, and bone management is mandatory. An individualized approach is required for provision of supportive care.

1.6.15 Multidisciplinary working in MM

Although MM is a hematological malignancy it may present to a variety of specialties and when diagnosed will often require the input of a multi-disciplinary team. Only when all members of this team are aware of MM as a potential diagnosis and recognize the urgency in prompt treatment, will optimal care be delivered. It is important to emphasize that involvement of a specialty is dependent on a specific indication and involvement can occur anywhere from diagnosis through to end-of-life care.

There is evidence that the diagnosis of MM may be delayed when the initial presentation of the patient is to a nephrologist [261]. The multi-disciplinary team who may be involved in the diagnosis and management of MM are shown in Table 1.5.

Table 1.5: Multi-disciplinary and medical specialty involvement in the diagnosis and management of MM

Multi-disciplinary specialists

- Haematology clinical nurse specialists
- Dialysis nurses
- Research nurses
- Pharmacists
- Physiotherapists
- Occupational therapists
- Social workers
- Psychologists

Medical specialties

- General Practitioners
- Haematologists
- Nephrologists
- Neurosurgeons (to manage spinal cord compression)
- Clinical oncologists (radiotherapy to bone lesions)
- Palliative care specialists
- Pain control specialist

1.6.16 Conclusions

The kidney is a major target organ in MM. Renal involvement with MM is the commonest paraprotein related renal disease. Patients with MM and severe AKI have poor outcomes, however with the introduction of bortezomib and other novel chemotherapy agents there are increased rates of renal recovery and long-term survival is improving dramatically. Prompt and accurate care in MM with disease specific management and supportive care is essential in achieving improved outcomes.

1.7 Waldenström's macroglobulinemia

WM is due to clonal proliferation of lymphoplasmacytic cells. Renal complications are less common than in patients with MM. Typically the renal lesion is due to intracapillary thrombi, secondary to IgM deposition, and may be associated with cryoglobulinemia. WM can also cause light chain cast nephropathy, nephrotic syndrome (due to amyloid deposition), non-amyloid nephrotic syndrome (with a minimal change-like picture). Treatment is generally rituximab plus chemotherapy. Single agent rituximab is less effective than other treatment options and often results in transient increase in serum IgM level that may worsen hyper viscosity symptoms. Commonly used regimens are bendamustine plus rituximab, dexamethasone, rituximab, and cyclophosphamide and bortezomib plus rituximab with or without dexamethasone.

The choice of the regimen is guided by toxicity profile, disease burden and disease status i.e., relapse.

1.8 Management of monoclonal gammopathy related kidney disease

General treatment principles

Treatment of monoclonal gammopathy related kidney disease is indicated to preserve or restore kidney function and prevent recurrence after kidney transplantation. The choice of chemotherapy regimen depends on the nature of the underlying clone i.e., lymphocytic or plasmacytic in origin. The renal response strongly correlates with the nature of the hematologic response and rapid suppression of MIg secretion with chemotherapy is required to improve outcomes. Effective treatment involves disease specific management and supportive care as in myeloma but there are specific considerations that are required as symptoms may be driven more from the renal effects.

Chemotherapy

The aim of treatment is to rapidly suppress MIg production, producing a deep and sustained clonal response while minimizing treatment-related mortality and morbidity. Response to treatment is assessed by measuring the change in the sFLC and paraprotein concentrations as in myeloma, however as the clone may be more subtle and the evidence base is not as robust the optimal treatment and how long it should be given for is often much more challenging. The choice of chemotherapy depends on the underlying hematological diagnosis, the degree of RI and/or other organ involvement and local availability of therapeutic agents.

Outcomes of kidney transplantation

MGRS can recur frequently and sometimes rapidly after kidney transplantation, and therefore achieving a complete hematologic remission is recommended prior to transplantation. Patients with LCDD who are treatment naïve prior to kidney transplantation have very high recurrence rates with recurrence often leading to graft loss and death due to hematologic relapse or other complications of therapy. Patients who experience a CR or better response prior to transplantation have few recurrences and good graft and overall survival [262-264]. In a large multicenter study of 237 patients with AL amyloidosis who underwent renal transplantation, the median OS from transplantation was significantly longer in patients with complete and very good hematologic responses compared to less than VGPR at transplantation [265]. Outcomes of kidney transplantation in patients with myeloma are very limited and relapse after transplantation is common [266] however selected MM patients i.e. with standard risk MM and adequate disease control (VGPR or CR or absence of minimal residual disease (MRD)) may benefit from transplantation.

1.9 The need for more research

Monoclonal gammopathy related kidney disease represents a group of heterogenous, relatively rare disorders with renal manifestation, secondary to a monoclonal nephrotoxic protein. These have high rate of recurrence post-transplant and although the patient survival is good in MGRS, kidney survival is poor. The understanding of these complex disorders is improving however standardized diagnostic and treatment strategies based on clinical trials are required to improve outcomes in this group. High quality research is a therefore a priority in this field.

The importance of research is demonstrated by the growing literature in this field and by the efforts of IKMG. The IKMG was founded in Bath, UK in October 2010 with a vision to bring together world experts in kidney disease caused by monoclonal proteins. The multidisciplinary team is made of hematologists, nephrologists, pathologists, immunologists, and scientists from around the world. The work of IKMG group led to the introduction of the term MGRS in 2012, to separate MM and MGUS from monoclonal gammopathy that result in renal damage. This has helped to clarify the seriousness of this condition and clarify the role of chemotherapy [267]. Since then the group has published recommendations for treatment [68], a classification scheme [268], updated the classification and refined the definition of MGRS [109].

Some of the potential benefits of further research in this field are:

1. Defining the burden of kidney disease in patients with a MIg can help generate evidence regarding the magnitude of problem and guide health care policy decisions.

2. The identification of factors associated with high risk of progression of MGRS to myeloma and other hematological malignancies, may allow development of risk stratification models that can help plan frequency of clinic visits and better counselling.

3. The identification of factors associated with outcomes in patients with monoclonal gammopathy related kidney disease would provide insight into the underlying pathophysiology and identify potential therapeutic targets.

4. Determining the incidence and prevalence of albuminuria in MGUS patients would guide decisions relating to cost effectiveness of screening programs in early detection of kidney disease versus consequences of undiagnosed kidney disease.

1.10 Summary and Research Aims

The introductory chapter has provided an overview of monoclonal gammopathy related kidney disease including MM and MGRS and made a case for more research in this field. ESRF is a significant determinant of poor outcome and recovery of kidney function is associated with better outcomes. Newer treatments have improved OS however data on renal outcomes in patients with MM and MGRS in the era of novel therapies is lacking.

The aims of this thesis are:

1. To review the diagnostic timeline in patients presenting with AKI and new myeloma and identify areas to improve the pathway.

2. To determine the renal outcome of patients presenting with MM and factors associated with outcome.

3. To determine recommendations for renal transplantation in patients with MGRS.

4. To evaluate albuminuria in patients with MM and MGUS.

1.10.1 Hypotheses

The hypotheses tested in this thesis are:

1. High levels of baseline involved LCs are independently associated with improvement in renal function at 12 months in the NDMM patients.

2. The depth of clonal response is independently associated with renal response and progression.

3. Albuminuria is an independent predictor of renal outcomes at 12 months in newly diagnosed MM patients.

1.11 Thesis structure

Chapter II provides an overview of the methods involved in the investigative strategy for monoclonal gammopathy. Methods specific to each analysis are included in the relevant chapter.

Chapter III reviews the diagnostic pathway of patients presenting with de novo myeloma and AKI at two large tertiary centres in the UK and provides recommendation for improvement.

Chapter IV reports on 12-month renal outcomes in the NDMM patients; results from the UK NCRI Myeloma XI trial.

Chapter V explores how specific treatments impact 12-month renal outcomes in the NDMM patients; results from the UK NCRI Myeloma XI trial.

Chapter VI evaluates albuminuria at baseline and 12 months in patients with NDMM from the UK NCRI Myeloma XI trial.

Chapter VII reports on a case with MGRS.

Chapter VIII reports on a single centre case series of post-transplant MGRS.

Chapter IX evaluates the role of screening for albuminuria in the MGUS cohort.

Chapter X provides a summary of the results, draws conclusions from this research, and ends with a discussion for future research.

Chapter II. Methodology

This chapter provides an overview of the general methods employed in the investigative strategy for the diagnosis of myeloma and MGRS.

2.1 Introduction

All patients require a detailed clinical evaluation (history and examination) to determine if there are any symptoms, signs and/or laboratory evidence to suggest a monoclonal gammopathy. The diagnosis of a monoclonal plasma cell disorder is made on the basis of IFE of blood and urine samples and bone marrow biopsy [269]. Most patients with myeloma develop bone disease [270] and as this is often heterogenous, a detailed assessment of the degree of skeletal involvement with imaging is crucial. The use of imaging modalities is influenced by the availability and affordability of techniques available [271].

If bone marrow evaluation does not reveal a clonal disorder the next step could be to perform imaging studies like computed tomography (CT) with or without positron emission tomography (PET) or whole-body magnetic resonance imaging (MRI) to look for localized plasmacytoma or for lymphadenopathy in lymphoma [68, 268]. Any suspicious lesions need to be biopsied for diagnosis and prognostication.

In patients with MGRS the evaluation starts with a kidney biopsy and if an MGRS associated lesion is identified, hematologic evaluation (MIg studies, bone marrow and cytogenetic analysis) should be performed [18].

Next generation flow cytometry has been used in minimal residual disease [272] and may be useful in patients with suspected MGRS with negative findings on cytology or flow cytometry of bone marrow samples [109]. In patients with CLL clone diagnosis could be made with peripheral blood flow cytometry. Patients with suspected myeloma or MGRS should be investigated using the tests listed in Table 2.1

Table 2.1: Initial investigations for patients with suspected MM/MGRS.
Adapted from Sive et al [273]

Screening tests
Full blood count with differential Serum creatinine, eGFR and serum calcium Serum immunoglobulins Serum protein electrophoresis with immunofixation Serum free light chain testing Urine albumin:creatinine ratio Urine albumin:creatinine ratio, Troponin and NT-proBNP for detecting amyloid
Tests to establish diagnosis
Bone marrow aspirate and trephine biopsy with plasma cell phenotyping Renal or tissue biopsy Imaging of the entire skeleton
Tests to estimate tumour burden and prognosis
Cytogenetic analysis Albumin, lactate dehydrogenase and beta 2 microglobulin levels

2.2 Laboratory methods for screening of a monoclonal gammopathy

Monoclonal gammopathies are characterized by clonal expansion of B cells that secrete intact MIg, monoclonal FLCs or both. SPEP followed by IFE tests are required to establish the presence of intact MIg.

LCs are readily filtered at the glomerulus, absorbed in the proximal tubule and the catabolized within the tubular cells. LCs will appear in the urine to be detected by conventional methods once the tubular absorption mechanism saturated [274, 275].

UPEP and IFE tests are required to detect monoclonal LC protein which is the only monoclonal product in 15% of myeloma cases, LCDD and in AL-amyloidosis [276, 277]. These LC diseases do not have sufficiently high concentrations of serum monoclonal LCs to be detected by SPEP or IFE [278] and even when IFE detects a monoclonal LC the amount of protein may be too low to be quantified and monitored by SPEP.

The sFLC assay is more sensitive than SPEP or UPEP and IFE for detection of monoclonal FLCs and is a suitable alternative to urine as a screening test for FLC monoclonal gammopathies except in AL amyloidosis. The sFLC assay has increased the diagnostic sensitivity of serum studies in LC diseases. Recently, MS techniques have been developed to identify M-proteins using high resolution molecular mass measurements that have superior sensitivity compared to traditional methods [279-281].

An overview of the different methods involved in the investigative strategy for screening of a monoclonal gammopathy is discussed below.

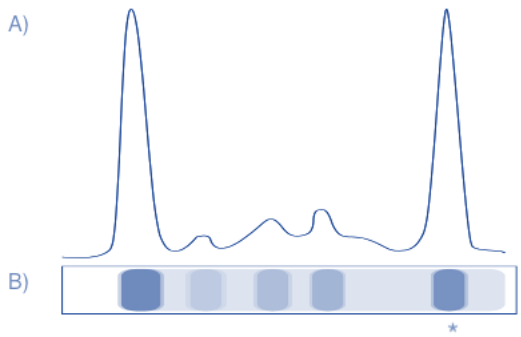
2.2.1 Screening for intact monoclonal gammopathy

2.2.1.1 Serum protein electrophoresis

SPEP is the most common screening tool to detect an intact Ig paraprotein. It is inexpensive and easy to perform screening test. It is usually done by the agarose gel method. Here serum is placed on a specific medium and a charge is applied. Electrophoresis separates proteins based on physical properties like charge, size and shape [282]. The different protein subtypes are placed in separate physical locations.

The proteins are classified by their final position after electrophoresis is complete into five general regions: albumin, alpha-1, alpha-2, beta, and gamma. These regions only refer to mobility through the support medium and do-not refer to the Ig class. The proteins are stained and their densities calculated to provide graphical data on the absolute and relative amounts of the various proteins [282]. A MIg usually presents as a homogenous spike in the gamma, beta, or alpha-2 region of the densitometer tracing or as a dense, discrete band on the agarose gel, shown in figure 2.1[282]. A more sensitive method of performing SPEP is by capillary zone electrophoresis [283, 284].

Figure 2.1: Densitometer tracing and agarose gel electrophoresis.



A) Densitometer tracing showing a spike in the gamma globulin zone. B) A localized band shown by asterisk, represents a MIg in the gamma globulin zone on agarose gel.

A polyclonal increase in Igs usually presents as a broad-based peak or band, typically of gamma mobility.SPEP helps to detect the presence or absence of an M-spike in the serum and enables quantification of the MIg. It is sensitive for detection of an Ig clone to a concentration of 500 mg/L [285]. SPEP is unhelpful in patients with non-secretory MM and with LCO MM. The initial SPEP should always be performed in conjunction with serum IFE; to determine and confirm monoclonality, and to determine the Ig class if a MIg is identified.

Therapeutic human monoclonal antibodies like daratumumab and elotuzumab can be detected on SPEP and IFE. This is important as standard SPEP, and IFE cannot differentiate between the therapeutic monoclonal antibody and the endogenous MIg. Laboratories should be notified if a patient has received one of these antibodies. In contrast infliximab, rituximab, eculizumab do not produce quantifiable monoclonal spikes on SPEP and false positive on IFE [286].

2.2.1.2 Immunofixation electrophoresis

IFE is more sensitive than SPEP for MIg detection. It can detect a clone to a concentration of 120 mg/L for an intact M protein and 20-50 mg/L for FLC (depending on the anti-sera used) [287] and characterize the heavy and light class of an M-protein.

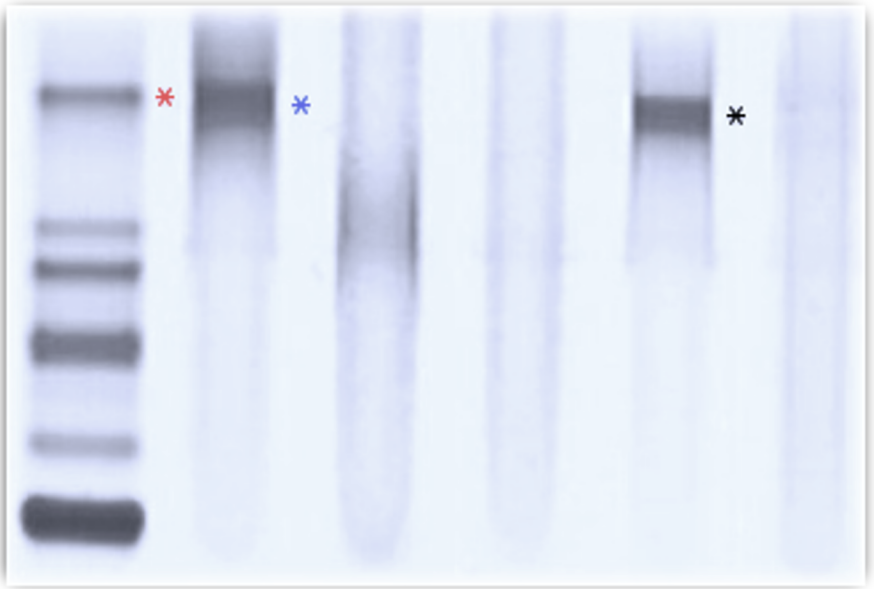
It is particularly useful for identifying and monitoring a pathological clone when there is more than one clone and for differentiating a monoclonal from a polyclonal increase in Igs.

IFE is not a routine first line test for assessment of monoclonal gammopathies [288]. It is performed if there is abnormality on SPEP or when MM, WM, primary amyloidosis, plasmacytoma, or a related disorder is suspected, despite a normal SPEP pattern [289].

IFE involves electrophoretic separation of the serum proteins, each sample is overlaid with different monospecific antibodies usually three for the heavy chain component and two for the light chain component (eg, anti-gamma, anti-mu, anti-alpha, anti-kappa, and anti-lambda, respectively). Precipitation of proteins occurs; this is followed by washing out of non-precipitated proteins and staining of the remaining precipitates.

An M protein is characterized on IFE by the presence of a discrete band associated with a single heavy chain class and a sharp, well-defined band that reacts with either kappa or lambda light chain antisera, but not both. Figure 2.2 shows SPEP and IFE pattern of a serum sample indicative of an M-protein.

Figure 2.2: The serum protein electrophoretic and immunofixation pattern indicative of an IgG kappa M protein.



SPEP *IgG* *IgA* *IgM* *Kappa* *Lambda*

Bands are represented by asterisk.

Limitations of IFE are that it is labour intensive, and cannot quantify MIg because of precipitating antibodies [290]. It is therefore not practical for measurement of disease response. A further limitation is that like with SPEP human IgG kappa monoclonal antibodies like daratumumab and elotuzumab can be detected on immunofixation assays.

The immunosubtraction procedure is an alternate procedure to IFE and is technically less demanding, automated, and is therefore a useful alternative to IFE [283, 284].

2.2.2 Screening for FLC monoclonal gammopathy

A clinically relevant clone can be present in the serum which is below the threshold for detection by SPEP and IFE, hence additional screening tests are required. These are discussed below.

2.2.2.1 Urine assays

Monoclonal urinary FLCs are known as Bence Jones proteins [291]. Urine testing for light chains by UPEP and urine IFE have been the long-standing method of choice for FLC detection. Urine dipsticks are unable to detect Bence Jones protein.

Urine is relatively deficient in total protein compared with plasma, however, can be concentrated many times which increases the sensitivity for detection of FLCs; most labs can work to detection limit of 10-40 mg/L. Quantitation of the urine M protein also requires a simultaneous measurement of 24-hour total urine protein.

24-hour UPEP

The UPEP detects M-protein in the urine by electrophoretic method like SPEP. A 24-hour urine collection is required to determine the total amount of protein excreted in the urine per day. The quantity of M protein excreted is determined by measuring the size of the M spike in the densitometer tracing and multiplying it by the total 24-hour urinary protein excretion. With evolving techniques this is now rarely used in clinical practice.

Urine IFE

Urine IFE is more sensitive than UPEP and allows the determination of isotype of the urinary M-protein. It does not quantitate the M-protein and hence must be done in conjunction with UPEP. IFE is sufficiently sensitive to detect a urine M protein of ≥0.004 g/dL [292]. The presence of urinary M-protein is characterized by a discrete band with reactivity to either κ or λ antisera but not both. κ and λ antisera that are monospecific, potent, and able to recognize both free and combined LCs should be used when performing IFE.

Limitations of urine assays

The urine assay in clinical practice has limitations and these include:

1. A few patients with a monoclonal gammopathy may have undetectable levels of urinary FLCs due to the way LCs are metabolized by the kidney, reducing the sensitivity of this method [293].

2. The process of concentrating the urine can result in both loss of protein [294] and false positive bands [295, 296].

3. The frequent failure to provide an appropriate 24-hour urine collection or a paired serum sample with urine collection [297] [14] [298].

4. UPEP and IFE are labour intensive even with modern automated analysis and not simple to interpret.

5. Other factors relate to the IFE method [299], affinity of polyclonal antibodies used in the IFE assay [299].

6. The excretion of LCs is affected by alterations in renal function and as a result urine LCs do not correlate with serum concentration and therefore are not directly related to tumor size and LC production [300].

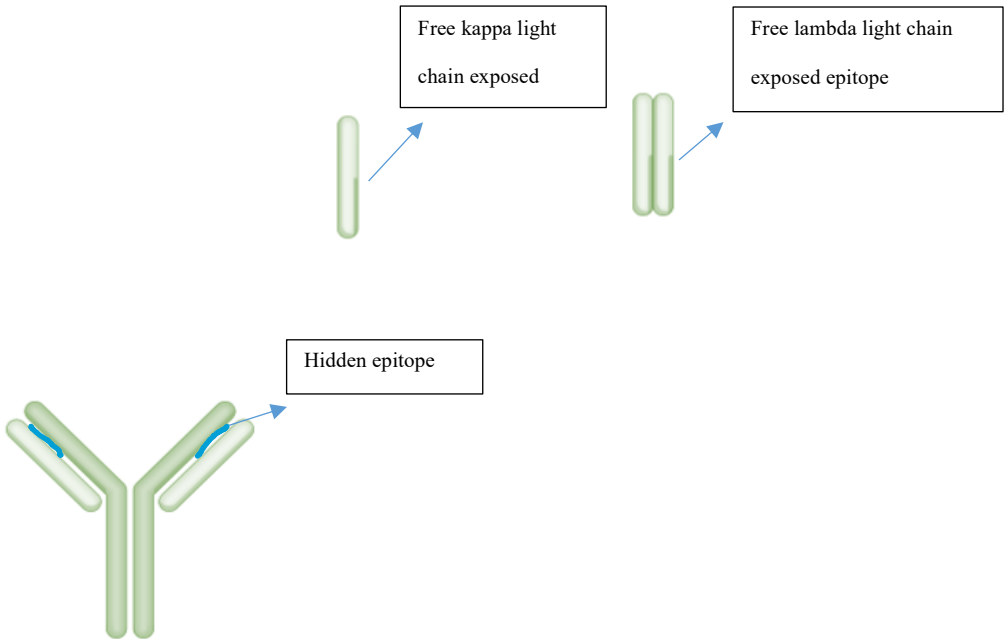
2.2.2.2 Serum free light chains

The serum FLC assay is automated, quantitative, and more sensitive than IFE for detection of monoclonal FLCs. The assay can detect both isotypes of light chain, to levels that are below the normal physiological range, which was not possible previously.

The Freelite assay (The Binding Site Group Ltd, Birmingham, UK) was the first marketed assay for quantifying serum FLC. It comprises of two sensitive and specific immunodiagnostic tests to measure κ and λ FLCs.

It is a nephelometric serum assay based on the principle of measuring the intensity of scattered light and uses rabbit/sheep anti human polyclonal antibodies against epitopes that are hidden when the LC is present in the intact Ig molecule and exposed when LCs are present in a free form. The assay reacts only with exposed free light chain epitopes which are hidden when the light chain is bound to the heavy chain (Figure 2.3).

Figure 2.3: Antibody molecule with hidden epitope and κ and λ FLC with the exposed epitopes.



The sFLC assay measures both isotypes of LC, κ and λ, and provides a ratio of the isotypes. When interpreting the results both the ratio and level of each isotype should be considered. Each cell of the B-cell lineage produces only one isotype of LC Ig and in clonal proliferation excess production of LC will usually occur compared with the other isotype. This leads to abnormal FLC ratio.

An elevated ratio is due to presence of κ FLC clone, and a low ratio indicates a λ FLC clone. Monoclonality can be identified by demonstration of an abnormal ratio of k:λ FLCs which is a consequence of excess production of a single FLC isotype by a clone of B cell lineage [301].

The assay is particularly useful in monitoring LCO MM, AL amyloidosis, non-secretory and oligo-secretory MM associated with production of low levels of FLC not routinely detected by IFE techniques. Using the FLC assay, the normal ranges are κ light chain: 3.3-19.4 mg/L, λ light chain: 5.7-26.3 mg/L, with a κ/λ ratio of 0.26-1.65. Using these reference ranges the immunoassay has a sensitivity of 97% and a specificity of 100% [301].

A sFLC κ/λ ratio of >1.65 suggests presence of κ M-proteins and a ratio of <0.26 suggests λ M-proteins. In CKD patients modifying the κ/λ reference range to 0.37-3.1 improves the diagnostic specificity.

Measurement of serum FLCs is important in both diagnosis and monitoring of patients with non-secretory, oligo secretory, LCO myeloma, primary systemic amyloidosis [24, 302], intact Ig MM [303] and is prognostic in MGUS [304], SMM [305], myeloma and solitary plasmacytoma of bone [306]. The FLC assay has contributed to the introduction of stringent CR as a new criterion of MM response [307] and defining response criteria in LCDD and AL-amyloidosis [10, 308, 309].

In patients with a monoclonal gammopathy; the serum FLC assay is more sensitive than urine testing [310-315] for detection of monoclonal FLCs, and the combination of SPE-IFE and serum FLC analysis identifies the majority of patients requiring treatment obviating the need for urine analysis which can be cumbersome and is often incomplete [311, 315, 316].

The limitations of FLC assays include; antigen excess which can lead to false negative results and manual dilution may be required in suspicious samples [317], underestimation, overestimation due to extreme polymerization, significant lot-to-lot variation (co efficient of variability 10-20%) between batches of polyclonal FLC antisera which may result in variable immunoreactivity of monoclonal FLCs and non-linearity [318]. The use of polyclonal antibodies enables broader LC recognition but may lead to higher lot to lot variation, monoclonal antibodies on the other hand may increase the risk of antigen excess and some paraproteins may escape detection [319]. Finally, a change in amino acid sequence of LC may render some epitopes unrecognizable to FLC reagents but are apparent on IFE or electrophoresis [320].

In addition to Freelite two other assays are available; N latex FLC and ELISA based Sebia FLC assay. The N latex FLC assay is a nephelometric assay based on monoclonal antibodies and was introduced in 2011 [321]. The Sebia FLC assay was launched in 2018 and is an ELISA based assay using polyclonal antibodies [322].

Higher total values for κ FLC and κ/λ ratio are seen with Freelite compared to N latex FLC and Sebia FLC assay and despite strong correlation and good agreement rates the FLC assays cannot be used interchangeably [319].

Seralite (Abingdon health) is a rapid lateral flow test for measurement of κ and λ FLCs. It can be offered in all clinical laboratories and provide a FLC results in ~10 minutes rather than days or weeks. This assay may improve the diagnostic pathway of patients with MM.

The IMWG recommends that SPEP, IFE, and serum FLC assay is a sufficient screening panel for monoclonal gammopathy except for AL amyloidosis, where screening should include urine IFE [320]. NICE now recommends sFLC assay rather than urine LCs to confirm the presence of an M-protein and studies have validated this [323]. The sFLC cannot replace SPEP in screening tool for monoclonal gammopathy as it is less sensitive for detection of intact Ig [303].

The most comprehensive and inclusive way to screen for monoclonal gammopathies is SPEP-IFE and FLC assays combined with UPEP-IFE.

2.2.3 Mass spectrometry

Electrophoretic separation of serum and urine proteins remain the front-line tests for screening, diagnosis, and monitoring of monoclonal gammopathies. However, these are limited in resolution, sensitivity and additional testing is required.

MS based methods that can detect low levels of M-protein in the serum and urine, have been developed. These assays are based on the principle that each M-protein has a unique amino acid sequence and therefore, has a unique mass which can be used to monitor the disease over time [324] [279].

The MS based methods provide a mass/charge for a given M-protein and are highly sensitive with limits of detection that are 100 times lower than that of IFE [281, 325]. The improvements in detection limits arises from the superior resolving power and mass accuracy enabling separation of the M-protein from the polyclonal background [280]. The monoclonal Ig rapid accurate mass measurement (mi-RAMM), is a MS based technique that uses the accurate molecular mass of the intact LC as a marker of disease [325] [279].

In the mi-RAMM method Igs are purified from the serum using camelid derived nanobodies directed against the HC constant domains of IgG, IgA and IgM or the LC constant domains of κ and λ [279, 326]. Then the heavy and LCs are dissociated using a reducing agent. The sample is analyzed by LC-MS. Here the LCs are used as a marker of disease as they produce a much higher signal. When an M-protein is present, a “spike” above the polyclonal background is observed.

The accurate molecular mass of the M-protein LC can be used as a surrogate marker of disease since it is specific to the patient and is constant over time [325]

A recent study has shown that MS of blood outperforms serum and urine immunofixation, sFLC and six colour flow cytometry of the bone marrow in detecting residual disease in AL amyloidosis [327]. A study of myeloma patients in stringent CR has shown greater sensitivity for MS techniques [280]. Also, the mass/charge of the monoclonal LC in the urine is not altered in renal failure as compared to that of serum making this a helpful tool in different patient populations [328].

At the Mayo Clinic, IFE is being replaced by MS for identification of M-proteins. At present, MS based assays are still relatively new and not a standard part of the evaluation of M-proteins in most centres. Work is ongoing to evaluate the clinical utility of these assays as preliminary studies are encouraging.

2.3 Bone marrow

A bone marrow aspirate and trephine biopsy are required to confirm a clonal disorder and provide prognostic information. Morphological assessment (for plasma cell percentage, presence of atypical lymphoid or lymphoplasmacytic aggregates i.e., lymphoma clones), flow cytometry immunophenotyping, cytogenetic and genetic evaluation are helpful for identification of small clones, prognostication, and treatment recommendations.

Fluorescence in-situ hybridization analysis on CD138-selected bone marrow plasma cells helps to identify the adverse risk abnormalities t(4;14), t(14;16), 1q gain, del(1p) and del(17p)(TP53 deletion), t(14;20), standard risk abnormalities t(11;14), hyper diploidy and guide treatment strategies [329-331].

Occasionally pathological clones can be difficult to detect in MGRS diseases especially those that do not have a detectable monoclonal Ig on IFE studies [96] and in patients with PGNMID [95, 96]. Here treatment can be very challenging as treatment depend on the nature of underlying clone i.e., plasmacytic or lymphocytic nature.

2.4 Renal biopsy

A kidney biopsy is essential to diagnose MGRS and is recommended in patients with unexplained kidney and monoclonal gammopathy, CKD and atypical course or those age <50 years with kidney disease and monoclonal gammopathy [109]. An integration of patient's history, laboratory findings and changes seen on LM, IF and EM are required to diagnose monoclonal gammopathy related kidney disease.

To confirm monoclonal nature of Ig deposits IF staining for LC restriction should be performed. EM is required to identify specific MGRS associated lesions.

Ultrastructural immunogold labelling can help in histopathological diagnosis of MGRS associated lesions by confirming the location and composition of MIg deposits but is not widely available [332, 333].

Laser microdissection followed by LC-MS is gold standard for amyloid typing but is available only in specialized centres. MS is crucial for diagnosis of rare hereditary forms of renal amyloidosis that cannot be typed by IF but also important to distinguish AL amyloidoses from non-Ig amyloidosis associated with non-specific entrapped Ig (AA amyloidosis) and from fibrillary glomerulonephritis [334, 335]. It can also aid in MGRS where IF studies are negative or are not available [336].

2.5 Flow cytometric immunophenotyping

Is a technique that evaluates individual cells in suspension for the presence and absence of specific antigens (phenotype) and thereby can help in identification and characterization cell types in a peripheral or bone marrow sample including types of cancer cells.

This technique can provide a rapid screen for hematologic neoplasms and play a key role in diagnosis and classification i.e., distinction between plasma cell neoplasms and mature B-lymphoid malignancies with plasmacytic differentiation [337].

Neoplastic plasma cells frequently show aberrant loss of CD45 and CD19, as well as aberrant expression of CD56 and CD117; therefore, these markers (in addition to κ and λ light chains and CD38) are useful in identifying small plasma cell clones and including CD5 and CD20 in the immunophenotyping of B cells can help separate small clones from polytypic cells [320]. The sensitivity of flow cytometry immunophenotyping depends on the total number of collected cells, the number of antibodies used to find an aberrant phenotype, the phenotype of the abnormal clone and sample quality [337].

2.6 Imaging

The IMWG recommends that in people with NDMM or SMM whole-body CT is the imaging of choice and fluorodeoxyglucose positron emission tomography CT can be used in place of whole-body CT. If whole-body CT is negative, and no other myeloma-defining events are present, IMWG recommends the use of whole-body MRI (or MRI of the spine and the pelvis, if whole-body MRI is not available) as the next diagnostic step because of its high sensitivity and the necessity to exclude focal lesions as myeloma-defining events. In MGRS imaging may not be helpful and in AL amyloidosis patients need to have an SAP scan to quantify amyloid. SAP is a normal circulating plasma protein that is deposited on amyloid fibrils because of its specific binding affinity for the amyloid fibrils. Scintigraphy after the injection of ^{123}I-SAP can be used for diagnosing, locating, and monitoring the extent of systemic amyloidosis [338].

Chapter III. Improving the Diagnostic Pathway in Patients Presenting with Acute Kidney Injury Secondary To De Novo Multiple Myeloma: A Two Centre Experience

The work presented in this chapter aims to review the diagnostic pathway in NDMM patients with AKI at two large tertiary centres in the UK, with a view to identify and improve areas where delays occur.

3.1 Extent of personal contribution

This study was a two-centre study, and I was responsible for the Birmingham cohort. I was responsible for the regulatory documents, audits, and correspondence with the Oxford site. I collected data with the help of informatics at the Birmingham site and colleagues at the Oxford site. I maintained the site file and led the data analysis, statistical plan, and interpretation of the dataset. The first draft of the manuscript was written by myself, and I was responsible for all the edits and revisions.

This chapter is based on the following published first author original article [339].

Rana, R., Pratt, G., Cook, M., Drayson, M.T., Ramasamy, K., Sadler, R., Zhu, D., Connor, T. and Pinney, J.H., 2021. Improving the diagnostic pathway in patients presenting with acute kidney injury secondary to de novo multiple myeloma: a short report. *BMJ open quality*, *10*(3), p.e001085 [339].

This work has been presented as an oral presentation at the Myeloma Early Diagnosis Working Group meeting, 2018, Birmingham (U.K); IKMG meeting, 2019, Montreal (Canada) and as a poster at UK Kidney week, 2019 Brighton (U.K).

3.2 Abstract

MM has one of the longest pathways to diagnosis of any cancer in the UK. Most patients are diagnosed in the outpatient setting; however, some present acutely to hospital as in-patients with AKI. These patients require prompt diagnosis and treatment to prevent long term kidney dysfunction. To identify and improve the areas where delays occur; the diagnostic pathway was reviewed in NDMM patients with AKI at two tertiary centres in the UK. All patients who presented to the two centres with AKI (any stage) and a sFLC concentration ≥ 500 mg/L from 1 April 2015 to 31 Dec 2017, were included in the study. Patients already known to have MM were excluded. A total of 56 patients were included (28 patients from each centre). The results at the two centres were comparable. Delays occurred throughout the pathway; from requesting sFLC to delay in anti-myeloma therapy. The main delay often related to whether or not patients were directed to the correct specialty for disease specific management. Having analyzed causes of diagnostic delay an intervention was developed whereby all new sFLCs ≥ 500 mg/L were reported (by phone/email) directly to the on-call hematology team. A re-audit over a 36-month period (February 2018 to February 2021) at University Hospitals Birmingham (UHB) identified 19 patients with AKI, sFLC <500 mg/L and new myeloma; the median time to bone marrow was 3 days (range 1–7) and the median time to dexamethasone was also 3 days, nearly a 50% improvement in the timeline. This low-cost simple intervention can be modelled by other trusts to speed the diagnostic pathway for patients with MM and this may improve patient outcomes.

3.3 Introduction

MM has a prolonged diagnostic pathway, with a median time to diagnosis (from onset of symptoms to diagnosis) of 163 days (IQR 84-306) [340]. RI is a common complication of MM and is associated with worse outcomes. AKI is a medical emergency and the time to initial treatment with anti-myeloma therapy is pivotal for renal recovery and patient outcome. Patients can present to various specialties and initial symptoms can be vague, this can make the diagnosis challenging resulting in diagnostic delay. To identify where delays occur; the diagnostic pathway in de novo MM patients with AKI was reviewed at two tertiary centres in the UK. Delays were identified within this clinical pathway and a change to the pathway was implemented to speed up the time to disease specific treatment. The aim was to improve the timeline to diagnosis by nearly 50%.

3.3.1 The Two Centres

UHB NHS Foundation Trust is one of the largest teaching hospitals in England. It manages more than 2.2 million patient contacts every year across the various sites. It is a regional centre for cancer, renal dialysis and has expertise in bone marrow transplantation. It has over 20,000 members of staff.

Oxford University Hospitals (OUH) is a world-renowned centre of clinical excellence and one of the largest NHS teaching trusts in the UK. It has over 1 million patient contacts every year. OUH notably provide regional hematology and renal services to a population of 2.2 million. The myeloma service is based in the Cancer and Hematology Centre at the Churchill Hospital. The OUH have over 11,700 members of staff. Both centres see between 40-60 new myeloma patients every year. Patients are referred for RRT in the context of AKI from a variety of regional district general hospitals.

UHB has a defined clinical pathway for the diagnosis of MM (Table 6.1).

Table 3.1: The UHB trust pathway for AKI and suspected myeloma

AKI and suspected myeloma
This is a haematological emergency.
Samples should be sent for SPEP, IFE and sFLC.
Speak to laboratory for same day light chain test results.
Bone marrow should be performed within 24 hours of presentation.
Liaise with the renal team to ensure optimisation of AKI (management of sepsis, dehydration, hypercalcaemia and cessation of nephrotoxic drugs).
Patients should be started on dexamethasone if sFLC level is ≥ 500 mg/L, without waiting for bone marrow result. This includes weekends.

3.4 Aims

The aims of this study were:

To determine delays in the diagnostic pathway.

To identify areas for improvement and assess efficacy of the change

3.5 Methods

The electronic records of patients who presented to the two centres with AKI of any stage and sFLC concentration of ≥ 500 mg/L between 1 April 2015 to 31 December 2017 were retrospectively reviewed. Automated AKI detection system was embedded in the biochemistry laboratory of UHB in April 2015 and elucidates the start date for data collection period for this study. Patients known to have MM were excluded.

3.6 Results

In total 56 patients were identified, 28 from each centre. Table 3.2 summarizes the results of the two centres. Patients had mostly been referred to hospital from primary care (41%). The median time to sFLC request was 1 day and median time to initial treatment with dexamethasone was 5 days. The longest delay was waiting for a bone marrow biopsy, with a median time of 6 days. The blood test request for sFLC was sent but there was a significant delay from the test to administration of dexamethasone and bone marrow biopsy. The median time from sFLC request to dexamethasone was 4.5 days (range 0-30) at UHB, and 4 days (range 0-28) at OUH. At UHB, the median time to hematology referral following admission was 3 days (range 0-39). There were delays in reporting of sFLC results, lack of communication between teams and slow referral to hematology.

Table 3.2: The pathway of investigations for patients presenting with de novo MM and AKI at two tertiary referral centres.

	First Centre	Second Centre
Referral pathway (number of patients)	GP 12 (43%) A and E 10 (36%) Other 6 (21%)	GP 11 (40%) AMU 9 (32%) A and E 2 (7%) Other 6 (21%)
Median time to first treatment with dexamethasone (days)	5 (IQR 13-3)	5 (IQR 16-3)
Median time to sFLC test request (days)	1 (IQR 2-0)	1 (IQR 1-0)
Median time from sFLC request to dexamethasone (days)	4.5 (IQR 12-2)	4 (IQR 14-3)
Median time to bone marrow (days)	6 (1QR 11-3)	6 (IQR 13-4)
Dexamethasone prior to bone marrow		
Number of patients	6 (21%)	10 (36%)
Median time (days)	2 (IQR 5-1)	4 (IQR 6-2)
Dexamethasone same day as bone marrow (number of patients)	10 (36%)	4 (14%)
Dexamethasone post bone marrow		
Number of patients	9 (32%)	7 (25%)
Median time (days)	8 (IQR 14-3)	7 (IQR 14-1)
Dexamethasone not received	3 (11%)	7 (25%)
Renal biopsy (patients)	6 (21%)	5 (18%)
Median time (days)	2 (IQR 7-2)	4 (IQR 10-2)

Other, presentation to any other specialty.

3.7 Intervention

The intervention was to call through all new sFLC results ≥ 500 mg/L by the immunology laboratory directly to the on-call hematology team via phone/email. The intervention aimed to reduce the time to diagnosis by shortening the delay between requesting and reporting of sFLC results ≥ 500mg/L to the hematology team.

A re-audit over a 36-month period (February 2018 to February 2021) at UHB identified 19 patients with AKI, sFLC ≥ 500mg/L and new myeloma; the median time to bone marrow was 3 days (range 1-7) and the median time to dexamethasone was also 3 days (1-12). The re-audit showed nearly a 50% improvement in the timeline.

The study also reviewed the timeline of all patients with a new sFLC result ≥ 500 mg/L (irrespective of whether patients had AKI or not) whose results were communicated to the on-call hematology team from February 2018 to January 2019 at UHB. Of the 35 patients whose results were called through by the immunology laboratory; 9 were new myeloma patients, 4 were managed as in-patients and 5 were outpatients. For the 4 in-patients; median time to bone marrow was 3 days (range 1-7), time to dexamethasone was 2.5 days (range 1-7); these showed a 50% improvement compared to baseline audit. Median time from collection of sFLC test to phone call/email was 2.5 days (range 1-5). For the 5 new myeloma patients managed as outpatients; the median time to bone marrow was 15 days (range 3-36) and median time to treatment was 29.5 days (range 4-49).

3.8 Discussion

MM has a prolonged diagnostic pathway and delayed diagnosis is associated with worse outcome [341]. Delays occur throughout the pathway from patient presentation to final diagnosis. A sFLC level ≥ 500 mg/L is associated with a high risk of cast nephropathy and requires urgent hematological assessment [14, 144, 342] without the need for a kidney biopsy [342]. Patients who have a prompt diagnosis of cast nephropathy and rapid intervention with anti-myeloma therapy have better outcomes [259, 343, 344].

The baseline audit showed prompt requests of the sFLC test, however there was delay in referring to the hematology team, a requirement for progression to bone marrow biopsy and start of disease specific treatment. Another finding at UHB was that, although the immunology laboratory has a system of in-house reporting of sFLC results within 24 hours 5 days a week, there was often a delay in the results being reported onto the electronic patient record. Also, there was no system of flagging up the abnormal sFLC results to ensure the team had noted and acted on the abnormal result. sFLC testing remains a specialist test and many clinicians may not be aware of the importance of such an abnormal result.

Following this a change in the pathway at UHB was implemented, and any new sFLC test result ≥ 500 mg/L was reported to the on-call hematology team with a note on the patient electronic record of the communication to the relevant team member.

By shortening the time to hematology referral, delays in directing appropriate patients to hematology in need of disease specific intervention were minimized. As a result of this intervention, the time to treatment in the Birmingham cohort was reduced by nearly 50%.

There have been various initiatives to improve the diagnostic pathway for myeloma patients [345-347] including the establishment of The Myeloma Early Diagnosis Working Group in 2016 [348]. Alternative flagging systems such as auto generated emails to hematology and/ or other push notifications can be used by trusts to improve the timeline to diagnosis in myeloma patients, although these may not be as targeted as the intervention of direct communication with the relevant team.

This project demonstrates that targeted communication has the potential to improve the time to diagnosis in MM patients.

3.9 Strengths and limitations

This project is the first of its kind to provide insight regarding time to diagnosis in the NDMM patients in tertiary centres in the U.K (with availability of in-house sFLC testing). It demonstrates that targeted communication has the potential to improve the diagnostic pathway in myeloma.

A limitation of this study is that it not representative of the pathways in smaller hospitals which are likely to incur a longer time to diagnosis. Another limitation is the small number of patients in this study.

3.10 Conclusions

In conclusion, delays occur throughout the diagnostic pathway however the main delay appears to be in referring to hematology for disease specific management.

Targeted communication is a low-cost intervention that has the potential to improve the time to diagnosis in MM patients and can be modelled by other trusts to improve the diagnostic pathway.

Chapter IV. Renal Outcome in Patients Presenting with Multiple Myeloma; results from the UK NCRI Myeloma XI Trial.

The work presented in this chapter will evaluate renal outcome at 12 months in the NDMM patients from the Myeloma XI trial.

4.1 Extent of personal contribution

The Myeloma XI trial was a phase III multicentre, open label randomized controlled trial and I was responsible for the secondary analysis, looking at renal outcomes and factors related to outcomes in the NDMM patients. The central laboratory in Birmingham assessed the samples (i.e., for creatinine, immunoglobulins, paraprotein, sFLC) and I utilized the core dataset to investigate renal outcomes. I led the data analysis, statistical plan, and interpretation of this secondary dataset. Following analysis, I wrote the first draft of the manuscript, and I was responsible for all the edits and revisions.

This work in this chapter formed the basis for a peer reviewed publication [349].

Rana, R., Cockwell, P., Drayson, M., Cook, M., Pratt, G., Cairns, D.A., Pawlyn, C., Jackson, G., Davies, F., Morgan, G. and Pinney, J.H., 2020. Renal outcome in patients with newly diagnosed multiple myeloma: results from the UK NCRI Myeloma XI trial. *Blood advances*, *4*(22), pp.5836-5845.

This work has been presented as an oral presentation at the UK Kidney week, 2019, Brighton (UK) and as a poster in the 4th IKMG meeting, 2019, Montreal (Canada).

4.2 Abstract

RI in myeloma is common; however, the natural history of renal injury in this cohort remains uncertain, especially in the context of intensive therapy and novel therapies. To address this lack of data, renal function of 2,334 patients from the UK Myeloma XI trial were evaluated at baseline and at 12-months, to assess renal function over time and the factors associated with change. Patients who had severe AKI after initial fluid resuscitation or requirement for dialysis were excluded. At 12-months of the 1,450 evaluable patients planned for autologous transplantation; 204 (14%) patients had a decline in eGFR ≥ 25% from baseline, 341 (23.5%) had an improvement, and 905 (62%) had no significant change in eGFR. Renal outcome at 12-months for the 884 evaluable patients who were not planned for transplant was similar. Improved renal function was more likely if patients were <70 years old, male, with an average eGFR <60 ml/min/1.73 m^2, had higher baseline FLC level >1000 mg/L, and/or a FLC response of >90%. In conclusion, this study shows that with current therapies a significant decline in renal function in the first 12 months in patients receiving either intensive or non-intensive treatment is low. The greatest relative improvement in eGFR is seen in patients with high FLC at baseline and a high LC response.

4.3 Introduction

Around 50% of patients with MM have RI at presentation as defined by an eGFR <60 ml/min/1.73m^2. Severe RI (eGFR <30ml/min/1.73m^2) affects up to 20% and is associated with poorer outcomes [115, 350-352]. Many patients with severe RI have severe AKI and some of these patients are dialysis dependent at or shortly after presentation: in this group of patients one-year survival improves if there is recovery of renal function [344, 353, 354]. Many of these patients have MCN caused by high levels of sFLC. With rapid reduction of sFLC many patients with MCN will recover kidney function. However, most patients with a low eGFR have CKD and mild to moderate RI (eGFR 30-59 ml/min), and in this group the impact of RI on long-term outcomes is uncertain. In addition, it is not known what proportion of patients with mild to moderate RI have MCN as a component of their RI.

Understanding the natural history of renal function in patients with myeloma including the impact of mild to moderate RI on clinical outcomes in patients with MM will become increasingly important as long-term outcomes continue to improve. In many long-term diseases the baseline level of kidney function is a major determinant of patient survival. Defining the contribution of kidney disease stratified by severity on outcomes will inform discussions around future therapy choices and overall long-term prognosis. In addition, there has been no large-scale assessment of the relationship between renal function change post induction and sFLC at presentation and the change in sFLC post induction in patients with mild to moderate RI at presentation.

To address this lack of knowledge of the impact of renal disease on outcomes in the era of novel therapies and transplantation, data from the Myeloma XI trial (the largest trial worldwide of NDMM patients) was utilized to assess the effects of renal function at presentation and sFLC pre- and post-induction on 12-month renal function and patient survival.

4.4 Hypotheses

The work presented in this chapter aimed to address the following hypotheses:

1. Baseline FLC level is independently associated with renal outcomes at 12 months.

2. Depth of clonal response is independently associated with renal outcomes at 12 months.

4.5 Methods

4.5.1 Myeloma XI trial patients

Myeloma XI was a phase III, randomized, multi-centre, parallel group design, open-labelled trial of thalidomide, lenalidomide, carfilzomib and bortezomib induction combinations, and maintenance lenalidomide +/- vorinostat in newly diagnosed patients with symptomatic myeloma (International Standard Randomised Controlled Trial Number 49407852).

A multicentre research ethics committee and local ethics committees approved the protocol, and all patients gave written informed consent in accordance with the Declaration of Helsinki. The main trial results have been published elsewhere [217, 355, 356]. The trial had two distinct treatment pathways, the intensive pathway, which included younger patients where HDT with stem cell support was deemed appropriate, and the non-intensive pathway which included patients who were deemed unsuitable for a transplant.

In both pathways patients were randomized to induction therapy with CTD or cyclophosphamide lenalidomide and dexamethasone (CRD) and +/- cyclophosphamide, bortezomib, dexamethasone (CVD) intensification in patients with suboptimal response to CRD or CTD and treated to maximum response. In a modification of the trial carfilzomib, cyclophosphamide, lenalidomide, and dexamethasone (KCRD) was used as induction compared to the response adapted approach with CTD or CRD +/- CVD. Post-transplant (or after the completion of induction if transplant ineligible) patients were randomized to lenalidomide, lenalidomide and vorinostat or no maintenance treatment.

Patients aged 18 years or above with symptomatic new myeloma were eligible to participate. The exclusion criteria notably included acute renal failure (unresponsive to up to 72 hours of rehydration, characterized by creatinine >500 μmol/L or urine output <400 ml/day or requirement for dialysis) [217]. The treatment pathway was decided on an individual basis based on a combination of the patient's performance status, clinical judgement, and participant preference.

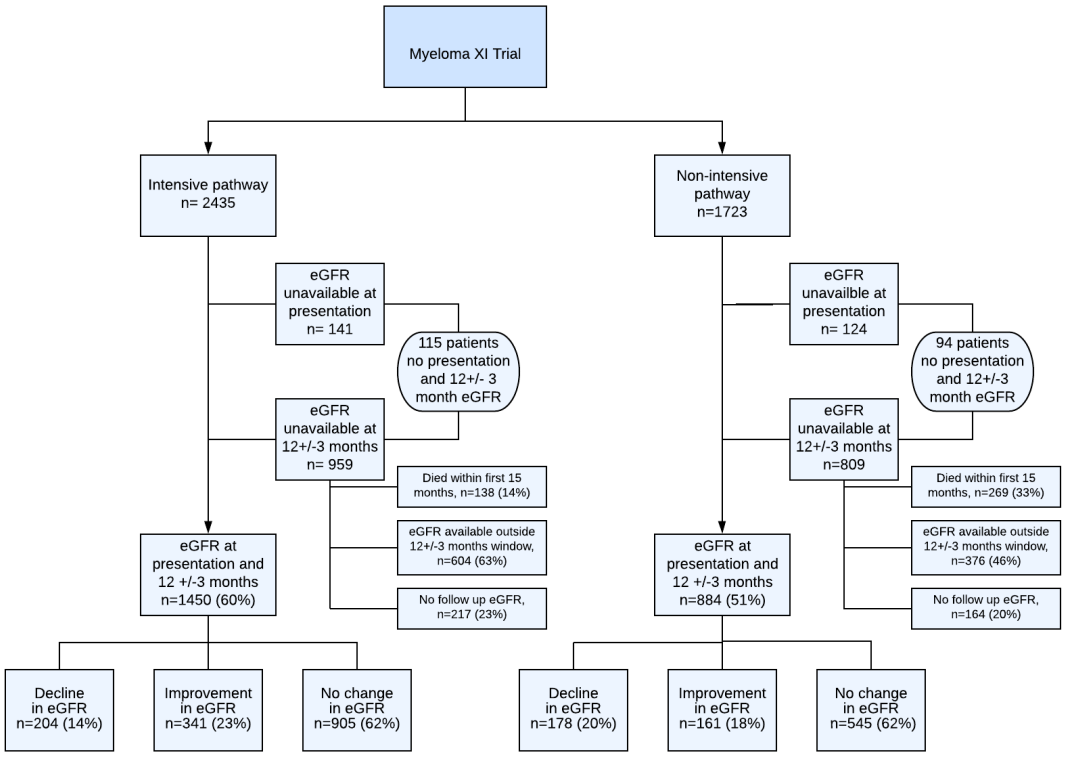
4.5.2 Baseline characteristics

The baseline variables included age, ethnicity, gender, paraprotein type (Sebia), serum creatinine (Roche) at baseline and at 12 +/- 3 months, sFLC assay (Freelite assay from The Binding Site Ltd, Birmingham, UK) at baseline and at 12 +/- 3 months. eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula. Renal function was stratified according to level of eGFR; eGFR of >59 ml/min/1.73m^2, eGFR of 30-59 ml/min/1.73m^2 or an eGFR <30 ml/min/1.73m^2 [357].

4.5.3 Renal outcome and Hematological response assessments

Patients who had baseline and 12-month eGFR results available at the central laboratory database were included in the renal response assessment (n=2334) (Figure 3.1). If a 12-month eGFR was not available; then the sample nearest to 12 +/- 3 months was taken as a 12-month eGFR. Patients who did not have a baseline eGFR (n=265) or a 12-month follow-up sample (n = 1768) were excluded from this analysis (Figure 4.1).

Figure 4.1: NCRI Myeloma XI trial consort diagram showing patient distribution with a baseline and 12 +/- 3-month eGFR.



Any positive or negative change in eGFR <25% at 12 months compared to baseline was considered no change, change in eGFR ≥ 25% at 12 months compared to baseline, was considered significant. This was based on the UK Kidney association criteria for change in kidney function [358]. Patients were categorized into three renal outcome groups; patients who had a decline in eGFR (≥ 25%), those who had an improvement in eGFR (≥25%) and patients who maintained their renal function at 12 months (<25% change).

Polyclonal sFLC concentrations increase progressively with declining renal function [320]. To provide a meaningful assessment of the hematological disease response, only patients who had a dFLC level of greater than 100 mg/L were included in the measurement of the serum dFLC response [359].

4.5.4 Statistical Methods

Statistical analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc, Chicago, IL). Categorical variables were summarized as frequencies and percentages. Comparison for categorical variables among different groups was made with chi square test and Fisher's exact test where appropriate. Continuous variables were expressed as median with interquartile range (IQR) for data that was not normally distributed.

The Mann Whitney U test was used to compare differences in age, baseline eGFR and baseline dFLC. The Kruskal Wallis test was used to compare differences in age, baseline eGFR, 12-month eGFR, baseline dFLC level in the three renal outcome groups defined based on change in eGFR. The association between different baseline dFLC categories, dFLC response level and the three renal outcome groups defined by change in eGFR was assessed by chi-square test. Logistic regression analysis was used to determine factors associated with renal outcome.

The outcome categories were based on changes in eGFR from baseline to 12 months; and so, problems associated with mathematical coupling would have been introduced, had baseline eGFR level been included as an explanatory variable. For continuous variables, under the assumption of equality of variances of initial and final values, change and initial value are correlated, but change and mean of initial and final values are not [360]. For this reason, eGFR based on an average of baseline and 12-month eGFR was used in the regression model.

4.6 Results

4.6.1 Baseline characteristics

The Myeloma XI study enrolled 4,420 patients; for 4,158 patients the central laboratory analysis for these patients have been used in the study.

In total 55% of patients presented with an eGFR ≥ 60 ml/min/1.73 m^2. 2,435 (58.5%) patients were assigned to the intensive pathway and 1,723 (41.4%) to the non-intensive pathway (Figure 4.1).

Baseline characteristics are shown (Table 4.1). Compared to the non-intensive pathway, patients in the intensive pathway were younger, with a median age of 65 years (IQR 70-59) vs 79 years (IQR 75-83) (*P* <0.001) and had better renal function at presentation, median eGFR 68 ml/min/1.73m^2 (IQR 54-85) vs 58 ml/min/1.73m^2 (IQR 44-75) (*P* <0.001). Patients in the intensive pathway had a significantly better dFLC response at 12 months; dFLC response >90% of 36% compared to 21% in the non-intensive pathway (*P* <0.001).

Table 4.1: Baseline characteristics of patients recruited to Myeloma XI by pathway

Characteristics	Total	Intensive pathway	Non-intensive pathway	*P*
N or n (%)	**4158**	**2435 (58.5)**	**1723 (41.4)**	
Age, y				
Median (IQR)	71 (78-64)	65 (70-59)	79 (83-75)	<.001
Sex, n (%)				
Male:female	2439 (59):1719 (41)	1462 (60):973 (40)	977 (57):746 (43)	.031
Ethnicity, n (%)				
White: others	3880 (93):278 (7)	2255 (93):180 (7)	1625 (94):98 (6)	.199
Myeloma type, n (%)				
IgG	2252 (54)	1296 (53)	956 (55.5)	.001
IgA	919 (22)	519 (21)	400 (23.2)	
LCO	491 (12)	325 (13)	166 (9.6)	
No paraprotein	32 (0.8)	20 (0.8)	12 (0.7)	
IgD	42 (1)	33 (1.4)	9 (0.5)	
IgM	8 (0.2)	6 (0.2)	2 (0.1)	
Missing	414 (10)	236 (9.7)	178 (10.4)	
FLC isotype, n (%)				
κ	2449 (59)	1421 (58.4)	1028 (60)	.177
λ	1260 (30)	757 (31)	503 (29)	
Missing	449 (11)	257 (10)	192 (11)	
dFLC κ, mg/L				
Median (IQR)	320 (44-984)	292 (40-901)	370 (72.5-1124)	.008
dFLC λ, mg/L				
Median (IQR)	406 (69-1127)	429 (69-1143)	380 (63-1098)	.575
Baseline eGFR, ml/min/1.73 m^2				
Median (IQR)	64 (49-81)	68 (54-85)	58 (44-75)	<.001
Baseline eGFR, n (%), ml/min/1.73 m^2				
eGFR >59	2271 (55)	1514 (62)	757 (44)	<.001
eGFR 30-59	1366 (33)	658 (27)	708 (41)	
eGFR <30	256 (6.2)	122 (5)	134 (7.7)	
Missing	265 (6.4)	141 (6)	124 (7)	
Baseline dFLC, n (%), mg/L				
<500	2314 (56)	1379 (57)	935 (54)	<.001
500-1000	596 (14)	356 (15)	240 (14)	
≥1001	995 (24)	559 (23)	436 (25)	
Missing	253 (6)	141 (6)	112 (6.5)	
dFLC response, n (%)				
<50%	125 (3)	34 (1.4)	91 (5.2)	<.001
50%-90%	290 (7)	86 (3.5)	204 (12)	
>90%	1239 (30)	879 (36.1)	360 (21)	
Baseline dFLC <100				
Baseline dFLC <100	1160 (28)	718 (29.4)	442 (25.6)	
Missing	1344 (32)	718 (29.4)	626 (36.3)	

For this analysis the patients in the intensive and non-intensive pathways were analyzed separately due to the significant differences in baseline renal assessments. Overall RI defined by eGFR <60 ml/min/1.73m^2 at presentation was reported in 39%; 32% patients in the intensive arm and 49% patients in the non-intensive pathway (Table 4.1). 89 (2.4%) patients died with renal failure (myeloma related) as the primary cause of death and 52% of these had an eGFR ≥ 45ml/min/1.73m^2 at diagnosis.

Renal function at recruitment for patients in the intensive pathway, stratified according to eGFR were as follows; 1,514 (62%) patients had eGFR >59 ml/min, 658 (27%) had eGFR of 30-59 ml/min and 122 (5%) patients had an eGFR <30 ml/min. In the non-intensive pathway, 757 (44%) patients had eGFR >59 ml/min, 708 (41%) had eGFR of 30-59 ml/min, and 134 (7.7%) had an eGFR <30 ml/min (Table 4.1).

4.6.2 Renal outcome

Baseline and 12-month eGFR results were available for 2334 patients: 1,450 in the intensive pathway, and 884 in the non-intensive pathway (Table 4.2).

Table 4.2: Baseline characteristics stratified by renal outcome in patients in the intensive and non-intensive pathway of the Myeloma XI trial.

	Intensive pathway				**Non-intensive pathway**			
Characteristic	**Decline in eGFR (≥25%)**	**No change in eGFR (<25%)**	**Improvement in eGFR (≥25%)**	***P***	**Decline in eGFR (≥25%)**	**No change in eGFR (<25%)**	**Improvement in eGFR (≥25%)**	***P***
N (%)	204 (14)	905 (62)	341 (23.5)		178 (20)	545 (62)	161 (18)	
Age, y								
Median (IQR)	66 (70-59)	65 (70-58)	66 (70-60)	.184	78 (75-82)	78 (75-82)	78 (75-82)	.706
Baseline GFR, median (IQR), ml/min/1.73 m²	80 (68-90)	75 (62-89)	51 (38-59.5)	<.001	72 (58-87)	62 (48-77)	45 (33-57)	<.001
12-month GFR, median (IQR), ml/min/1.73 m²	51 (43-57)	73 (60-89)	76 (60-90)	<.001	44 (35-52)	60 (47-76)	67 (51-83)	<.001
Baseline dFLC, n (%), mg/l								
<500	142 (70)	575 (64)	163 (48)		114 (64)	332 (61)	86 (53)	
500-1000	25 (12)	143 (16)	51 (15)		27 (15)	86 (16)	20 (12)	
>1000	36 (18)	181 (20)	124 (37)		36 (20)	124 (23)	55 (34)	
Missing	1 (0.5)	6 (0.7)	3 (0.9)	<.001	1 (0.6)	3 (0.6)		.07
Baseline κ dFLC median (IQR), mg/L (in κ patients)	241 (26-637)	264 (37-817)	426 (83-1531)	<.001	240 (33-781)	374 (80-1027)	477 (103-1483)	.017
Baseline λ dFLC median (IQR), mg/L (in λ patients)	289 (49-694)	294 (47-801)	689 (174-2629)	<.001	213 (25-782)	279 (47-755)	450 (131-2131)	.07
dFLC response, n (%)								
<50%	9 (4.4)	15 (1.6)	6 (1.8)		28 (15.6)	48 (8.7)	11 (6.8)	
50%-90%	9 (4.4)	58 (6.4)	17 (5)		28 (15.7)	128 (23.5)	41 (25.5)	
>90%	109 (53.4)	516 (57)	236 (69.2)		57 (32)	216 (39.6)	72 (44.7)	
Baseline dFLC <100	75 (37)	310 (34)	79 (23)		63 (35)	152 (27.8)	36 (22.3)	
Missing	2 (1)	6 (0.66)	3 (0.9)	.001	2 (1)	1 (0.1)	1 (0.6)	.002

In the intensive pathway at 12 months, 204 (14%) patients of the 1,450 evaluable patients had a decline in eGFR (≥25%) with a median decline of 26 ml/min/1.73m^2 (IQR 22-32), 341 (23.5%) had an improvement in eGFR (≥ 25%) with a median rise of 24 ml/min/1.73m^2 (IQR18-29), and 905 (62%) did not have a significant change (Table 4.2).

Patients who had an improvement in eGFR at 12 months had a significantly lower baseline eGFR (51 ml/min/1.73m^2 vs 80 ml/min/1.73m^2 in the decline group and 75ml/min/1.73m^2 in the no change group, *P* value <0.001). Patients who had improvement in eGFR also had a significantly higher proportion of patients with baseline dFLC of more than 1000 mg/L (37% versus 18% in the decline group and 20% with no change, (*P* value <0.001)), and significantly more patients achieved >90% dFLC response (69.2% versus 53% in the decline group and 57% in the no change group, *P* 0.001) (Table 4.2).

Age was not significantly different in the three renal outcome groups in the intensive arm. In the 14% of patients who had a significant decline in their renal function, the majority (70%) had a baseline dFLC of less than 500 mg/L (Table 4.2) and the decline was not associated with higher FLC levels or a lack of FLC response (Table 4.2).

In the non-intensive pathway, the changes in renal function identified at 12 months were like those in the intensive pathway (Table 4.2). 178 (20%) of 884 evaluable patients had a decline in their eGFR with a median decline of 26 ml/min/1.73m^2 (IQR 19-33), 161 (18%) had an improvement in their eGFR with a median rise in this group of 19 ml/min/1.73m^2 (IQR 15-25), and in 545 (62%) patients' renal function was maintained. The factors associated with renal outcome in the non-intensively treated patients were also like those in the intensive pathway.

Patients who had an improvement in their eGFR at 12 months had; a lower starting eGFR (45 ml/min/1.73m^2 compared to 72 ml/min/1.73m^2 in the decline group, and 62 ml/min/1.73m^2 in the no change group, P <0.001), a higher proportion of patients with baseline dFLC of more than 1000 mg/L (34% versus 20% in the decline group and 23% in the no change group, P=0.07), and had a higher proportion of patients achieving >90% dFLC response (44.7% in the improvement group compared to 32% in the decline group and 39.6% in the no change group, P=0.002) (Table 4.2).

In the 20% patients who had a significant decline in their renal function, the majority (64%) had a baseline dFLC of less than 500 mg/L (Table 4.2).

4.6.3 Multivariate analysis

In a multivariate analyses using a logistic regression model (Table 4.3) patients were more likely to have had an improvement in renal function if they were; <70 years old (odds ratio 1.58, 95% CI 1.23-2.04, *P* <0.001), male (odds ratio 1.62, 95% CI 1.26-2.09, *P* <0.001), had an average eGFR <60 ml/min (odds ratio 2.23, 95% CI 1.73-2.87, *P* <0.001) compared to eGFR ≥ 60 ml/min, baseline dFLC >1000 mg/L (odds ratio 1.69, 95% CI 1.28-2.22, *P* <0.001) compared to baseline dFLC <500 mg/L and dFLC response >90% (odds ratio 1.92, 95% CI 1.11-3.34, *P*=0.02) compared to dFLC response <50%.

Factors associated with a decline in eGFR at 12 months were dFLC response <50% (odds ratio 2.54, 95% CI 1.64-3.94, *P* <0.001) and female sex (odds ratio 1.42, 95% CI 1.08-1.88, *P*= 0.01).

Table 4.3: Univariate and Multivariate analysis of factors associated with improvement and decline in renal function at 12 months.

Variables	Univariate analysis		Multivariable analysis	
	OR (95% CI)	***P***	**OR (95% CI)**	***P***
Improvement				
Age, y				
<70	1.31 (1.07-1.60)	.007	1.58 (1.23-2.04)	<.001
≥70	Reference		Reference	
Sex				
Male	1.37 (1.08-1.75)	.009	1.62 (1.26-2.09)	<.001
Female	Reference		Reference	
Baseline dFLC, mg/L		<.001		<.001
>1000	1.98 (1.53-2.57)	<.001	1.69 (1.28-2.22)	<.001
500-1000	1.06 (0.77-1.46)	.70	0.98 (0.70-1.36)	.91
<500	Reference		Reference	
Average eGFR, mL/min		<.001		<.001
<30	2.90 (1.82-4.64)	<.001	3.35 (1.96-5.73)	<.001
30-59	1.69 (1.37-2.08)	<.001	2.23 (1.73-2.87)	<.001
≥60	Reference		Reference	
dFLC response, %		.012		.04
>90	2.01 (1.18-3.42)	.010	1.92 (1.11-3.34)	.02
50-90	1.53 (0.84-2.75)	.15	1.55 (0.84-2.84)	.15
<50	Reference		Reference	
Decline				
Age, y				
<70	0.70 (0.54-0.84)	.001	0.74 (0.55-1.00)	.05
≥70	Reference		Reference	
Sex				
Female	1.41 (1.12-1.75)	.002	1.42 (1.08-1.88)	.01
Male	Reference		Reference	
Baseline dFLC, mg/L		.14		.22
<500	1.37 (0.99-1.88)	.05	1.33 (0.95-1.86)	.08
500-1000	1.18 (0.80-1.73)	.39	1.23 (0.82-1.83)	.30
>1000	Reference		Reference	
Average eGFR, mL/min		.15		.78
<30	1.17 (0.65-2.13)	.59	1.12 (0.55-2.26)	.74
30-59	1.25 (0.99-1.58)	.05	1.10 (0.82-1.49)	.50
≥60	Reference		Reference	
dFLC response, %		<.001		<.001
>90	0.34 (0.22-0.52)	<.001	0.39 (0.20-0.60)	<.001
50-90	0.32 (0.19-0.55)	<.001	0.34 (0.20-0.57)	<.001
<50	Reference		Reference	

4.6.4 Missing data

Baseline data and/or 9-15 months eGFR was missing for 985 patients in the intensive pathway, and these were excluded from analysis (Figure 4.1). There were 242 (24.5%) deaths in this group. 236 (24%) of these 985 patients in the intensive pathway had an available eGFR at more than 15 months; in this group there were 37 (17%) deaths, 199 were censored. Of the 236 patients, 203 patients had both a baseline and an eGFR result at >15 months; compared to baseline 60% had no change in renal function, 19% had a decline and 22% had an improvement. In the 749 patients who did not have a >15-month available eGFR; there were 205 (27%) deaths, 544 were censored.

In the non-intensive pathway 839 patients did not have a baseline and/or 9-15month eGFR (Figure 4.1). There were 401 (48%) deaths in this group. 155 of 839 patients had an available eGFR at more than 15 months; in this group there were 42 (27%) deaths, 113 were censored. Of the 155 patients, 110 had a baseline and an available eGFR at >15 months; compared to baseline 60% had no change in renal function, 20% had a decline and 20% had an improvement. In the 684 patients who did not have >15-month eGFR there were 359 (52%) deaths, 325 were censored.

4.7 Discussion

The work presented in this chapter shows that the renal prognosis for patients presenting with NDMM is reassuring with relatively few patients having a significant decline in renal function at 12 months. In this large study which included patients with all levels of renal function down to an eGFR of 15 ml/min, RI (eGFR <60ml/min/1.73m^2) was common and affected 40% of the NDMM patients.

The analysis took account of patients in the two treatment pathways separately as there were significant differences in the approach to treatment and difference in overall patient survival. This study is important as it is informative of the factors associated with the renal outcome at 12 months, for MM patients not requiring dialysis.

Patients presenting with LCs more than 500 mg/L are more likely to develop cast nephropathy [14, 342] . This is due to a heavy load of filtered free light chains that escape tubular reabsorption and co-precipitate with Tamm–Horsfall protein in the distal nephron causing intratubular obstruction, and rapid treatment can result in improvement in renal function [259].

In this study patients who had an improvement in renal function at 12 months started with higher baseline dFLC and had lower mean eGFR suggesting that these patients were more likely to have reversible cast nephropathy with a treatment response.

With the current approaches to treatment, decline in eGFR at 12 months was seen in relatively few patients with only 14% in the intensive arm and 20% in the non-intensive arm. Factors associated with decline in eGFR at 12 months were a dFLC response <50% and female sex. The lack of timely clonal response is known to result in renal fibrosis and subsequent CKD. Gender differences are not explained as the clonal responses were not different between men and women. The Myeloma IX study reported on gender-dependent differences in the prevalence of the primary genetic events of myeloma; and secondary genetic events were more frequent in women. Female gender was also associated with inferior OS. The study concluded that higher prevalence of lesions associated with poor prognosis in the female myeloma population may adversely affect clinical outcome [361].

4.8 Strengths and limitations

The strengths of this study comprised: broad inclusion criteria ensuring that the data is useful for the interpretation of the natural history of myeloma patients on a population basis; biochemical data derived from a single central laboratory ensuring the accuracy and reproducibility of the results generated; and detailed sFLC data.

There are several potential limitations of this study, which may impact conclusions about the proportion of patients affected by CKD. One of the limitations of this study was that the criteria for renal response prepared by the IMWG [237, 362-365] could not be used. The IMWG criteria defines a complete renal response (CR renal) as a sustained (i.e. at least 2 months') improvement of baseline eGFR from <50 ml/min to ≥ 60 ml/min, renal partial response (PR renal) as sustained improvement of baseline eGFR from <15 ml/min to 30-59 ml/min, renal minor response (MR renal) as an improvement from <15 ml/min to 15-29 ml/min or if baseline eGFR was 15-29 ml/min and improved to 30-59 ml/min [362].

In this dataset using the IMWG criteria only 5% in the intensive and 4.5% in the non-intensive arm met the criteria for a CR renal and as patients with severe RI and those on dialysis were excluded from the trial, conclusions regarding the proportion of patients with PR renal and MR renal could not be made. Therefore a ≥ 25% change in eGFR was used to define change in renal function [358]. The definition of a renal response may change with time as the relationship between renal function and long-term outcomes are better defined and this study may contribute to this. Another limitation is the volume of missing data. Data was missing for 985 patients in the intensive pathway, and 839 patients in the non-intensive pathway who did not have a baseline and/or 12-month eGFR result and were excluded from analysis. A further shortfall is that current diagnostic guidelines for MM do not include the presence of albuminuria, and this was not available for this study. Albuminuria data may help to differentiate between AKI due to cast nephropathy and CKD.

4.9 Conclusions

In summary, renal prognosis in patients who present with MM and an eGFR >15ml/min is reassuring. In patients with an eGFR of ≥30 ml/min renal function is not an independent determinant of 12-month outcome. Improvement in renal function is more likely in patients who present with very high LCs and in those who achieve a deep clonal response.

Chapter V: Impact of specific treatments on 12-month renal outcome and relation between renal function and patient survival; Myeloma XI Trial.

The work presented in this chapter will explore how specific treatments impact renal outcome and review the impact of renal function on patient survival.

5.1 Extent of personal contribution

This work is an extension of chapter IV and has utilized the Myeloma XI trial data for secondary analysis. Please refer to chapter IV section 4.1 for further details relating to extent of personal contribution.

This chapter is based on the supplementary file and survival data from the following published first author original article [349].

Rana, R., Cockwell, P., Drayson, M., Cook, M., Pratt, G., Cairns, D.A., Pawlyn, C., Jackson, G., Davies, F., Morgan, G. and Pinney, J.H., 2020. Renal outcome in patients with newly diagnosed multiple myeloma: results from the UK NCRI Myeloma XI trial. *Blood advances*, *4*(22), pp.5836-5845.

Parts of this work have been presented as a poster in the 4th IKMG meeting, 2019, Montreal (Canada).

5.2 Abstract

Novel and intensive therapies have dramatically improved survival in myeloma. However, the impact of these on renal outcomes is not well defined. In this study the role played by hematological response, transplantation and/or bortezomib based chemotherapy on12-month renal outcome was investigated, and the impact of renal function on patient survival was evaluated. Data of 2,334 patients from the UK Myeloma XI trial, who had a baseline and 12-month renal function result available, were analyzed. The M-protein response in the 3 renal outcome groups at different treatment points across the 2 pathways was similar. Of the 1,450 evaluable patients in the intensive pathway; 890 (61%) underwent stem cell transplantation and 560 (38.6%) did not. The renal outcome in the two groups was similar and not statistically significant. At 12 months of the 186 patients who received bortezomib based chemotherapy, 121/186 (65%) had a no change in eGFR, 46/186 (25%) had an improvement and 19/186 (10%) had a decline in eGFR compared to baseline. The median survival was significantly better for patients with eGFR $\geq$ 30 ml/min/1.73m^2 compared to those with eGFR<30 ml/min/1.73m^2. In conclusion, M-protein response, transplantation or bortezomib based chemotherapy did not correlate with 12-month renal outcome in NDMM patients.

5.3 Introduction

Severe RI in myeloma is associated with worse outcomes and early death [113, 366]. The median survival of patients who remain dialysis dependent is 18-22 months versus 63 months who become dialysis independent [114, 367]. Historically survival of myeloma patients requiring dialysis was poor [114] and this group was excluded from intensive therapies because of increased risk of morbidity and mortality. ASCT is feasible in dialysis-dependent renal failure and can improve disease response [368] however data relating to recovery of renal function is heterogenous [369-371].

The last few decades have seen a dramatic improvement in the survival of myeloma patients including those with RI due to novel therapies, transplantation, and best supportive care. However, the impact of these on 12-month renal outcomes in patients with mild to moderate RI is not known. Understanding this is important for patients and clinicians as they set goals of therapy.

To appraise this, we utilized data from the Myeloma XI trial to assess the impact of hematologic response, transplantation and bortezomib therapy on 12-month renal outcome and evaluated patient survival.

5.4 Hypotheses

The work presented in this chapter aimed to address the following hypotheses:

1) Hematologic response has an impact on 12-month renal outcome.

2) Bortezomib based therapy has an impact on 12-month renal outcome.

5.5 Methods

The study design, protocol for the NCRI Myeloma XI trial, baseline characteristics and renal outcome assessment have been explained in chapter IV. The outcome categories were based on changes in eGFR from baseline to 12 months. Patients were categorized into three renal outcome groups; patients who had a decline in eGFR (≥ 25%), those who had an improvement in eGFR (≥25%) and patients who maintained their renal function at 12 months (<25% change). Please refer to chapter IV for more details. Hematological response was reviewed post induction, post CVD and at day 100 post ASCT across the three renal outcome groups in the two treatment pathways.

5.5.1 Hematological response criteria

Definitions of response and progression were based on the international uniform response criteria of response and progression [372-374].

Complete Response: a CR required all the following

1) Absence of the original M-protein in serum / urine by routine electrophoresis and IFE.

2) <5% plasma cells in bone marrow (repeat bone marrow was not required).

3) No increase in the size or number of lytic lesions on radiological investigations if performed.

4) Disappearance of soft tissue plasmacytomas.

5) A normal FLC ratio in LCO myeloma.

Very Good Partial Response: a very good partial response (VGPR) required

1) Serum and urine M-protein detectable by IFE but not on SPEP

Or

2) ≥90% reduction in the serum M-protein plus urine light chain excretion < 100 mg/24 hours if measured.

3) No increase in the size or number of lytic lesions on radiological investigations if performed.

4) For patients with LCO myeloma >90% decrease in the dFLC.

Partial response: a partial response (PR) required

1) ≥50% reduction in serum M-protein and

2) Reduction in 24-hour urinary LC excretion by ≥90% or to < 200 mg/24 hours if measured.

3) For LCO myeloma a ≥50% reduction in the dFLC levels.

4) For non-secretory myeloma only, ≥50% reduction in plasma in bone marrow provided baseline percentage was ≥ 30%.

5) For soft tissue plasmacytoma ≥50% reduction in size of soft tissue plasmacytoma.

6) No increase in size or number of lytic lesions on radiological investigations if performed.

Minimal response: a minimal response (MR) required all the following

1) 25-49% reduction in the serum M-protein level.

2) 50-89% reduction in 24-hour urinary LC excretion, still exceeding 200 mg/24 hours if measured.

3) For non-secretory myeloma a 25-49% reduction in plasma cells in bone marrow

4) 25-49% reduction in size of soft tissue plasmacytomas.

5) No increase in size or number of lytic lesions on radiological investigations if performed.

No change: no change (NC) was defined as not meeting the criteria for either MR or progressive disease.

Progressive disease: a progressive disease (PD) required one or more of the following

1) ≥25% increase in the serum M-protein from the lowest response level, which must also be an absolute increase of 5g/L and confirmed with at least one repeat investigation.

2) ≥25% increase in 24-hour urinary LC excretion from the lowest response level, if measured, which must also be an absolute of at least 200 mg/24 hour and confirmed with at least one repeat investigation.

3) For LCO myeloma a ≥25% increase in the dFLC levels from the lowest response level, which must be an absolute increase of > 100mg/L and confirmed with at least one repeat investigation.

4) ≥25% increase in plasma cell percentage in bone marrow, which must also be an absolute percentage of at least 10%.

5) Increase in size of size of existing lytic bone lesions or plasmacytomas or development of new lesions.

6) Hypercalcemia not attributable to other causes.

5.5.2 Statistical Methods

Statistical analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc, Chicago, IL). Categorical variables were summarized as frequencies and percentages. Comparison for categorical variables among different groups was made with chi square test and Fisher's exact test where appropriate. The association between different M-protein response categories and the three renal outcome groups defined by change in eGFR was assessed by chi-square test. Kaplan-Meier analyses were used to report patient survival, and the curves were compared using the log-rank test. *P* value of <0.05 was considered statistically significant.

5.6 Results

5.6.1 Hematological response and renal outcome

The proportion of patients achieving, CR vs PR vs NC at post induction (PI), post CVD and ASCT across the three renal outcome groups in the two treatment pathways was similar and not statistically significant (Table 5.1).

Table 5.1: Response at different treatment points across the renal outcome groups in the intensive and non-intensive pathway.

	Intensive pathway				**Non-intensive pathway**			
Response	**Decline in eGFR (≥25%) N (%)**	**No change in eGFR (<25%) N (%)**	**Improvement in eGFR (≥25%) N (%)**	***P***	**Decline in eGFR (≥25%) N (%)**	**No change in eGFR (<25%) N (%)**	**Improvement in eGFR (≥25%) N (%)**	***P***
PI								
CR/VGPR	104 (50.8)	437 (48.3)	162 (47.5)	0.712	87 (48.9)	236 (43.3)	73 (45.3)	0.191
PR/MR	36 (17.6)	195 (21.5)	82 (24.0)		35 (19.7)	135 (24.8)	49 (30.4)	
NC/PD	1 (0.5)	6 (0.7)	3 (0.9)		5 (2.8)	1 (2)	4 (2.5)	
Missing	63 (30.9)	267 (29.5)	94 (27.6)		51 (28.7)	163 (29.9)	35 (21.7)	
Total	204 (100)	905 (100)	341 (100)		178 (100)	545 (100)	161(100)	
Post CVD								
CR/VGPR	5 (50)	34 (42.5)	19 (61.3)	0.111	4 (44.4)	16 (39)	9 (60)	0.606
PR/MR	4 (40)	45 (56.3)	11 (35.5)		4 (44.4)	22 (53.7)	5 (33.3)	
NC/PD	1 (10)	1 (1.3)	1 (1.3)		1 (11.1)	3 (7.3)	1 (6.7)	
Total	10 (100)	80 (100)	31 (100)		9 (100)	41 (100)	15 (100)	
100 HDM-ASCT								
CR/VGPR	103 (91)	483 (85.3)	179 (84.8)	0.252				
PR/MR	9 (8)	76 (13.4)	26 (12.3)					
NC/PD	1 (0.9)	7 (1.2)	6 (2.8)					
Total	113 (100)	566 (100)	211(100)					

5.6.2 ASCT and renal outcome

Of the 1450 evaluable patients in the intensive pathway; 890 (61%) underwent ASCT and 560 (38.6%) did not. The renal outcome in the two groups was similar and not statistically significant (Table 5.2).

Table 5.2: Renal outcome at 12 months in patients who ASCT versus those who didn't get a transplant in the intensive pathway.

	ASCT **N (%)**	**No ASCT** **N (%)**	*P* value 0.161
Decline in eGFR	113 (12.7)	91 (16)	
Improvement in eGFR	211 (23)	130 (23)	
No change in eGFR	566 (63.6)	339 (60)	
Total	890 (100)	560 (100)	

In a univariate analysis, patients who underwent ASCT, were less likely to have a decline in kidney function at 12 months (odds ratio 0.637, 95% CI 0.50-0.81, $P<0.001$) compared to those who did not. However, this was not of significance in a multivariate analysis.

5.6.3 Value of Bortezomib

Patients with a sub-optimal response to standard induction treatment; CTD / CRD were randomized to CVD vs nothing. Of the 2334 evaluable patients, 367 were randomized to CVD, 226 from the intensive pathway and 141 from the non-intensive pathway. The proportion of patients who achieved a CR vs PR and the proportion of patients achieving improvement vs decline in eGFR was similar in the intensive and non-intensive groups (Table 5.3).

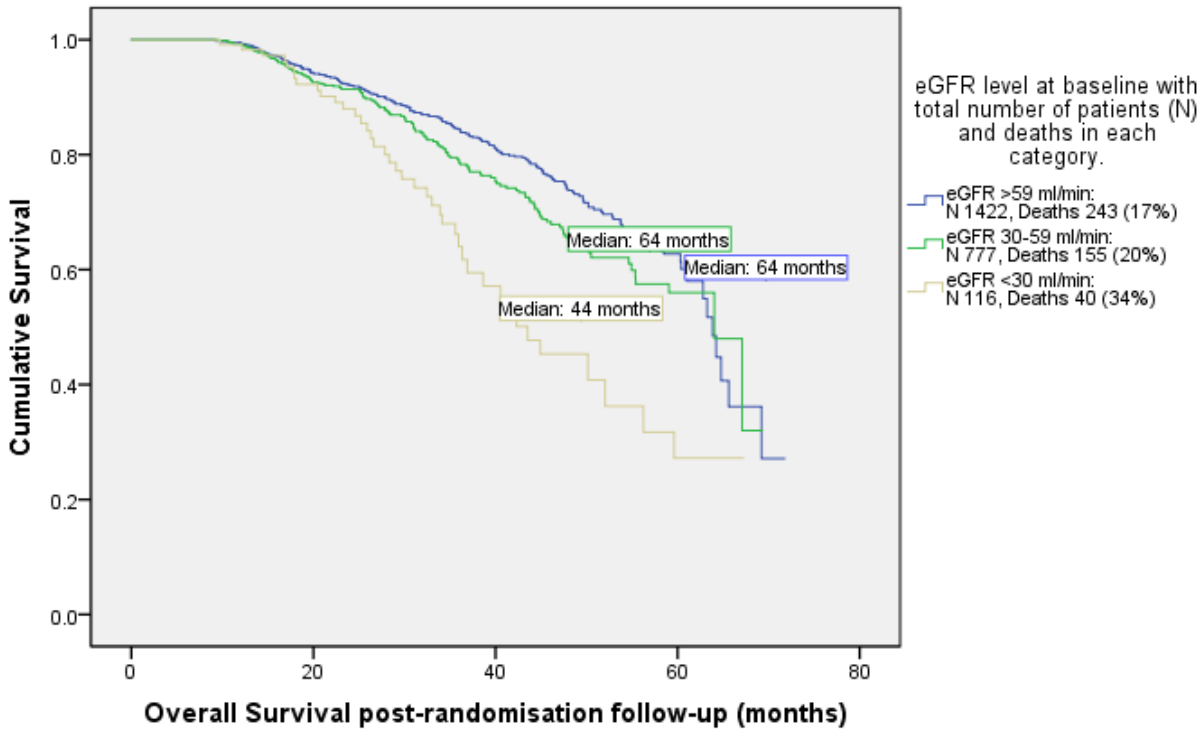
Table 5.3: Response post CVD and renal outcome at 12 months in the two treatment pathways.

Characteristic	**Intensive N (%)**	**Non-intensive N (%)**	***P* value**
Response			
CR/VGPR	58 (47.9)	29 (44.6)	0.260
PR/MR	60 (49.6)	31 (47.7)	
NC/PD	3 (2.5)	5 (7.7)	
Total	121 (100)	65 (100)	
Renal outcome			
Decline in eGFR	10 (8.3)	9 (13.8)	0.515
Improvement in eGFR	31 (25.6)	15 (23)	
No change in eGFR	80 (66.1)	41 (63.1)	
Total	121 (100)	65 (100)	

5.6.4 Survival analysis

The median follow-up for the series as a whole cohort was 30.5 months (95% CI 29.38-31.658). The median OS for the series was 60 months (95% CI 57-63), 407 (10%) died in the first 12 months. Median survival was longer in patients recruited to the intensive pathway compared to the non-intensive, 64 months (95% CI 61 to 67) vs 44 months (95% CI 41 to 48), P <0.01 respectively. The median survival by baseline eGFR was: 64 months (95% CI 61 to 67) for baseline eGFR > 59 ml/min; 64 months (95% CI 59 to 69) for eGFR 30-59 ml/min and 44 months (95% CI 34 to 53) for eGFR <30ml/min, P <0.001 (Figure 5.1).

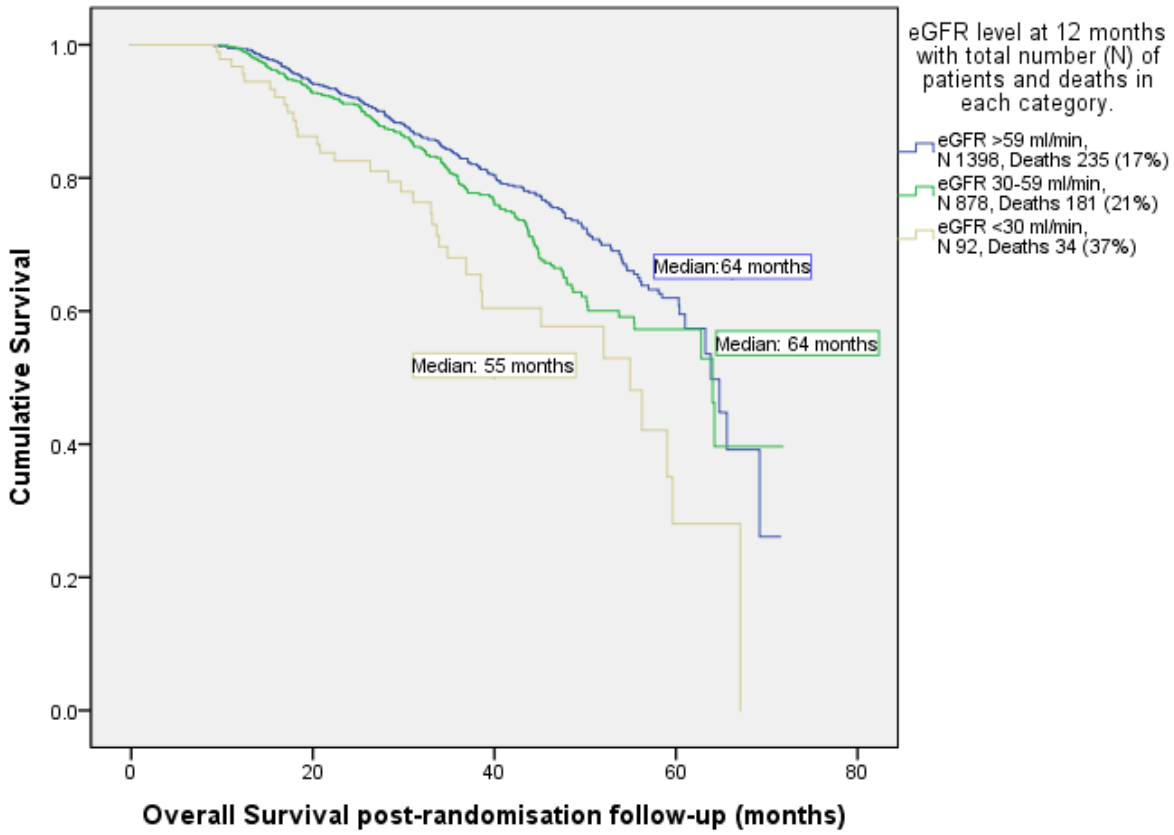
Figure 5.1: Kaplan-Meier plot comparing overall survival between patients with different eGFR levels at baseline.



Overall survival was significantly longer among those with baseline eGFR ≥30 ml/min compared to those with eGFR <30 ml/min (P<0.001).

The median survival in patients stratified by eGFR level at 12 months was significantly better in those with eGFR ≥ 30 ml/min compared to patients with eGFR < 30ml/min; 64 months (95% CI 57-72) vs 55 months (95% CI 43-67), *P* <0.001 (Figure 5.2).

Figure 5.2: Kaplan-Meier plot comparing overall survival between patients with different eGFR levels at 12 months.



Overall survival was significantly longer among those with 12-month eGFR ≥30 ml/min compared to those with eGFR <30 ml/min (P<0.001).

5.7 Discussion

5.7.1 Hematological Response

Several studies have shown that hematological response is independent of kidney function [371, 375, 376] and RI has no impact on patients' responses to chemotherapy [377]. In this cohort as has been shown in previous studies, hematological response across the three renal outcome groups in the two pathways was similar.

5.7.2 ASCT

HDT followed by ASCT is associated with superior survival outcomes and is a standard of care for patients <65 years [378, 379]. Retrospective studies suggest that ASCT is feasible in those with advanced RI and could lead to good hematological response outcomes but is associated with higher transplant-related mortality and greater toxicity [370, 371, 375, 380-382]. The data relating to improvement in renal function after transplant is heterogenous with some studies suggesting improvement in nearly 50% patients [375, 383-390]. A recent review indicated that patients with myeloma and renal failure who undergo ASCT have good improvement of renal function but have a lower survival rate [382].

In this study which consisted of patients with relatively good baseline renal function (eGFR>15 ml/min), undergoing HDT and ASCT did not have an impact on renal outcomes at 12 months, and renal outcomes in patients who underwent ASCT (890 patients, 61%) vs those randomized but did not undergo ASCT (560 patients, 39%) in the transplant eligible pathway, were similar. These results are reassuring and help in understanding the trajectory of renal function in patients undergoing intensive therapy.

5.7.3 Bortezomib

Bortezomib based regimens are very effective in the treatment of patients with MM and RI and are associated with a significant probability of renal function recovery [175, 391, 392]. In this study which included patients with mild to moderate RI, most patients who received bortezomib because of poor response to immunomodulatory drugs had no change in their renal function at 12 months compared to baseline and bortezomib therapy resulted in a hematological response in 178/186 (96%) patients.

5.7.4 Survival

Overall survival was significantly longer among those with an eGFR ≥30 ml/min compared to those with eGFR <30 ml/min (P<0.001) and is consistent with previous studies [376, 381]. When comparing the three renal outcome groups no advantage in overall survival was found for patients with improvement in renal function.

The current findings enhance the results of a study that looked at impact of RI on outcomes after ASCT in MM and reported no difference in overall and progression free survival in the three groups [376]. In this study patients were categorized into 3 groups: no RI at diagnosis and ASCT, RI at diagnosis with normalization before ASCT and RI both at time of diagnosis and ASCT.

5.8 Strenghts and Limitations

The strenghts of this study comprise: an adequate sample size, this is the largest cohort to date of NDMM patients, biochemical data derived from a single central laboratory ensuring the accuracy and reproducibility of the results generated, and detailed response data. ASCT can be associated with AKI. This study did not look at renal function around the time of ASCT and hence could not comment on when AKI occurred in context to transplantation. Another limitation of this study was that data on patients with relapse of myeloma was not avaialble, which could have helped to explain survival analysis in the three renal outcome groups.

5.9 Conclusions

In conclusion, M-protein response, transplantation, immunomodulatory or bortezomib based chemotherapy do not impact 12-month renal outcome in the NDMM patients. Patients with an eGFR ≥30 ml/min survive longer compared to those with eGFR <30 ml/min.

Chapter VI: Albuminuria at baseline and 12-months in patients with newly diagnosed multiple myeloma; results from the UK NCRI Myeloma XI trial.

The work presented in this chapter will evaluate albuminuria in relation to 12-month renal outcomes in the NDMM patients from the Myeloma XI trial.

6.1 Extent of personal contribution

This study utilized the Myeloma XI trial data for a secondary analysis. I was responsible for identifying relevant urine samples from the central laboratory in Birmingham and arranging urine ACR test on the samples with the help of Immunology Laboratory Manager and Principal Clinical Scientist. I collected and assembled the data, maintained a site file and was responsible for all the electronic data input. I led the data analysis, statistical plan, and interpretation of this secondary dataset. Following analysis, I wrote the first draft of the manuscript, and I was responsible for all the edits and revisions. This chapter is based on unpublished first author original article that has been submitted for peer review.

This work has been presented as a poster; at the IKMG meeting, 2023, Milan (Italy) and at the UK Kidney week, 2023, Newport (U.K).

6.2 Abstract

Kidney disease in myeloma is heterogenous and associated with worse outcomes. Albuminuria is a powerful adverse determinant of outcomes in kidney disease but there are limited data on patients with MM. This study therefore evaluated the Myeloma XI trial at diagnosis and at a year for (i) albuminuria incidence and prevalence by urinary ACR; and (ii) the relationship between ACR and excretory kidney function as categorized by eGFR. Patients were matched for age, sex, baseline FLC level, 12-month clonal response and 529 patients met the criteria. At presentation 305 patients (58%) had albuminuria (ACR >3 mg/mmol). In 15 patients (3%) this approximated to ≥1 gram/24 hours (ACR ≥70 mg/mmol). Albuminuria at baseline was not independently associated with a lower eGFR category at 12 months. At 12 months 162 patients (30%) had albuminuria. Further studies are needed to identify the impact of persistent albuminuria on long-term outcomes.

6.3 Introduction

Excretory kidney function is measured in clinical practice by eGFR, calculated from serum or plasma creatinine (eGFR creatinine). A further measurement of kidney function is increased albuminuria, a marker of kidney damage which is strongly associated with progression of kidney disease, irrespective of the underlying cause [357]. In clinical practice albuminuria is usually measured as an ACR in a spot urine sample. Kidney disease associated with myeloma is common [366] and heterogenous, and at presentation can range from moderate impairment of excretory kidney function (AKI stage one or two and/or eGFR 30-59 ml/min/1.73m^2) that often recovers to normal function, to severe AKI (stage 3) often due to myeloma cast nephropathy [259, 349, 354]. Most patients with myeloma and RI subsequently recover kidney function, but some patients have ongoing low excretory kidney function and will be at increased risk of early mortality and increased morbidity.

Despite the incidence and prevalence of kidney disease in patients with myeloma, there is limited data on albuminuria, both at presentation and at follow-up. This is an important shortfall as in other settings albuminuria is an adverse determinant of clinical outcomes such as accelerated progression of kidney disease and early mortality. Therefore, this study evaluated patients in the Myeloma XI trial at baseline and 12 months, to assess the incidence and prevalence of albuminuria and the relationship of albuminuria to eGFR.

6.4 Hypothesis

The work presented in this chapter aimed to address the following hypothesis:

1. Albuminuria in myeloma is associated with decline in kidney function at 12 months.

6.5 Methods

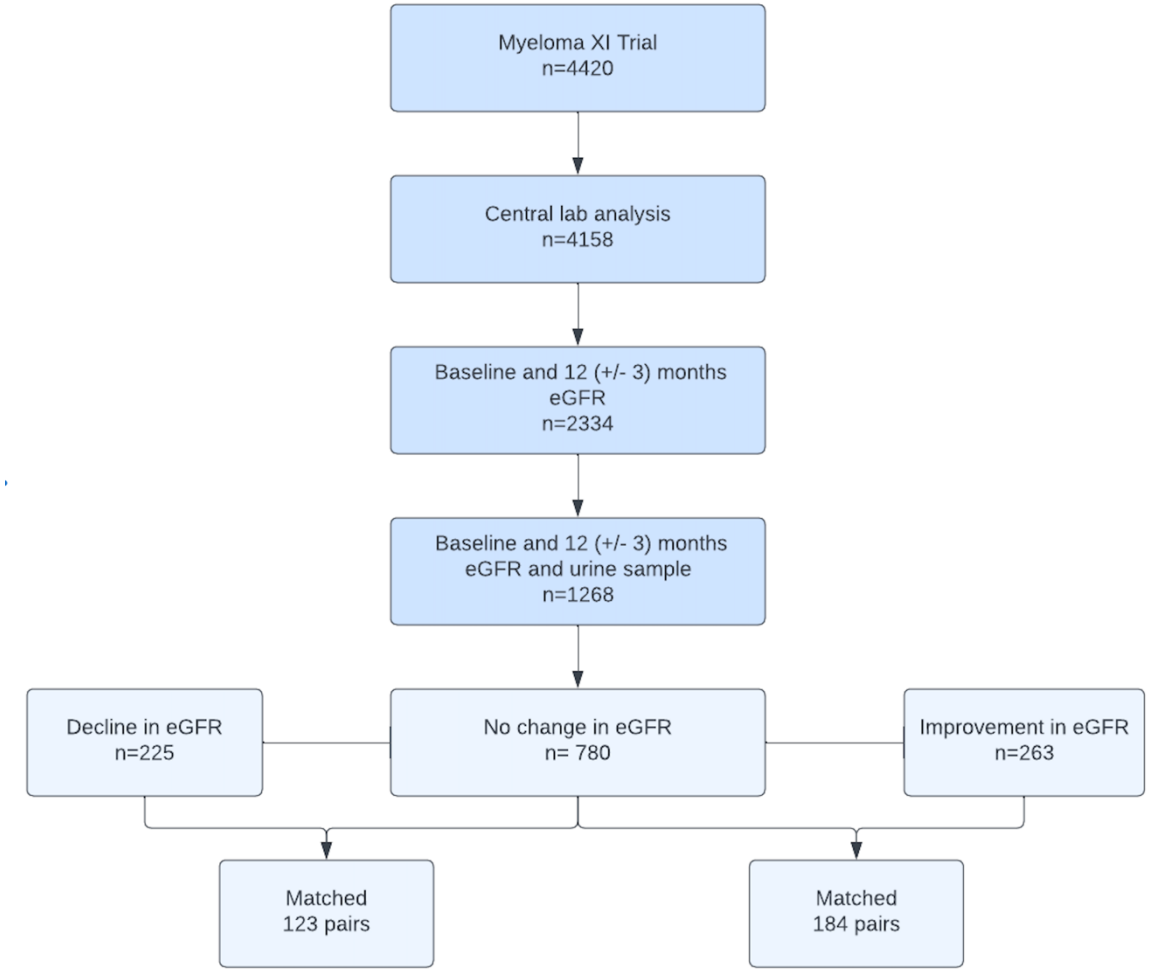
6.5.1 Myeloma XI trial

Details of the Myeloma XI trial protocol, the main results and renal outcomes at 12 months have been reported previously [217, 349, 355, 356]. A multicentre research ethics committee and local ethics committees approved the protocol, and all patients gave written informed consent in accordance with the Declaration of Helsinki.

6.5.2 Study population and procedure

4420 patients were enrolled. 1268 patients had both baseline and 12 (+/- 3) month serum creatinine and baseline and 12 (+/- 3) month stored frozen (-20°C) urine samples available (Figure 6.1). eGFR was calculated by MDRD equation. Patients were categorized into three renal outcome groups from baseline and 12-month eGFR. This categorization was based on the National Institute of Health and Care Excellence (NICE) criteria for change in kidney function and comprised (1) improvement in eGFR (≥25%) (n=263) (2) decline in eGFR (≥ 25%) (n=225) and (3) stable function (<25% change) (n=780).

Figure 6.1: Flowchart showing patient selection in this study



Patients who at 12 months had an improvement in eGFR or a decline in eGFR were matched with those who had no change in eGFR. The two groups comprised (i) improvement matched with no change (184 pairs) and (ii) decline matched with no change (123 pairs). These groups were matched for age categories (41-50, 51-60, 61-70, 71-80, 81-90 years), sex, baseline dFLC (<500, 500-1000, >1001 mg/L) and 12-month dFLC response (<50, 50-90, >90%).

To calculate ACR, urine samples were thawed and processed on the Alinity c analyser (Abbott) for quantification of albumin and an enzymatic assay was used to measure creatinine. Albuminuria was categorized according to the KDIGO classification system as follows: ACR <3 mg/mmol - normal to mildly increased, ACR 3-30 mg/mmol - moderately increased and ACR >30 mg/mmol - severely increased [393].

The following variables were available as part of the study dataset and included in the analysis; demographic data, paraprotein type, FLC isotype, baseline, and 12-month ACR, eGFR, dFLC and urine light chain level. Patients who had a dFLC level of greater than 100 mg/L were included in the measurement of the serum dFLC response [359].

6.5.3 Statistical Analyses

Statistical analysis was performed using SPSS for Windows, version 21.0 (SPSS Inc, Chicago, IL). Case control matching was carried out to build two matched datasets. Categorical variables were summarized as frequencies and percentages. Continuous variables were expressed as median with IQR for data that were not normally distributed. Variables with a *P* value of <0.05 were considered significant. To compare differences in the dependent variable at baseline and 12 months and amongst the matched pairs, Wilcoxon signed rank test was used. Logistic regression analysis was used to determine if ACR at baseline was independently associated with renal progression.

6.6 Results

6.6.1 Patient and disease characteristics

There were 529 evaluable patients grouped into (i) improvement vs no change (184 pairs) (ii) decline vs no change (123 pairs) in eGFR at 12 months compared to baseline. 85 patients in the no change group were common matches to both groups. The characteristics of the 529 patients are described in Table 6.1.

Table 6.1: Patient Characteristics

Variable	Summary statistic (n=529)
Age, years	
Median (IQR)	70 (63-77)
Gender, n (%)	
Male: Female	327 (62%): 202 (38.2%)
Pathway, n (%)	
Intensive: Non-intensive	338 (63.9%): 191 (36.1%)
Myeloma type, n (%)	
IgG	301 (56.9%)
IgA	110 (20.8%)
LCO	104 (19.7%)
IgD	11 (2.1%)
No paraprotein	2 (0.4%)
IgM	1 (0.2%)
FLC isotype, n (%)	
Kappa	346 (65.4%)
Lambda	183 (34.6%)
Baseline dFLC, n (%), mg/L	
<500	225 (42.5%)
500-1000	119 (22.5%)
>=1001	185 (35%)
dFLC response, n (%)	
<50%	10 (1.9%)
50-90%	91 (17.2%)
>90%	428 (80.9%)
12 month renal outcome, n (%)	
Decline in eGFR	123 (23.3%)
Improvement in eGFR	184 (34.8%)
No change in eGFR	222 (42%)

6.6.2 Baseline and 12-month characteristics

At presentation 305 (58%) patients had albuminuria (ACR >3 mg/mmol) and 224 (42%) had no albuminuria; 263 (50%) had an ACR of 3-30 mg/mmol and 42/529 (8%) had an ACR >30mg/mmol. 162 patients (31%) had albuminuria at 12 months (Table 6.2).

Of those who had albuminuria at baseline, 173/305 (57%) had no albuminuria at 12 months. Of those with no albuminuria at baseline, 35/188 (19%) had albuminuria at 12 months. A higher proportion (263/529 (50%)) of patients at baseline had moderate albuminuria (ACR 3-30mg/mmol) than at 12 months (137/529 (26%)) (Table 6.2).

Median ACR was 5.35 mg/mmol (IQR 1.64-15.83) at baseline and 1.23 mg/mmol (IQR 0.62-3.98) at 12 months (P <0.001) (Table 6.2).

Table 6.2: Kidney function and myeloma status at baseline and 12 months (n-529)

	Baseline	12 month	*P* value
ACR, mg/mmol			
Median (IQR)	5.35 (1.64-15.83)	1.23 (0.62-3.98)	<0.001
ACR, n (%), mg/mmol			
<3	188 (35.5%)	346 (65.4%)	<0.001
3-30	263 (49.7%)	137 (25.9%)	
>30	27 (5.1%)	17 (3.2%)	
≥70	15 (2.8%)	8 (1.5%)	
Missing	36 (6.8%)	21 (4%)	
GFR, n (%), ml/min/1.73m^2			
≥90	67 (12.7%)	88 (16.6%)	0.248
60-89	226 (42.7%)	207 (39.1)	
30-59	199 (37.6%)	213 (40.3%)	
15-29	32 (6%)	19 (3.6%)	
<15	5 (0.9%)	2 (0.4%)	
Clonal LCR, **LCR, gm/gm creatinine**			
Median (IQR)	0.17 (0.00-0.74)	0.00 (0.00-0.02)	<0.001
Clonal LCR n (%), gm/gm creatinine			
<0.2	97 (53%)	168 (92%)	<0.001
≥0.2	84 (46%)	14 (7.6%)	
Missing	2 (1%)	1 (0.5%)	
Clonal KCR **KCR, gm/gm creatinine**			
Median (IQR)	0.29 (0.02-1.35)	0.02 (0.00-0.07)	<0.001
Clonal KCR KCR, n (%), gm/gm creatinine			
<0.2	156 (45%)	298 (86%)	<0.001
≥0.2	190 (55%)	45 (13%)	
Missing	0 (0%)	3 (0.8%)	
Differences were calculated using Wilcokson sign rank test. Abbreviations: LCR, lambda creatinine ratio; KCR, kappa creatinine ratio.			

6.6.3 Renal outcome at 12-months and ACR in the matched groups.

184 patients with improvement in eGFR at 12 months were compared to 184 patients with no change in eGFR (Table 6.3). Median ACR at baseline and 12 months were not significantly different in the two groups.

123 patients with a decline in eGFR at 12 months were compared to 123 patients with no change in eGFR. Median ACR at baseline and at 12 months in the 2 groups was not significantly different (*P* value 0.433 and 0.555 respectively). Amongst those who had a decline in eGFR at 12 months, 6 (5%) patients had an ACR ≥ 70 mg/mol at baseline compared to 0 (0%) patients in the no change group (*P* = 0.573) and 5 (4.1%) patients had an ACR ≥ 70 mg/mol at 12 months compared to 0 (0%) patients in the no decline group (*P*=0.237) (Table 6.3).

Table 6.3: Albumin creatinine ratio in patients at baseline and 12 months in patients matched for age categories, sex, baseline dFLC and clonal response.

Improvement vs no change in eGFR							
	Baseline				**12 months**		
Characteristic	**Improvement** (n=184)	**No change** (n=184)	***P***		**Improvement** (n=184)	**No change** (n=184)	***P***
Baseline ACR, mg/mmol Median (IQR)	7.35 (2.2-17.2)	4.55 (1.3-16.5)	0.092	**12 month ACR,** mg/mmol Median (IQR)	1.35 (0.64-4.94)	1.12(0.54-3.82)	0.102
Baseline ACR, n (%), mg/mmol				**12 month ACR**, n (%), mg/mmol			
<3	54 (29.3%)	69 (37.5%)	0.100	<3	116 (63%)	124 (67.4%)	0.861
3 -30	97 (52.7%)	87 (47.3%)		3-30	55 (29.9%)	42 (22.8%)	
> 30	12 (6.5%)	14 (7.6%)		>30	7 (3.8%)	8 (4.3%)	
≥70	5 (2.7%)	4 (2.2.%)		≥70	1 (0.5%)	2 (1.1%)	
Missing	16 (8.7%)	10 (5.4%)		Missing	5 (2.7%)	8 (4.3%)	
Decline vs no change in eGFR							
	Baseline				**12 months**		
Characteristic	**Decline** (n=123)	**No change** (n=123)	***P***		**Decline** (n=123)	**No change** (n=123)	***P***
Baseline ACR, mg/mmol Median (IQR)	4.05 (1.49-10.8)	3.43 (1.21-11.2)	0.433	**12 month ACR**, mg/mmol Median (IQR)	1.23 (0.58-4.19)	1.21 (0.62-3.46)	0.555
Baseline ACR, n (%), mg/mmol				**12 month ACR**, n (%), mg/mmol			
<3	51 (41.5%)	54 (43.9%)	0.573	<3	79 (64.2%)	82 (66.7%)	0.237
3 -30	57 (46.3%)	53 (43.1%)		3-30	31 (25.2%)	33 (26.8%)	
> 30	1 (0.8%)	7 (5.7%)		>30	0 (0%)	4 (3.3%)	
≥70	6 (4.9%)	0 (0%)		≥70	5 (4.1%)	0 (0%)	
Missing	8 (6.5%)	9 (7.3%)		Missing	8 (6.5%)	4 (3.3%)	
Differences were calculated using Wilcokson sign rank test.							

6.6.4 Patients with ACR ≥ 70 mg/mol

Of 529 evaluable patients, 15 (2.8%) patients had a baseline ACR ≥ 70 mg/mol. At 12 months 6/15 (40%) had a decline in eGFR, 5/15 (33%) had an improvement in eGFR and 4/15 (27%) had no change in eGFR. Improvement in albuminuria was seen at 12 months in 9/15 (60%) patients; 4/15 (27%) continued to have an ACR ≥ 70 mg/mol and 2/15 (13.3%) were missing a 12-month ACR.

6.6.5 Univariate and multivariate analysis

An ACR ≥ 70 mg/mol at baseline was not associated with decline in eGFR at 12 months on univariate logistic regression analysis or when adjusting for other variables (odds ratio 2.257, 95% CI 0.786-6.481, *P*=0.130). ACR was re-run as a continuous variable and this was also not associated with a statistically significant decline in eGFR at 12 months (odds ratio 1.003, 95% CI 0.998-1.008, *P*=0.224).

6.7 Discussion

Quantification of albumin in the urine by ACR is a standard assessment for patients presenting with and undergoing monitoring for kidney disease. The presence of albuminuria is strongly associated with increased risk of kidney disease progression and other adverse outcomes [394-396]. This study found that albuminuria in patients with myeloma is common, almost 60% of patients had an ACR >3 mg/mmol at presentation and albuminuria levels approximating to >1g/24 hours (ACR ≥70 mg/mmol) was present in 3%.

At 12 months most patients showed improvement in albuminuria. There was no relationship between improvement in albuminuria and improvement in eGFR at 12 months. In addition, there was no association between albuminuria at diagnosis and eGFR at 12 months. In patients with myeloma, where kidney biopsies have been studied and albuminuria quantification is available, patients with myeloma cast nephropathy have lower albuminuria and patients with alternative renal lesions such as AL or LCDD may have higher albuminuria [397, 398]. However, these studies are not representative of all patients with myeloma and kidney disease, as they are selected based on knowledge of renal histology. It is not known what proportion of patients with myeloma and kidney disease have distinct renal lesions associated with myeloma and what proportion have AKI secondary to pre-renal causes and/or pre-existing CKD of a cause separate to myeloma.

Most patients with myeloma at presentation with albuminuria have moderate albuminuria and in many patients the significance of this at presentation is uncertain. In the setting of an acute illness renal tubular albumin handling may change and an elevated ACR may be common and transient. Furthermore, there is significant intra-assay variability in ACR testing and a limitation of this study was that repeat testing at each time point was not available. ACR testing at presentation may therefore have limited utility and ACR quantification should also be done when the patient is in disease remission and has no other intercurrent illnesses. Therefore, a check ACR between three and six months may screen out those patients who have sustained albuminuria. In respect of 12-month albuminuria, this is the first study that has identified the prevalence of this in patients with myeloma after disease treatment. 31% of patients had albuminuria, and this compares to an age-matched general population prevalence of 11.7% [399]. In patients with myeloma the impact of albuminuria on clinical outcomes with time is not known. In other settings the presence of albuminuria is associated with increased risk of progression of kidney disease and other adverse outcomes [400-402].

In this study albuminuria at presentation was not independently associated with decline in eGFR at 12 months. Further studies are needed to identify whether persistent albuminuria is independently associated with increased risk of adverse outcomes in patients with myeloma. With the improving long-term outcomes of patients with myeloma identifying modifiable disease-independent risk factors will become an increasing priority.

6.8 Strengths and limitations

This study is the first to report on the prevalence of albuminuria in the NDMM patients. The detailed ACR data at presentation and at 12 months in the NDMM patients is useful for understanding the natural history of this disease and the effects of treatment. A limitation of this study is that it had only a small number of patients with ACR >70 mg/mmol and not enough patients to show an association. Another limitation is that the 12-month time point may also be too early to determine longer term renal outcomes for patients. A further limitation is that single ACR measurement is subject to substantial variability i.e., day to day variability depending on endogenous and exogenous factors and lab variability; and serial ACR measurements were not available in those with abnormal ACR results to determine if changes reflected test variability or change in disease status.

6.9 Conclusions

In conclusion, this study shows that majority of patients with NDMM have albuminuria at presentation and almost one-third have albuminuria at 12 months. In this study albuminuria at presentation was not independently associated with decline in eGFR at 12 months.

Chapter VII: Successful Renal Outcome in Membranoproliferative Glomerulonephritis, Following Treatment of the Underlying Subtle Clone: A Case Report.

The work presented in this chapter reports a case of MPGN secondary to MGRS, which responded to treatment of the underlying clone with chemotherapy, resulting in improvement in renal function.

7.1 Extent of personal contribution

I wrote the first draft of the manuscript, and I was responsible for all the edits and revisions.

The work in this chapter formed the basis for a peer reviewed publication [349].

Rana, R., Cockwell, P., Vydianath, B., Cook, M., Pratt, G., Drayson, M.T. and Pinney, J.H., 2018. Successful Renal Outcome in Membranoproliferative Glomerulonephritis Following Treatment of the Underlying Subtle Clone: A Case Report. *Mayo Clinic Proceedings: Innovations, Quality & Outcomes*, *2*(3), pp.297-302.

7.2 Abstract

MPGN secondary to a monoclonal gammopathy is a rare entity and is an MGRS related glomerulonephritis. The disease is characterized by glomerular monoclonal Ig deposits and specific changes on light microscopy and EM. Monoclonality is demonstrated by immunochemistry and this finding requires investigation for an underlying clone. A case of MPGN secondary to MGRS is reported that responded to treatment with chemotherapy, resulting in improvement in renal function. Patients with MPGN and Ig deposition need to be evaluated for a M-protein to guide the management strategy.

7.3 Introduction

Treatment of MGRS associated lesions is often required as renal damage can have major clinical implications including progression to ESRF and a higher mortality risk [41, 289, 403]. Broadly MPGN can be classified into, immune-complex mediated and complement mediated. Immune complex mediated MPGN has been classified into three groups: chronic infections, autoimmune diseases, and MGRS. Although MGRS related MPGN does not classify as a true ‘immune complex’ disease, it is being recognized as a separate entity, immune complex mediated and a cause in some cases of complement mediated MPGN [8].

In MGRS related MPGN, an abnormal clone of cell of B cell lineage produces an excess of MIg or its components. The structural configuration of the clonal Ig causes the histological changes of MPGN, through either monoclonal Ig deposition or isolated complement deposition (C3 glomerulopathy) [100-102, 404-407]. An MPGN pattern on a kidney biopsy requires evaluation for an underlying B-cell clone. Due to the low-grade nature of the underlying B-cell clone, careful serum and urine studies are needed to establish the presence of MGRS; and in most patients a serum and or urine clonal Ig is not detected despite clear demonstration of MGRS related lesion on kidney biopsy [96, 268].

A myriad of hematological diagnoses have been revealed in patients undergoing bone marrow biopsies for MGRS associated MPGN. The most common finding is a low-grade clone which would previously have been described as MGUS [408, 409]. Other known associated conditions are low-grade B-cell lymphoma, lymphoplasmacytic lymphoma, chronic lymphocytic leukemia, and MM [409]. Some patients have no evidence of clonal plasma cell/B-cell lineage proliferation in the bone marrow, and the clone is being produced at an extra-medullary site.

Evidence regarding renal outcome following treatment of patients with MGRS related MPGN is lacking. There is a strong justification for clone specific therapy in MGRS related MPGN as renal outcomes are improved in other renal diseases that are associated with a clonal protein (e.g., AL amyloid, MIDD) [9, 44, 68], It is now accepted that when there is evidence of MGRS, treatment with plasma cell-based therapy is indicated.

7.4 Hypothesis

The work presented in this chapter aimed to address the following hypothesis:

1. In a patient with MGRS a deep clonal response is associated with improved renal outcomes.

7.5 Methods

A case of MGRS related MPGN was diagnosed by the collaborative efforts of the hematology, nephrology, and pathology department at the Queen Elizabeth Hospital, Birmingham, UK. Kidney function monitoring, serial kidney biopsies and bone marrow biopsies were carried out to monitor disease progression and subsequent chemotherapy treatment and response.

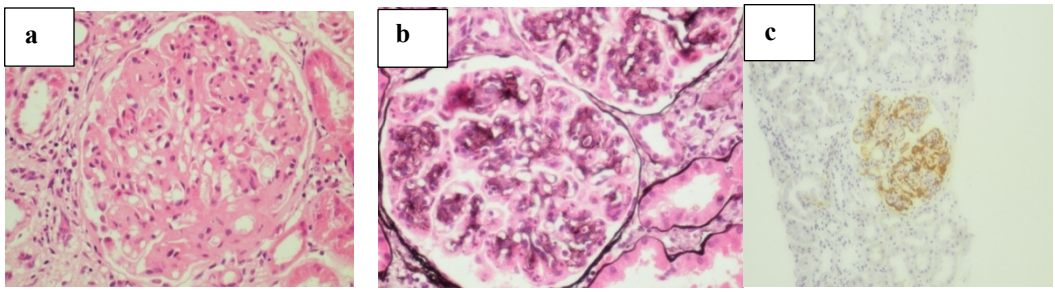
7.6 Case

A 66-year-old lady was referred to the nephrology clinic with hypertension and an active urinary sediment. She had previously been investigated for microscopic hematuria with normal prior renal tract ultrasound and cystoscopy. Her blood pressure was 160/84 mmHg, weight 82.1 kg, and clinical examination was normal. Urinalysis showed 3+ protein and 3+ blood. ACR was 268 mg/mmol; MDRD eGFR 64 ml/min/1.73 m^2, albumin 36 g/L, calcium and alkaline phosphatase were normal, hemoglobin was 10.9 g/dL with normal hematinics.

IFE showed an IgG λ paraprotein, clonal Ig quantitation was 5.40 g/L. Serum Ig levels were normal (IgG was 9.96 g/L, IgA 1.49 g/L, IgM 1.31g/L); sFLC results showed no evidence of FLC clonality with κ LC of 26.30 mg/l, λ LC of 23.55 mg/l and a κ:λ ratio of 1.1. Urine κ LC level was 0.02 g/l, urine λ LC level was 0.01 g/l. Urine was negative for FLCs on IFE. Immunology profile revealed a negative ANCA, ANA titre was 1:10 (homogeneous pattern) with a negative ds-DNA. Complement levels (C3 and C4) were normal. Her Hepatitis B and C serology was negative.

She underwent a renal biopsy which showed type 1 MPGN (Figure 7.1a-c). There was diffuse accentuation of the nodular pattern, associated with mesangial matrix expansion. Double contours were evident on silver staining, and there was focal endocapillary proliferation. On immunostaining, there was faint IgG and strong diffuse C3d along the peripheral capillary loops. EM showed marked expansion in mesangial matrix which demonstrated a filamentous appearance. There were no mesangial, no sub epithelial deposits but several capillary loops contain scattered subendothelial deposits. Marked effacement of the foot processes were noted. There were no casts and Congo red stain was negative.

Figure 7.1: Pathology of MPGN with monoclonal IgG deposits.

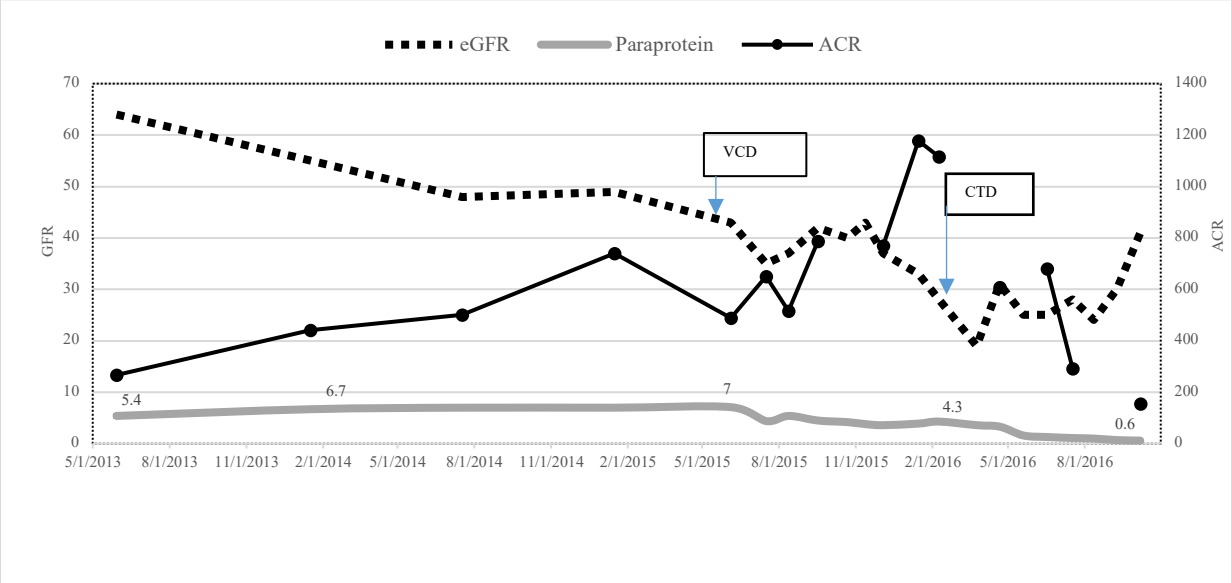


First biopsy *(a-c)* ***(a)*** *Glomeruli exhibit diffuse accentuation of the nodular pattern, with mesangial matrix expansion (H&E, X 200).* ***(b)*** *Silver stain; showing thickening of the membranes with double contours and endocapillary proliferation.* ***(c)*** *Immunohistochemistry highlights faint IgG in the peripheral capillary loops.*

Further investigations showed a small rise in IgG λ paraprotein quantitation at 7.2 g/L. Skeletal survey was negative and bone marrow biopsy showed a clonal population of plasma cells with a skewed κ:λ ratio of 1:5, these cells were CD117, cyclin D1, CD20 negative and CD56 positive, accounting for 2-3 % the total nucleated cell count. She had a full body CT scan which demonstrated small volume adenopathy both above and below the diaphragm; this was felt to be longstanding and related to her previous history of sarcoid. She was diagnosed with MGUS and given the uncertainty as to whether there was a direct connection with her renal disease, a period of monitoring was felt to be appropriate to determine whether there was a need for treatment.

Over the next two years, her proteinuria worsened (ACR 268 mg/mmol to ACR 741 mg/mmol) and she developed nephrotic syndrome (Albumin 30 g/L). Discussion between her treating nephrologist and hematologist resulted in repeat staging of her plasma cell dyscrasia. A further bone marrow biopsy showed an increase in clonal plasma cells to 10% with lambda light chain restriction, consistent with plasma cell myeloma. Her M-protein level over this period increased from 5.4 g/l to 7.1g/l, and there was a 20% decline in her eGFR (64ml/min to 51ml/min) (Figure 7.2).

Figure 7.2: Graph depicting change in eGFR (ml/min per 1.73 m^2), ACR (mg/mmol) and paraprotein (g/L) with time.



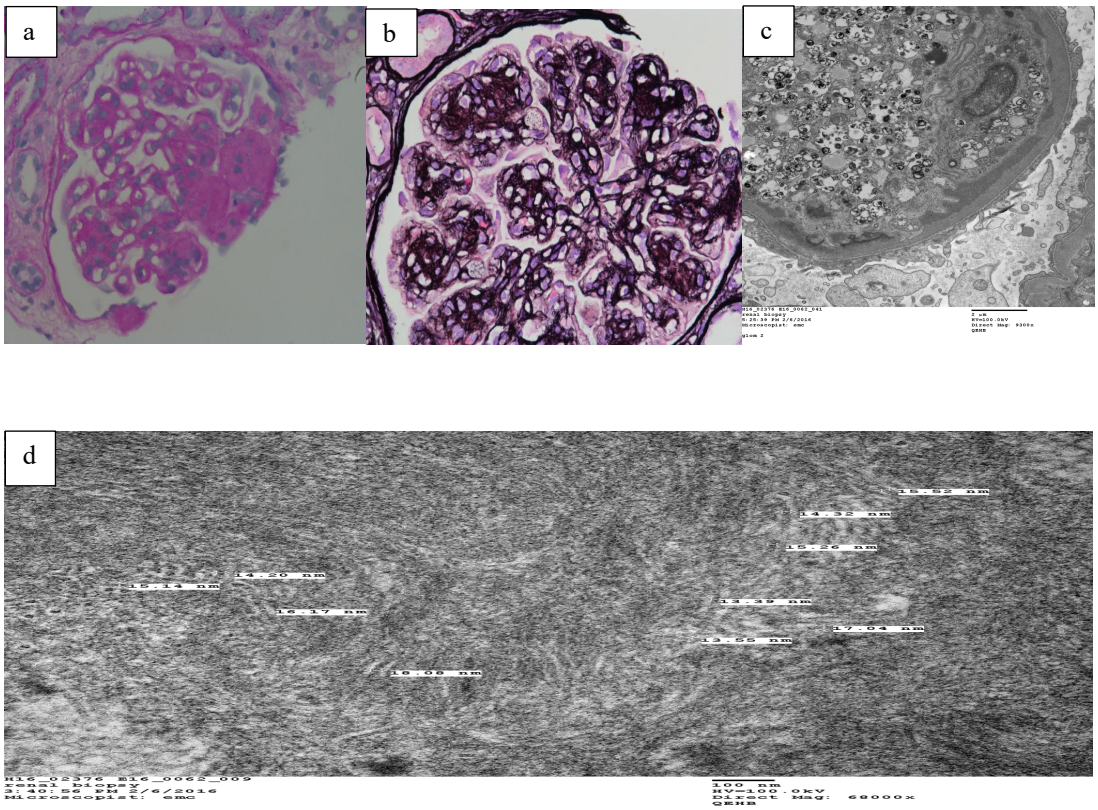
Arrows indicate initiation of treatment with VCD and later with CTD.

She received 4.5 cycles of attenuated VCD chemotherapy. Her treatment was complicated by fluid retention and macroscopic hematuria from cyclophosphamide, requiring mesna. A repeat flexible cystoscopy did not reveal any abnormality. The patient initially had a partial response to chemotherapy, the paraprotein level fell from 7.1 g/L to 3.5 g/L and subsequently plateaued (Figure 7.2).

Despite a partial hematological response, her renal function and proteinuria continued to deteriorate (eGFR 64ml/min to 42ml/min and ACR 268 mg/mmol to 769 mg/mmol, Figure 7.2). A repeat renal biopsy was performed to assess for evidence of renal disease progression; this again showed a membranoproliferative pattern (Figure 7.3 a-d) but there was more chronic damage, and an increase in extravasation of Tamm-Horsfall protein.

On immunostaining, there was peripheral capillary loop staining with IgG and with C3d and C1q. On EM, there was marked mesangial expansion with granular sub endothelial deposition and moderate effacement of podocyte foot processes. The renal biopsy taken two years previously was reviewed again with LC immunostaining. There appeared to be preferential λ staining of the membranous deposits with negative κ staining.

Figure 7.3: Findings of the second biopsy



(a) *Hypercellular glomerulus with nodular mesangial expansion, endocapillary proliferation, thickening of glomerular basement membranes and double contours (H&E, X 200) (**b**) On silver staining, double contours of the GBMs and mesangial deposits are seen. (**c**) Electron microscopy showing marked expansion of the mesangium with granular sub endothelial deposits and moderate effacement of podocyte foot processes. (**d**) Electron microscopy showing mesangial areas with a fibril deposition.*
H&E; Hematoxylin and eosin, IgG; Immunoglobulin G, GBM; Glomerular Basement Membrane.

As the renal biopsy was consistent with ongoing active MIDD; it was decided to target the clone further with a thalidomide-based regimen, to aim for a deeper clonal response. She received 6 cycles of attenuated CTDa and her paraprotein fell to 0.6 g/dl (Figure 7.2). Following second line treatment, the renal parameters improved (ACR 1115.8 mg/mmol to 154 mg/mmol and eGFR 19 ml/min to 47 ml/min) resulting in a favorable renal outcome (Figure 8.2). The patient has had a 2 year follow up since completing chemotherapy, with a latest eGFR of 54 ml/min//1.73 m^2 and ACR 25 mg/mmol.

7.7 Discussion

MGRS related MPGN is rare [410] and is by characterized by glomerular nonorganized, granular, clonal Ig deposits (commonly IgG3k); without any deposits along the tubular basement membrane [268]. A theory to elucidate disease pathogenesis suggests that MIg rapidly deposits in glomeruli through entrapment and or interaction with the negatively charged glomerular constituents and form definable electron dense deposits [268]. Other theories suggest the secretion of various biological factors or autoantibody activity of MIg [411].

Diagnostic workup includes 1) a renal biopsy 2) screening for a M-protein and 3) a complete hematological work up. Monoclonality in the renal biopsy can be determined by IF. It is important to correlate the specific MIg found on biopsy with that found during the hematologic work up to ensure a direct link is established between the MIg and type of nephropathy [95]. MGRS related MPGN is often challenging to diagnose as many patients don't have an identifiable clone in the bone marrow or peripheral blood [95]. Serum and urine MIg have been reported to be found in only one-third of patients; and a monoclonal proliferation of plasma cells in the bone marrow is found in less than 10% of patients [68, 94, 410, 412].

The literature surrounding renal outcome is very limited due to the rare nature of MGRS related MPGN. There are, however, studies of other conditions within the MGRS group which have looked at renal outcomes. A study of patients with MIDD, reported that most patients who didn't receive treatment, progressed to ESRF. In those treated with chemotherapy, a third progressed to ESRF [413]. These results highlight the importance of an early diagnosis to enable treatment, to preserve and potentially restore kidney function. Another study in patients with LCDD reported that patients who achieved a CR or VGPR with chemotherapy had a mean improvement in GFR of 6.1mL/min/year compared with a mean GFR loss of 6.5mL/min/year in patients with partial or no response [10].

Factors predictive of a renal response in MIDD are pre-treatment eGFR over 30ml/min per 1.73m2, and post treatment dFLC under 40mg/l [9]. The goal of therapy in MGRS related MPGN as with other MGRS lesions, should be to target a deep clonal response. Currently there is no standard treatment strategy for patients with MGRS related MPGN. Various treatment regimens including immunosuppressive regimes have been reported in the literature with variable outcomes [94, 95, 409].

Table 7.1 provides a summary of the literature reporting on treatment strategies and outcome in MGRS related MPGN.

Table 7.1: Summary of literature reporting on treatment of MGRS associated MPGN.

Series	Number of patients	Treatment	Renal outcome
Sethi [409] (Total-16 patients)			
	3	Prednisolone + Cyclophosphamide	Not reported
	3	Prednisolone + Rituximab	Proteinuria decreased from 4.3, 9.8, 0.5 g/24 to <0.3 g/24 Creatinine improved from 3.1 to 1.3 mg/dl, 2.4 to 1.4 mg/dl and 1.8 to 1.1 mg/dl.
	1	Prednisolone	Not reported
	1	Prednisolone +MMF	Not reported
	1	MMF	Not reported
	1	Dexamethasone	Not reported
Follow up at 2 years: In 6 patients' renal function was stable, 2 had a decline in renal function, 2 patients were on haemodialysis, no follow up was available for 6 patients.	1	Prednisolone 6 months Followed by MMF for 12 months Followed by Cyclosporine for 3 weeks Finally, Rituximab	No response No response Discontinued because of adverse effects After 5 months, proteinuria decreased from 10 to 4g/24h, creatinine decreased from 1.6 to 1.4 mg/dl, subsequently lost to follow up.
	5	No specific treatment	Not reported
Nasr [95] (Total-32 patients)			Mean follow up 30.3 months
	5	No treatment	3/5 developed ESRD, 1/5 persistent microhaematuria, 1/5 PRD [e]
	9	RAS blockade	2/9 CR [e], 2/9 PR [e], 2/9 PRD [e], 1/9 (who had severe tubular atrophy and interstitial fibrosis) progressed to ESRD
	6	Prednisolone	1/6 CR, 1/6 PR, 2/6 PRD, 2/6 ESRD
	3	Prednisolone + Cyclophosphamide/chlorambucil	2/3 PR, 1/3 ESRD
	1	Thalidomide + Prednisolone	PRD
	1	Thalidomide + Bortezomib	PRD
	3	1/3 MMF alone 1/3 MMF+ Prednisolone+ Cyclophosphamide 1/3 MMF +Cyclosporine	1/3 CR, 1/3 PR, 1/3 PRD
	2	Rituximab	2 PR
	2	Rituximab + MMF and or Cyclosporine	2 PRD
Yun [414]	1	Bortezomib + Dexamethasone	Progressed to ESRD.
Fatima [415]	1	Prednisolone for 3 months Repeat Prednisolone for 3 months	Improvement in renal function, but relapsed at 6 months At 1year, stable renal function (creatinine 1.7 mg/dl)

MMF: mycophenolate mofetil; ESRD: end stage renal disease; CR: remission of proteinuria to <500 mg/d with normal renal function; PR: reduction in proteinuria by at least 50% and to <2g/d with stable renal function (no more than a 20% increase in serum creatinine); PRD: failure to meet criteria for either CR or PR but not reaching ESRD

To conclude, in patients with MGRS related MPGN specific therapy targeting B cell/plasma cell clone is required to improve outcomes [96]. The requirement for treatment with chemotherapy needs to be carefully balanced against the potential risks associated with chemotherapy. Collaborative efforts are required of both nephrologists and hematologists to guide management strategy in this rare condition.

7.8 Strengths and Limitations

This case report highlights the challenges associated with the management of MGRS-related MPGN and demonstrates that targeting a deep clonal response with chemotherapy can lead to marked improvement in kidney function and proteinuria.

Limitation of this work relate to case report limitations i.e., may be risk of over interpretation, lack of ability to generalize and publication bias relating to reporting of positive outcome findings.

7.9 Conclusions

A deep clonal response needs to be achieved to get a renal response. The patient showed both improvement in renal function and proteinuria following successful suppression of the underlying clone.

Chapter VIII: Post-Transplant Monoclonal Gammopathy of Renal Significance: A Case Series

The work presented in this chapter aims to evaluate and characterize patients with post-transplant MGRS. Each case highlights the challenges in diagnosis and management of this rare condition.

8.1 Extent of personal contribution

This project was registered as an audit and with the help of UHB informatics team, I retrospectively identified patients who had undergone a bone marrow and renal biopsy. I reviewed the electronic data records of all the identified patients and developed a dataset of patients with post renal transplant MGRS and led interpretation of this data. I wrote the first draft of the manuscript, and I was responsible for all the edits and revisions.

The work in this chapter formed the basis for a peer reviewed publication [416].

Rana, R., Cockwell, P., Pratt, G., Cook, M., Drayson, M., Vydianath, B., Neil, D. and Pinney, J.H., 2020, April. Post-transplant Monoclonal Gammopathy of Renal Significance: A Case Series. In *Transplantation proceedings* (Vol. 52, No. 3, pp. 857-864). Elsevier.

8.2 Abstract

MGRS in the transplant kidney is a rare cause of graft dysfunction and can progress to graft loss. Most cases of post-transplant MGRS are due to recurrent disease. Clone specific chemotherapy is required to target the underlying clone, and this may improve graft survival; however, this can be challenging as many patients are elderly with age related comorbidities and may have complications associated with increasing immunosuppression. To better characterize this condition, a retrospective review of clinical records over a 19-year period was conducted and this identified a total of 3 patients diagnosed with allograft MGRS. The clinical presentation, renal pathology, and clinical course of post-transplant MGRS in these patients is discussed.

8.3 Introduction

The term MGRS was introduced by the IKMG Research Group in 2012 [408]. MGRS-associated kidney diseases characteristically tend to be progressive [41, 61, 95, 417], with high recurrence rates in the transplant kidney. An estimated 80 % of post-transplant MGRS is reported to be due to recurrent disease or probable recurrent disease [60, 98, 263]. The incidence of 'de novo MGRS' post-transplantation is not known. In a few transplant patients the cause of ESRF is not known and whether an MGUS was investigated for and/or excluded at the time of transplantation, making it difficult to know whether the allograft disease is recurrent or not.

As MGRS encompasses a wide spectrum of different pathological processes the natural history of these diseases vary and the risk and time to recurrence in a graft differs according to the underlying disease; from 3 months in patients with PGNMID [98, 418], to a median time to recurrence of 33 months in LCDD [263] and 71 months in AL amyloidosis [51, 263].

Outcomes following transplantation have been described in conditions such as LCDD, AL amyloidosis, PGNMID and in patients with monoclonal gammopathy associated fibrillary glomerulonephritis (Table 8.1) [51, 60, 98, 263] although these reports are limited due to the rare nature of these diseases.

Table 8.1: Summary of literature reporting on outcome of renal transplantation in patients with MGRS.

Histology	Patient number	Recurrence	Median time to recurrence (months)	Graft loss due to recurrence	Time to graft loss (months)	Median graft survival (months)	References
PGNMID	26	17 (85%)	5.5 (1-116)	11 (44 %)	Mean 63 (9-132)	92	[419]
AL amyloid	25	7 (28%)	71	0	N/A	70	[51]
LCDD	7	5 (71%)	33.3 (2-45)	5 (71%)	10.9 (0.9-15.5)	37.3	[263]
MGFD	7	4 (57%)	24 (3-87)	3 (43%)	17 (10-46)	38 (4-131)	[60]

Abbreviation: MGFD, monoclonal gammopathy with fibrillary deposits.

The risk of recurrence and graft loss is higher in MGRS patients who have not achieved a complete hematologic response prior to transplantation [51, 68](7, 8) [419]. It has been suggested therefore that kidney transplantation should be reserved for those patients whose LC production has been controlled and remission sustained [263].

This work illustrates the clinical challenges of MGRS post-transplantation through three case reports. The cases highlight the need to evaluate for MGRS lesions in those who have unexplained kidney dysfunction, the importance of screening and rescreening for a paraprotein and detailed haematological evaluation when a MIg is detected in kidney biopsy but a peripheral clone is not obvious and the need for serial biopsies when the kidney lesion is subtle but progressive.

The cases emphasize the central role of EM and IF in the diagnosis of MGRS on biopsy samples both before and after transplantation and finally the complexity of decisions in the elderly with a heavy comorbid load.

8.4 Hypothesis

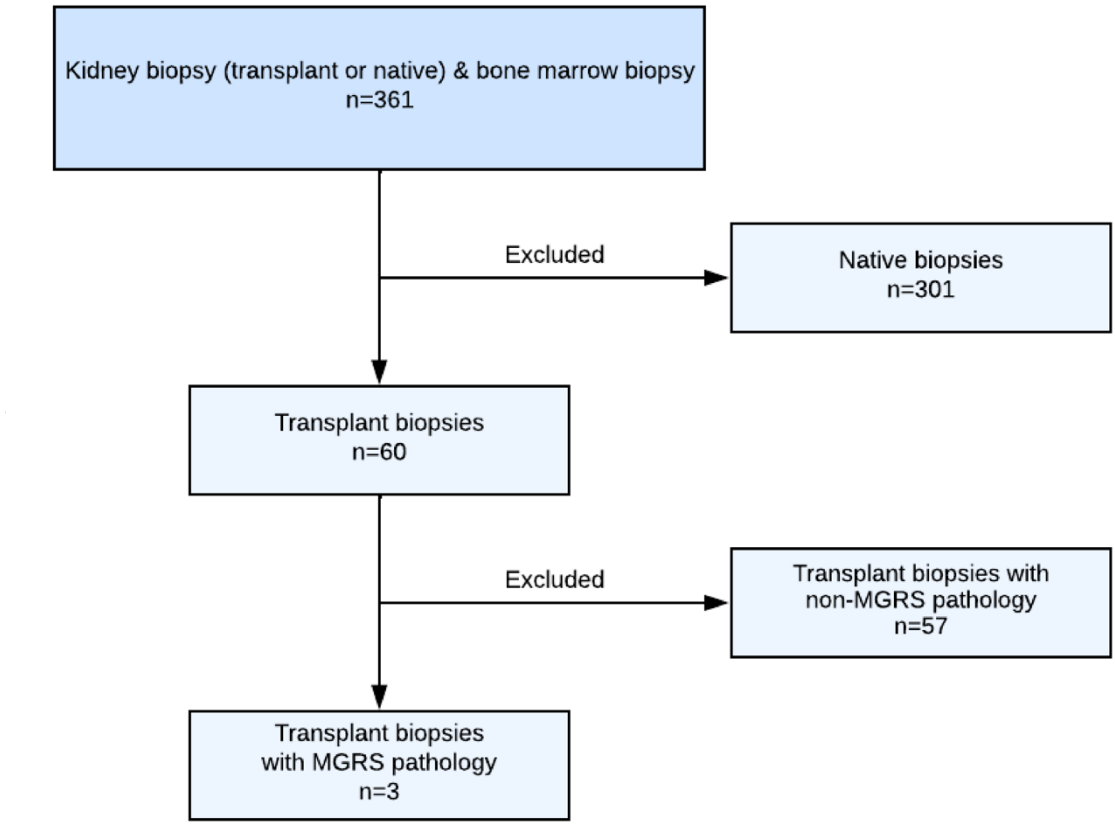
The work presented in this chapter aimed to address the following hypothesis:

1. In the absence of clone specific treatment MGRS is associated with progressive graft dysfunction and risk of death.

8.5 Methods

The institution's electronic database was searched over a 19-year period (from 1997 to 2016) for patients who had undergone a renal biopsy (native or transplant) and a bone marrow biopsy at any time point. 361 patients were identified in total, of which 60 had a transplant biopsy and met the criteria for this study. All the available renal transplant biopsy data and medical records were reviewed, and this identified 3 patients who had MGRS in renal transplant biopsies (Figure 8.1).

Figure 8.1: Flow diagram for inclusion and exclusion in case series



8.6 Cases

8.6.1 First Case

A 51-year-old female presented in 1994 with advanced CKD (eGFR 7ml/min/1.73 m^2). A renal USS scan showed small kidneys. Her soluble immunology tests were normal (ANA, ANCA, complement levels). SPEP and or IFE on serum and or urine was not done. She received a pre-emptive, live related renal transplant from her sister in 1997. The graft failed in 2009 with chronic allograft nephropathy and she was treated with continuous ambulatory peritoneal dialysis (CAPD). She received a second cadaveric kidney transplant in February 2012, after spending three years on CAPD. She was not screened for an MGUS during the work up for a kidney transplant.

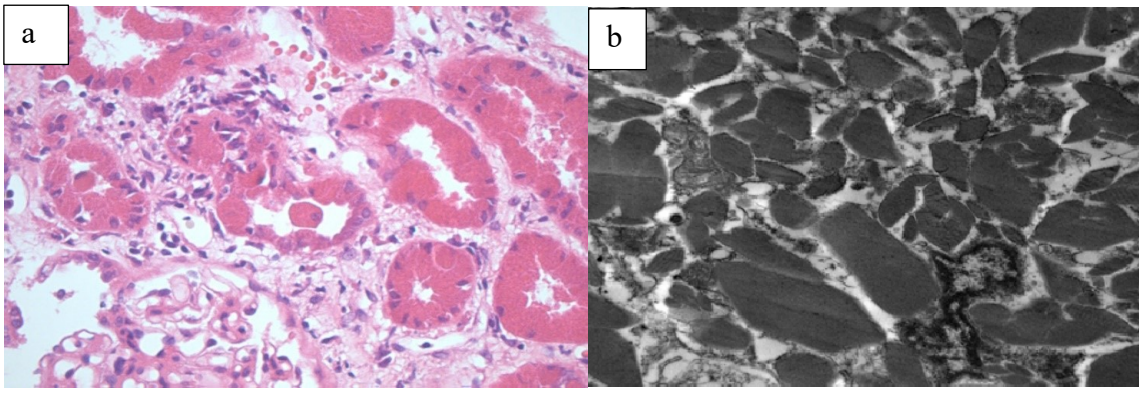
At three months post-transplant, she had a decline in kidney function (creatinine from 70 μmol/L to 130 μmol/L) and albuminuria (ACR 25 mg/mmol). A renal biopsy showed features of acute tubular injury with isometric vacuolation, raising the possibility of calcineurin inhibitor (CNI) toxicity. Her tacrolimus level was within range (7.7 pre-biopsy). There was no evidence of rejection and no chronic damage. Her SV40 and C4d staining were negative. Anti-HLA antibodies were negative and CMV, BK, EBV PCR were negative. Renal imaging showed no transplant renal artery stenosis and no obstruction.

The kidney function continued to decline and 3 weeks later a repeat biopsy showed borderline cellular rejection. She was pulsed with intravenous methylprednisolone, and her immunosuppression was optimized. Her renal function marginally improved to a creatinine of 157 μmol/L. Five weeks later there was a further decline in kidney function, and she was re-biopsied. This showed no rejection however the proximal tubular epithelium appeared very pink and granular, it lacked a brush border and was disrupted in places. There were odd tubular epithelial cell inclusions. EM was undertaken, and this showed organized deposits contained within the lysosomes of the tubular epithelium.

A myeloma screen identified an IgG kappa MIg on IFE. sFLC results showed a high κ: λ ratio at 29.6 (0.37-3.1), with a pathological (involved) sFLC level of 258 mg/L (normal range 3.3- 19.4 mg/L). She was referred to hematology for a bone marrow biopsy. Her renal function continued to decline and eight weeks later she underwent a fourth renal biopsy. There were densely packed hyper-eosinophilic granules in the tubular epithelium, seen on H&E staining (Figure 8.2a), and trichome stains.

On EM the glomeruli were ultra-structurally normal; however, there were organized deposits within the lysosomes of the tubular epithelium which were crystal in appearance, consistent with reabsorbed abnormal LCs (Figure 8.2b). There was electron dense immune-complex like deposition also seen in the tubular basement membrane.

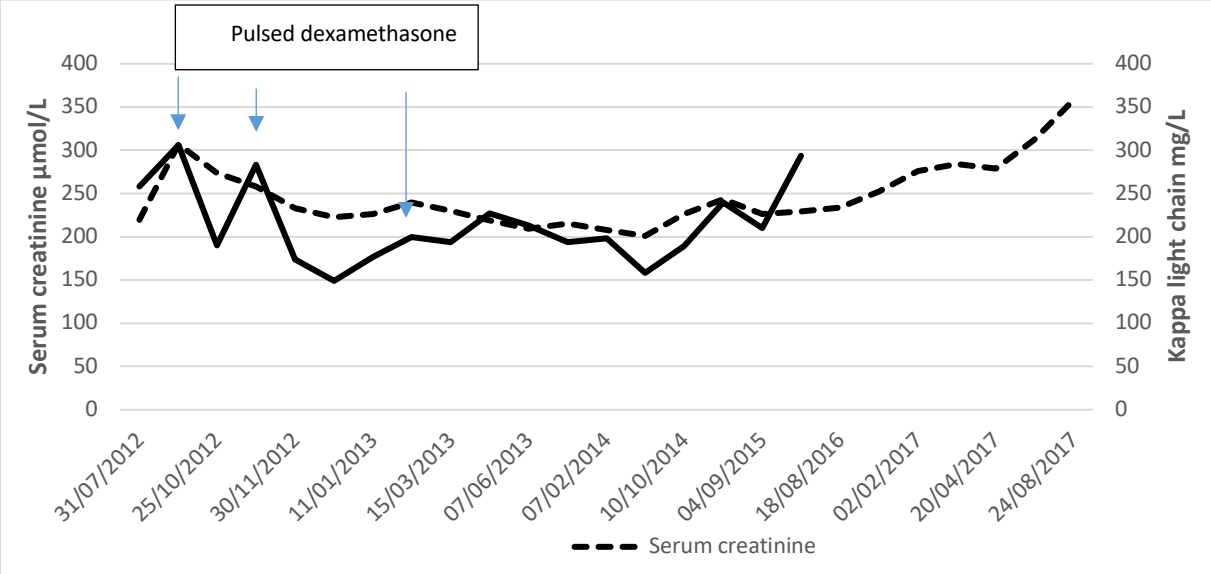
Figure 8.2: Second allograft biopsy findings.



(a) Light microscopy showing hyper eosinophilic granules in the tubular epithelium (H&E; x 10). (b) EM (high power) showing crystalline structures with tightly packed linear substructure in proximal tubules consistent with LCPT (x 20000).

A trephine bone marrow biopsy in October 2012, showed 5% κ LC restricted monoclonal plasma cells. Together, the findings of the bone marrow and transplant biopsy were consistent with a LC tubulopathy secondary to MGRS. She was treated with dexamethasone 40 mg for four days with a partial reduction in sFLC (Figure 8.3).

Figure 8.3: Serum creatinine (µmol/L) and κ LC (mg/L) over time.



Arrows indicate treatment with pulsed dexamethasone.

Treatment options were limited due to comorbidities. She received pulsed dexamethasone in November 2012 and February 2013. Over the next four years, she had a slow progressive decline in renal function and a rise in κ LC level (Figure 8.3). During this period, she had developed progressive dementia and because of this she received no further chemotherapy treatment, and a decision was taken to manage her kidney condition conservatively. She died 68 months following her second transplant from frailty and cognitive decline, with an eGFR of 11 ml/min/1.73m^2 and ACR of 32 mg/mmol.

8.6.2 Second Case

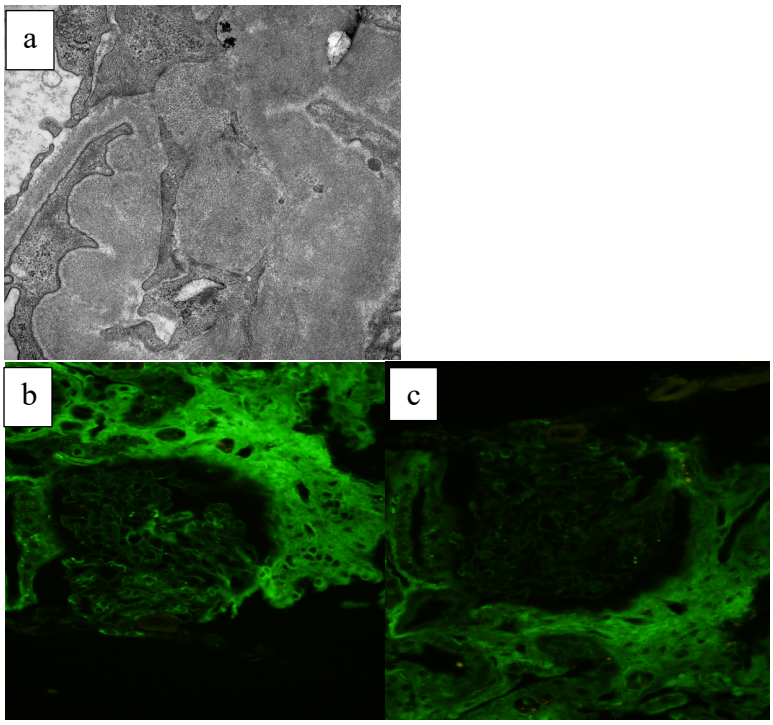
A 65-year-old male underwent a second cadaveric renal transplant in March 2016, for ESRF secondary to adult polycystic kidney disease. His first cadaveric renal transplant lasted for 19 years and failed due to severe damage from chronic vascular rejection, and he returned to hemodialysis in 2001. In Oct 2003, he underwent investigations for leukopenia and thrombocytopenia which showed IgG κ and IgM λ M-proteins (IgG kappa at 5 g/L and IgM at 2.4 g/L). A skeletal survey was normal. The bone marrow trephine showed reactive plasmacytosis, with plasma cells less than 5% which were morphologically normal.

By June 2013, as he had been active on the transplant list for 12 years and the IgG MIg had increased to 24 g/L; a bone marrow biopsy was undertaken. This was suboptimal for diagnostic purposes but there was no discernible increase in the number of lymphoid and plasma cells on immunohistochemistry. A skeletal survey was again normal. He was maintained under regular follow up for the MGUS and during this time the IgG MIg remained stable at 19 g/L.

He received a second cadaveric renal transplant in March 2016. One-week post-transplant, he underwent a transplant biopsy for delayed graft function. The biopsy showed isometric vacuolar change in some tubules, suggesting CNI toxicity (tacrolimus level was 12.6 ng/ml). Immunohistochemistry for C4d and SV40 was negative, and no abnormalities were found on EM. The tacrolimus dose was reduced, and his creatinine stabilized at 120 μmol/L.

He underwent a further biopsy two months later for a rising creatinine (163 μmol/L), this showed no features of rejection. However, EM now showed fibrillary areas within the mesangium (Figure 8.4), and on IF, performed on the formalin fixed paraffin embedded (FFPE) sections, there was κ restriction consistent with monoclonal fibrillary glomerulonephritis (Figure 8.4).

Figure 8.4: Renal transplant biopsy findings.



(a) EM showing fibrillary areas within the mesangium (x 23000). (b-c) IF staining for κ and λ on protease digested sections. (b) IF staining showing κ restriction. (c) IF staining showing no λ restriction.

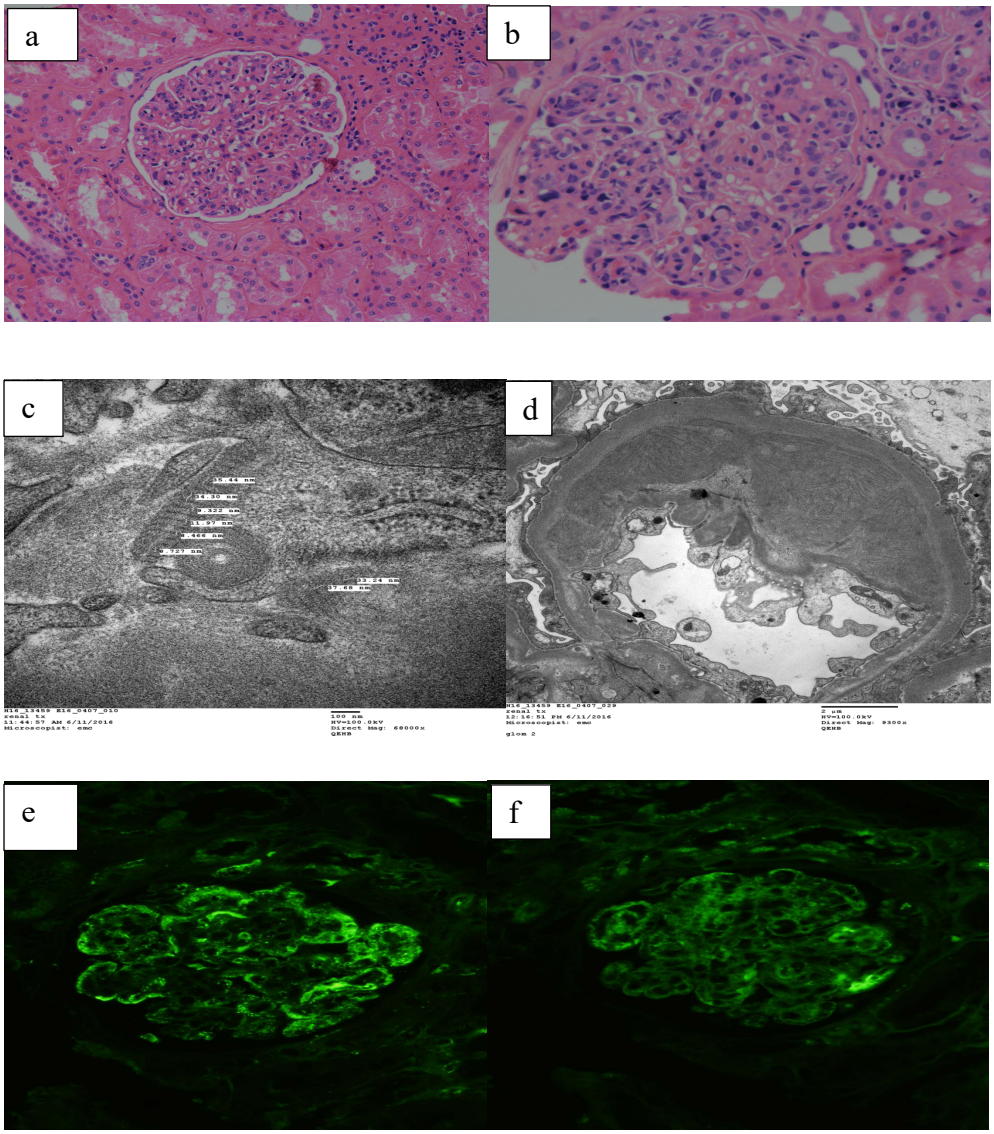
His kidney function subsequently plateaued without requiring any specific treatment. Interestingly post renal transplant, his MIg fell from 17.6 g/L (pre- transplant) to 6.5 g/L (post- transplant), and the κ LCs fell from 461 mg/L (pre-transplant) to 51 mg/L (post-transplant); this may reflect the effect of induction immunosuppression with methylprednisolone.

His most recent blood results from March 2019 show stable function with a creatinine of 102 μmol/L, ACR of 0.7 mg/mmol; MIg 7.7 g/L, and κ LC at 90 mg/L. Currently no treatment is proposed for MGRS unless kidney function declines and/or he develops proteinuria, and a transplant biopsy shows progression of MGRS.

8.6.3 Third Case

A 69-year-old male was referred to nephrology clinic with proteinuria. A renal USS revealed an 8 cm mass in the lower half of the left kidney. In February 2003 he underwent a left radical nephrectomy, histology of the mass revealed a moderately differentiated renal cell carcinoma; there was also evidence of a glomerular disorder noted in the surrounding tissues (Figure 8.5a). The glomeruli were enlarged with mesangial hypercellularity and there was glomerular subendothelial deposition of IgG, IgM and C9. The features were of a MPGN with no chronic damage (Figure 8.5a).

Figure 8.5: Membranoproliferative glomerulonephritis secondary to monoclonal immunoglobulin deposition.



(a) Light microscopy showing MPGN in native nephrectomy tissue in 2003 (b) Further renal biopsy of native right kidney in 2004 showing mesangiocapillary proliferation and moderate chronic damage. (c-d) EM showing non-organised electron dense deposits. The deposits are present in the paramesangium, mesangium, subendothelial position. (e-f) IF showng staining for κ is much brighter than for λ, in a granular segmental capillary wall and mesangial pattern.

As his kidney function continued to rapidly decline, he underwent a biopsy of his right kidney. The biopsy revealed moderate chronic damage with an underlying mesangioproliferative pattern of glomerular abnormality (Figure 8.5b). There was IgM in the expanded mesangium but no convincing IgA or IgG deposition, EM was not done. No serum or urine testing for a M-protein was performed at this stage. Within two years of the initial diagnosis, he reached ESRF and was treated with CAPD. After 5 years of cancer surveillance with no recurrence, he was activated on the transplant waiting list and received a cadaveric renal transplant in 2009.

His initial post-transplant period was uneventful, and he achieved a baseline creatinine of 110-120 μmol/L. SPEP and sFLC analysis in 2013 showed no abnormality. In 2016, he developed nephrotic syndrome (ACR of 976 mg/mmol, albumin of 29), his kidney function declined from a creatinine of 140 μmol/L to 200 μmol/L. He was found to have an IgM kappa MIg quantified at 1.5 g/L, κ LC level was elevated at 42 mg/L (normal range 3.3-19.4 mg/L), λ LC was normal at 25 mg/L (normal range 5.7-26.3 mg/L), with a normal κ:λ ratio of 1.67 (renal normal range 0.37-3.1).

He underwent a renal transplant biopsy which reported recurrent MPGN. EM showed non-organized electron dense deposits in the para mesangium, mesangium, sub endothelium and sub epithelium (Figure 8.5 c-d). The original nephrectomy and native biopsy samples were reviewed again, and EM was undertaken which showed similar non-organised deposits (Figure 8.5). IFE on the FFPE sections confirmed κ restriction (Figure 8.5e) and a diagnosis of PGNMID was made. A bone marrow trephine found a small population of plasma cells with no demonstrable LC restriction but with aberrant CD56 and CD117 expression (approximately 4% of cellularity).

He was started on prednisolone 30 mg once a day for two weeks with a view to then taper the dose. The treatment with steroids was complicated by fluid retention and hyperglycemia. Although sFLC levels and renal function did improve initially, he remained grossly proteinuric (ACR 528 mg/mmol) with impaired renal function (creatinine 271 μmol/L, eGFR of 20ml/min/1.73 m^2). The patient continued to have a progressive decline in renal function and was being managed for fluid overload. He was referred for peritoneal dialysis catheter assessment in view of advanced CKD and was not treated with chemotherapy. He died in November 2017 at the age of 82 years due to sepsis secondary to cellulitis with an eGFR of 12ml/min/1.73 m^2, and ACR of 707 mg/mmol.

8.7 Discussion

The overall understanding of MGRS has grown in recent years, with access to clone directed therapy becoming more widely available due to the expanding literature showing benefit [15, 44, 68, 263].

The incidence of MGUS increases with age [3]. As graft and patient survival continue to improve more patients with advanced and ESRF are being considered for kidney transplantation, and patients from both populations will have a co-existing MGUS. Although rare, some of these patients will develop recurrent or de novo MGRS after kidney transplantation.

The three cases described here highlight different aspects for consideration. The first case describes a patient with de novo LCPT. The patient's primary renal disease was unknown, her first transplant lasted for 12 years and failed due to chronic allograft nephropathy. The diagnosis of MGRS took several biopsies as the renal lesion was subtle but progressive. This case highlights the importance of screening and rescreening for a paraprotein in patients with a decline in graft function, where there are findings consistent with MGRS. It also emphasizes the central role of EM in the diagnosis of MGRS.

The current European Renal Best Practice Guidelines suggest that work up for transplantation should include screening for tumors according to guidelines that are used in the general population [420]. M-protein screening is not currently done in the general population and therefore not done as part of the routine transplant work-up. Future studies need to evaluate if this would be a cost-effective strategy versus consequences of an undiagnosed MIg. Clinicians should consider screening for a MIg if the cause of kidney disease is unknown [109, 408].

In the first case the patient was not suitable for clone specific chemotherapy and was managed with pulsed dexamethasone only. This decreased the involved LC by 38% and thereafter increase from this was slow. FLC monitoring was discontinued after February 2016 as the patient had developed progressive dementia and knowledge of the sFLC level would not have changed management. She sustained a progressive decline in kidney function and was approaching ESRF when she died 5 years after the diagnosis of MGRS was made.

The second patient had an MGUS diagnosed prior to his second transplant. There are no consensus guidelines on solid organ transplantation in patients with an MGUS, however the current literature supports transplantation in this group [421, 422].

One large study of over 22,000 solid organ recipients reviewed the outcomes of 72 patients reported to have an MGUS. There was a higher risk of transformation to MM in patients with an MGUS compared to transplant patients without an MGUS. The rate of transformation was not however higher in MGUS patients who had solid organ transplants when compared to MGUS patients without a transplant [421].

In the second case the methylprednisolone as a component of the induction immunosuppression regime for kidney transplantation may have led to the dramatic fall in MIg concentration and LC levels. Interestingly the diagnosis of fibrillary glomerulonephritis was made very early post transplantation within the first two months. The ongoing immunosuppression for the transplant has suppressed the clone and over a follow-up period of 36 months there has not been any evidence of progressive renal decline or proteinuria. The number of reported cases of fibrillary glomerulonephritis post-transplant are limited. In one study of 7 reported cases of monoclonal gammopathy associated fibrillary glomerulonephritis, kidney transplantation was highly associated with significant post-transplant complications, including graft loss from recurrence and death from hematological malignancies. Recurrent disease developed in 5 allografts in 4 patients. The recurrence resulted in graft loss in 3 instances. Recurrence occurred as early as 3 months and as late as 7 years post-transplant with median allograft survival of 37 months [60].

The third patient is a case of recurrent MPGN in a transplant. This was diagnosed as an MGRS only after recurrence in the transplant. The patient did not have EM on the original native renal biopsy, thereby missing the opportunity to link the renal pathology with an underlying subtle clone. M-protein screen post-transplant in 2013 did not show any abnormality, making the diagnosis challenging. The bone marrow biopsy did not reveal κ restriction and therefore the diagnosis was dependent on the renal transplant histology.

The deposition of M-protein in the kidney is due to an underlying plasma cell or B cell clone, and sometimes this may not be easily detectable, making diagnosis and treatment challenging. In a study of 29 patients with recurrent MPGN after kidney transplantation, only 23% of patients had evidence of detectable serum M-proteins. The presence of M-proteins appeared to be associated with earlier more aggressive disease, and were more common in recurrent than non-recurrent disease [419]. A recent study of allograft PGNMID reported nearly one-half of patients losing their graft within 3 years of diagnosis [423]. Separately, Alasfar *et al* reported a post-transplant MPGN recurrence rate of 45% and MPGN recurrence led to graft loss in half of cases. The presence of monoclonal gammopathy was associated with a higher risk of recurrence [424]

A low-level IgM MIg was eventually detected in the third case, and this is consistent with the current literature. Once the clone was detected the decline in renal function did appear to accelerate and without targeted therapy to suppress the clone, the patient progressed to ESRF.

This case highlights the difficulties associated with diagnosing MGRS and the importance of EM and IF on biopsy samples both before and after transplantation. It also demonstrates the complexity of decisions regarding chemotherapy in the elderly with a heavy comorbid load, where there is a lack of evidence regarding the impact of chemotherapy on renal outcome and survival.

MPGN reported on native, or transplant biopsy should be evaluated with a M-protein screen (SPEP or IFE) in the serum and urine, and biopsy assessed with EM and IF to avoid a missed diagnosis of MGRS; and serial testing for a clone is recommended if this is a potential diagnosis. Patients with confirmed MGRS should be counselled regarding the risk of recurrence and reduced graft survival. The current approach in patients with MGRS prior to transplantation, is to give clone specific treatment prior to transplant listing to aim to suppress the clone to at least a sustained partial remission.

8.8 Strengths and Limitations

This chapter describes three cases of MGRS post-transplant diagnosed over a 19-year period at a large tertiary centre highlighting various aspects for consideration and contributes to understanding and management of this rare condition. Limitations of this study are that it is single centre retrospective cohort study with a small sample size due to rare nature of disorder.

8.9 Conclusions

Patients with unexplained renal dysfunction, atypical clinical course, risk factors for MGRS or those on transplant waiting list with unknown primary renal disease should be evaluated for MGRS; and their biopsy samples should undergo EM along with IF studies, to help characterize the deposits, establish monoclonality and guide a management strategy. Well-designed collaborative clinical trials of MGRS related kidney diseases are required to improve diagnosis and management.

Chapter IX: Screening for Albuminuria in Patients with Monoclonal Gammopathy of Uncertain Significance: A Single Centre Experience

The work presented in this chapter aims to evaluate the role of screening for albuminuria in the MGUS cohort for early diagnosis of MGRS.

9.1 Extent of personal contribution

This project was registered as an audit with the UHB audit department. I was responsible for writing the patient information sheets, questionnaires, and then posting these along with urine bottles (for ACR) to the patients. I collected and assembled the data, maintained a site file and was responsible for all the electronic data input. I led the data analysis, statistical plan, and interpretation of this dataset. Following analysis, I wrote the first draft of the manuscript, and I was responsible for all the edits and revisions. This chapter is based on unpublished first author original article that will be submitted for peer review in due course.

This work has been presented as a poster; at the IKMG meeting, 2023, Milan (Italy) and at the UK Kidney week, 2023, Newport (U.K).

9.2 Abstract

Albuminuria is biomarker of kidney damage and may be indicative of MGRS in patients with a monoclonal gammopathy. Guidelines relating to screening for albuminuria in patients with MGUS are not well defined and it is not known whether this should be a part of baseline assessment. This study evaluated 349 patients followed up in the nurse led telephone MGUS clinic. Patients were sent a questionnaire and screened for albuminuria with spot urine ACR. Overall, 281/349 (81%) patients provided a urine sample. 70/281 (25%) had evidence of albuminuria (ACR >3 mg/mmol). Of the 70 patients, 54 (77%) had moderate albuminuria (ACR 3-30 mg/mmol) and 16 (23%) had heavy albuminuria (ACR > 30 mg/mmol). 24/70 (34%) patients were already under renal follow up. Defining the prevalence of albuminuria in this cohort resulted in a new diagnosis of AL amyloidosis in 1 patient and identified 45/281 (16%) patients that may benefit from a repeat ACR test and/or on-going renal opinion.

9.3 Introduction

MGUS is an asymptomatic pre-malignant clonal plasma cell disorder characterized by the presence of an M protein <30g/L, <10% clonal plasma cells in the bone marrow and absence of end organ damage that can be attributed to MM or related lymphoplasmacytic malignancies [133]. MGUS is present in approximately 5% of the general population ≥ 50 years of age [425] and consistently precedes the diagnosis of MM, AL amyloidosis and WM for years [5, 28, 426, 427]. It is associated with 1% per year risk of progression to a lymphoplasmacytic malignancy and this risk does not disappear even after long-term follow up.

Albuminuria is an early biomarker of renal damage before a decline in eGFR has occurred and before clinical symptoms are evident [428]. An eGFR test can be indicative of kidney function decline whereas albuminuria testing estimates kidney damage. Therefore, in combination these two tests can help in an early detection and accurate understanding of the risk of renal progression [429]. The presence of albuminuria in the absence of long-standing diabetes or hypertension in patients with MGUS may be indicative of MGRS associated lesions [428]. Our understanding of the natural history of MGUS has much expanded and there is universal agreement on the criteria for diagnosis and risk stratification, however guidelines relating to practical aspects such as extent of evaluation at initial assessment and follow up are less well defined.

The British Committee for Standards in Hematology recommends the following tests at baseline and follow up for patients with MGUS: SPEP and UPEP, Igs, sFLC assay, full blood count, serum creatinine, corrected calcium and albumin, however, assessment of urine for albuminuria is less well defined [430].

MGUS is highly prevalent in the general population as is CKD, the prevalence of albuminuria is estimated to be around 6% [431, 432]. MGRS is challenging to diagnose and conditions such as AL amyloidosis are frequently identified late in the course of the disease [433] and early identification can result in better outcomes for patients.

There are well described pathways for diagnosis of MGRS now published [18] but this rests with the clinician having concerns regarding a particular symptom and looking for albuminuria. Without assessment of urine for albuminuria it is difficult to identify patients with MGUS who may benefit from an early renal input to exclude an MGRS lesion. To address this lack of data we characterized 349 patients under follow up at the nurse led Hematology MGUS Clinic at the Queen Elizabeth Hospital, Birmingham (U.K) and screened this cohort for albuminuria.

9.4 Hypothesis

The work presented in this chapter aimed to address the following hypothesis:

1) Actively screening for albuminuria in MGUS will help early identification of patients with MGRS.

9.5 Methods

9.5.1 Baseline assessment and routine care

Patients referred to the Queen Elizabeth Hospital (Birmingham, U.K) Hematology team with a MIg, are at presentation seen by a Consultant Hematologist. They undergo initial investigations and if confirmed to have MGUS are risk stratified [434] and frequency of follow up is planned accordingly. Patients are given the Myeloma UK information booklet on MGUS, a patient diary and CNS contact details.

Patients who consent to follow up in the telephone MGUS nurse led clinic have their blood samples taken in Hematology outpatients. The CNS collates the results, discusses any changes with Consultant Hematologist and sends out a letter with patient results, and arranges their next clinic appointment clinic. If patients' M protein and sFLC are stable they can continue follow up in nurse led telephone. If patients are deemed low risk and remain stable over the course of next few clinic appointments, they are referred to their GP for annual monitoring.

9.5.2 Study population and screening procedure

This study evaluated 349 patients from June 2021 to June 2022 due for routine follow up in the nurse led telephone MGUS clinic at the Queen Elizabeth Hospital Birmingham, U.K. Patients were posted a universal white top container for urine ACR and a questionnaire approximately 6-8 weeks prior to their clinic appointment. Patients were requested to bring their urine sample at the time of routine MGUS blood tests and hand in the completed questionnaires. Decision to participate was voluntary and if patients decided not to participate, their standard of care was not affected. This project was registered as an audit with the University Hospitals Birmingham audit department.

Spot urine samples were processed on the Alinity c analyser (Abbott), and creatinine was measured using an enzymatic creatinine method to calculate ACR. Albuminuria was assessed according to the KDIGO classification.

The electronic records of the patients were evaluated at baseline and screening, and the following variables were included in the analysis; demographic data, paraprotein type, M protein level, FLC isotype, sFLC levels, urine light chains, Igs, ACR, full blood count, serum creatinine, eGFR, calcium, albumin, and questionnaire responses.

9.5.3 Statistical Analyses

Categorical variables were summarized as frequencies and percentages. Continuous variables were expressed as median with IQR.

9.6 Results

9.6.1 Baseline characteristics

The baseline characteristics of the 349 evaluable patients are described in Table 9.1. The median age at MGUS diagnosis was 65 (IQR 73-55) years. Majority of patients were white, 201/349 (56%); IgG MGUS was the most common subtype, 205/349 (59%); and nearly half of the patients were referred to hematology via primary care, 172/349 (49%). The proportion of patients who had undergone various tests at baseline were as follows; sFLC test: 342/349 (98%), urine LC: 90/349 (26%), imaging: 186/349 (53%), bone marrow biopsy: 98/349 (28%) and urine ACR in 78/349 (22%) patients. RI as defined by an eGFR <60 ml/min was seen in 76/349 (22%) patients.

Table 9.1: Patient characteristics

Variable	Summary statistic (n=349)
Age, years	
Median (IQR)	70 (78-61)
Age at MGUS diagnosis, years	
Median (IQR)	65 (73-55)
Gender, n (%)	
Male: Female	196 (56%): 153 (44%)
Ethnicity, n (%)	
White	201 (56%)
Asian	40 (11%)
Black	35 (10%)
Mixed	5 (1%)
Unspecified	68 (19%)
Referral pathway, n (%)	
GP	172 (49%)
Other medical specialities	34 (10%)
Neurology	24 (7%)
Renal	21 (6%)
Rheumatology	18 (5%)
Thrombosis clinic	13 (4%)
Surgical	12 (3%)
Not known	55 (16%)
MGUS type, n (%)	
IgG	205 (59%)
IgM	68 (20%)
IgA	51 (15%)
LCO	14 (4%)
Biclonal	10 (3%)
IgD	1 (0.2%)
FLC isotype, n (%)	
Kappa	205 (59%)
Lambda	135 (39%)
Kappa and Lambda	9 (3%)
Tests at presentation, n (%)	
Urine light chains	90 (26%)
sFLC	342 (98%)
ACR	78 (22%)
Bone marrow	98 (28%)
Imaging	186 (53%)
Presentation, median (IQR)	
M protein, g/L	5.28 (9-2.8)
ACR, mg/mmol	1.30 (4.40-0.00)
IgG, g/L	12.25 (15.9-8.89)
IgA, g/L	1.9 (3.29-1.35)
IgM, g/L	0.81 (1.57-0.50)
Hb, g/L	134 (145-124)
Calcium, µmol/L	2.32 (2.39-2.25)
Albumin, g/L	44 (47-41)
GFR, ml/min	76 (88-62)
Presentation ACR, n (%), mg/mmol	
<3	53 (15%)
3-30	21 (6%)
>30	4 (1%)
No sample	271 (78%)
κ patients, median (IQR), mg/L	
κ sFLC	30.57 (59.5-21)
λ patients, median (IQR), mg/L	
λ sFLC	22.14 (41.02-15.35)
Duration of follow up, years	
Median (IQR)	5 (8-3)

At presentation of the 78 evaluable patients who had an ACR result: 53 (67%) had no albuminuria (ACR <3mg/mmol), 21 (26%) had moderate albuminuria (ACR 3-30mg/mmol), 4 (5%) patients had heavy albuminuria (ACR >30mg/mmol). Of the 21 patients with moderate albuminuria, 13 (62%) were previously known to renal; and of the 4 patients with heavy albuminuria, 2 (50%) were already under renal follow up.

Of the 2 patients with baseline ACR > 30mg/mmol not under renal follow up; one was 74-year-old male with background of type 2 diabetes with microvascular complications, CKD G3a A2 (baseline ACR 37 mg/mmol, no screening ACR available), and the second patient a 76-year-old male with background of non-alcoholic steatohepatitis, previous stroke, CKD G4 A2 (baseline ACR 58 mg/mmol and screening ACR 27 mg/mmol).

9.6.2 Screening ACR results

281/349 (81%) patients provided a urine sample for ACR testing prior to their clinic appointment. Of the 349 patients, 211/349 (61%) patients had no albuminuria (ACR<3 mg/mmol), 54/349 (16%) had moderate albuminuria (ACR 3-30 mg/mmol), 16/349 (5%) had heavy albuminuria (ACR> 30 mg/mmol) and in 68/349 (20%) a urine sample was not provided. Of the 70 patients who had evidence of albuminuria, 20/70 (29%) had a follow up ACR. Of the 54 patients with moderate albuminuria, 14 (26%) were known to renal team and 40 (74%) were not known.

Of the 16 patients with screening ACR > 30mg/mmol; 10 (62%) were previously known to renal team, 3 (18%) patients were new referrals to renal team because of screening and 3 (18%) were not referred to renal. Of the 3 referred to renal team, 1 was diagnosed with AL amyloidosis, 1 declined follow up and 1 was a patient previously discharged from renal care with stable CKD (previous biopsy showing immune complex glomerulonephritis) and now requiring optimization of BP control. The characteristics of 16 patients with ACR > 30 mg/mmol at screening are shown in Table 9.2.

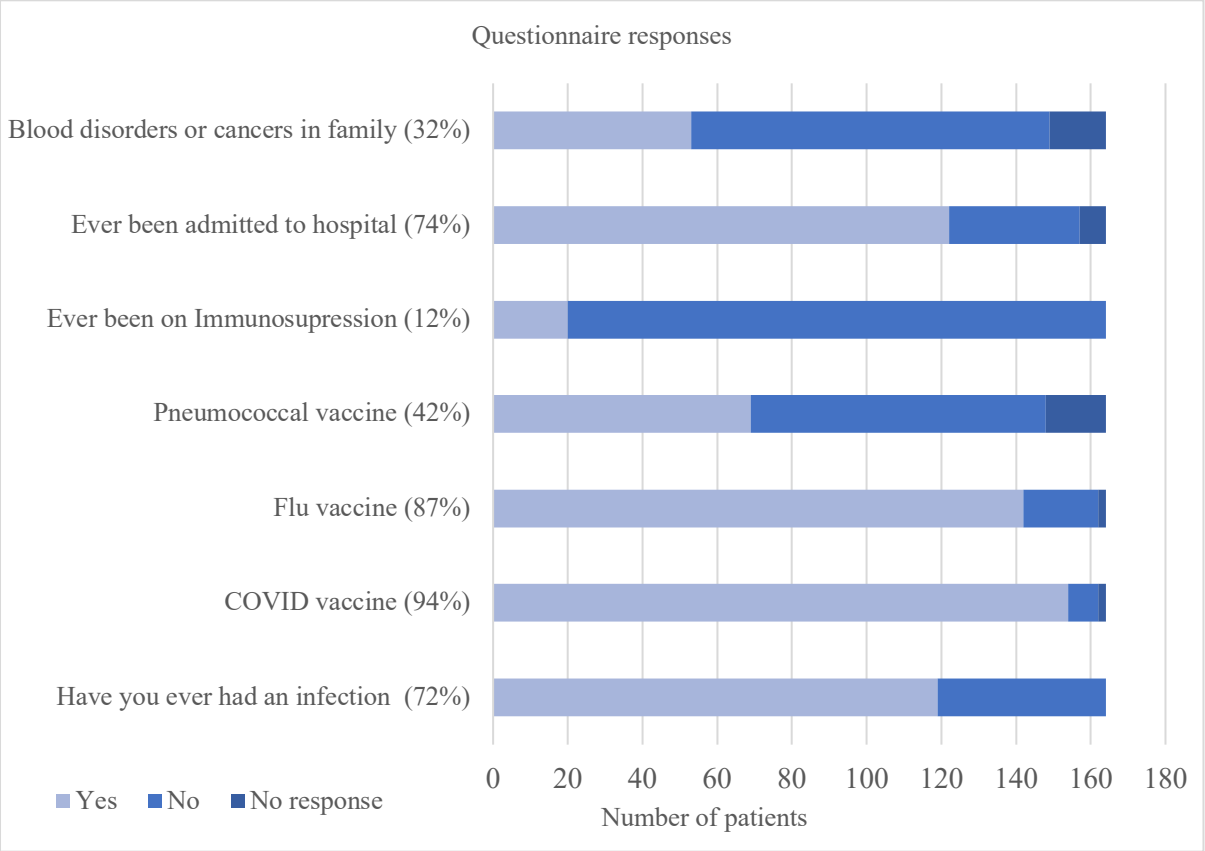
Table 9.2: Characteristics of the 16 MGUS patients with ACR > 30mg/mmol

Patient	Known to renal team prior to screening	Screening ACR mg/mmol	Repeat ACR mg/mmol	List of Diagnosis	Renal Biopsy	Referred to renal following screening
1. 53, Female	No	36	No	1. Diabetes 2.Cervical discectomy and fusion	No	No
2. 83, Female	Yes	40	Yes, 120	1. CKD G4A3 2. Previous AKI with diarrhoeal illness-biopsy acute tubulointerstitial nephritis 3. Hypertension 4. Polyarthritis	Previous biopsy acute tubulointerstitial nephritis	Already under follow up
3. 59, Male	Yes	64	No	1. CKD G3A3 2. Referred to NAC, no evidence of amyloidosis 3. Chronic left PUJ obstruction 4.Mettalic aortic valve replacement 5.Severe left ventricular hypertrophy 6. Hypertension	No	Already under follow up
4. 78, Male	Yes, and had been discharged from follow up	78	105	1. CKD G3bA3 2. Previous biopsy immune complex glomerulonephritis 3.Cirrhosis 4. Knee replacement	Previous biopsy immune complex glomerulonephritis	Yes
5. 91, Female	No	87	No	1. CKD G2A3 2. IgM MGUS	No	No
6. 84, Female Died	Yes	90	No	1. CKD G5A3 2. Lithium related CKD 3. Primary hyperparathyroidism 4. Hypertension 5. Previous Diverticulitis	No	Already under follow up
7. 70, Male	No	116	No	1. CKD G2A3 2. Rheumatoid arthritis 3.Heart Failure 4. Atrial fibrillation	No	Yes, but patient declined follow up
8. 71, Male	Yes	119	Yes, 118	1. CKD G4A3 2. IgA nephropathy 3. Alcoholic liver disease with portal gastropathy 4. Psoriasis 5. Malignant melanoma excised 6. Gout 7. Retinal detachment	Previous biopsy IgA nephropathy	Already under follow up
9. 66, Male	Yes	125	No	1. CKD G2A3 2. Diabetes 3. Hypertension 4. Bronchiectasis 5.Polyarthritis 5. Polymyalgia rheumatica	No	Already under follow up
10. 54, Male	Yes	144	Yes, 211	1. CKD G4A3 2. Renal transplant 2004 3. Primary renal disease focal segmental glomerulosclerosis 4. Diabetes 5. Ureteric stricture with stent placement	Previous 3 transplant biopsies- mild chronic damage	Already under follow up
11. 57, Female	Yes	174	Yes, 229	1. CKD G3aA 2. CKD secondary to thin basement membrane and focal segmental glomerulosclerosis 3. Hypertension	Previous biopsy thin basement membrane and focal segmental glomerulosclerosis	Already under follow up
12. 57, Male	Yes	180	Yes, 227	1. CKD G4A3 2. Diabetes 3.Hypertension 4. OSA	Previous biopsy diabetic nephropathy	Already under follow up
13. 85, Male	Yes	204	No	1. CKD G5A3 2. Diabetes 3. Hypertension	No	Already under follow up
14. 81, Male	No	221	129	1. CKD G2A3 2. AL amyloidosis no cardiac involvement 3. Hypertension 4. Previous renal stones	Yes, AL amyloidosis	Yes
15. 66, Male	Yes	221	65	1. CKD G4A3 2. Hypertension 3. Rheumatoid arthritis 4. Diabetes 5.Gout 6. Carpal tunnel 7. Diverticulosis	Previous biopsy hypertension related with possible IgA nephropathy	Already under follow up
16. 75, Male Died	No	234	No	1. High-grade glioma for supportive care 2. Hypertension 3. Chronic obstructive pulmonary disease 4. Dementia	No	No

9.6.3 Questionnaire responses

164/349 (47%) patients returned a questionnaire. A summary of the patient responses is shown in Figure 9.1. A table of detailed questionnaire responses is attached as a supplementary file in the appendix section (Appendix 4).

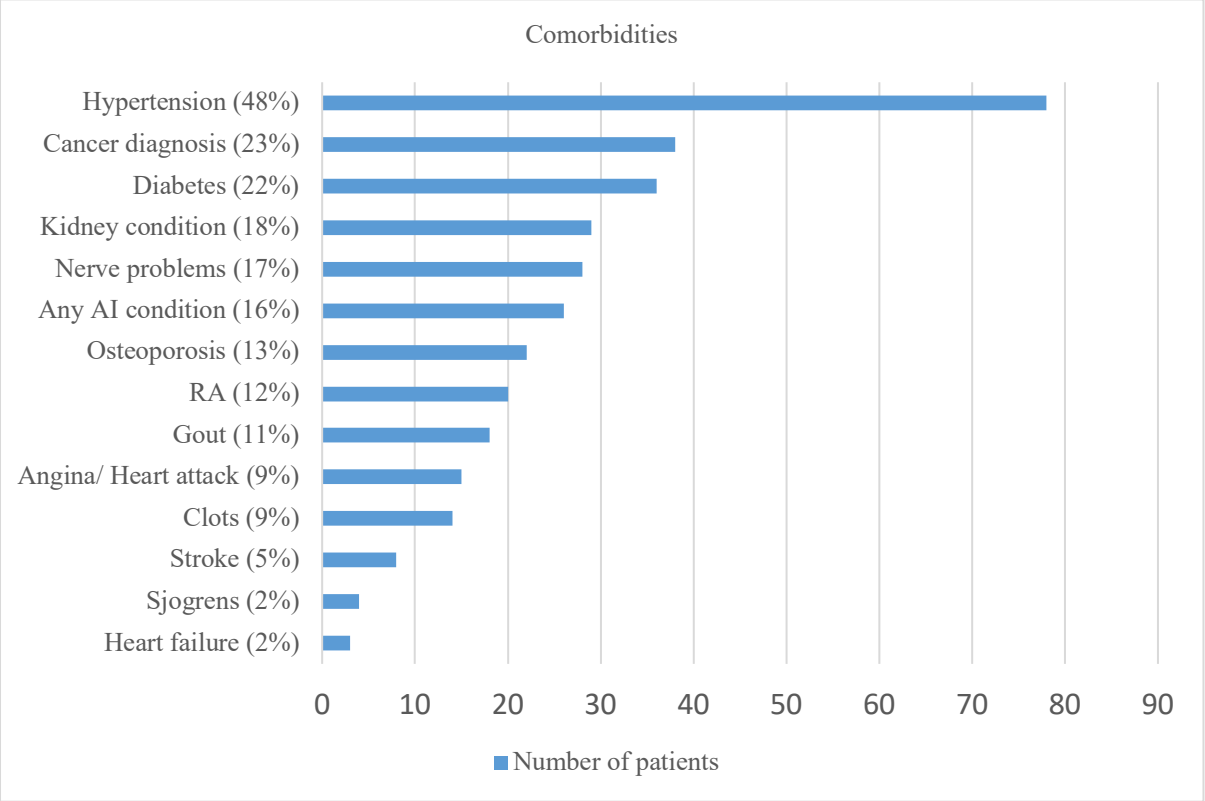
Figure 9.1: Questionnaire responses provided by 164 MGUS patients.



Infection was common with 119/164 (72%) patients reporting a prior infection. Most common site of infection was chest 69/119 (58%), with majority 90/119 (76%) patients not requiring hospitalization and oral antibiotics only; 34/119 (29%) patients reported having more than 2 infections in the last 3 years (Appendix 4).

Of the 164 patients; 154 (94%) reported having received COVID vaccine, 142 (87%) had undergone flu vaccination and only 69 (42%) had received a pneumococcal vaccine. Most 122/164 (74%) patients reported a previous episode requiring hospitalization and for majority 84/122 (69%) this was more than 2 years ago (Appendix 4). 53/164 (32%) patients reported a family history of blood disorders and/or cancers. The comorbidity burden reported in the 164 patients is shown in Figure 9.2.

Figure 9.2: Comorbidities reported as per questionnaire responses in 164 MGUS patients



Abbreviations: AI, auto immune; RA, rheumatoid arthritis.

9.7 Discussion

Testing for albuminuria is not currently a part of routine baseline assessment for MGUS patients. The aim of this project was to characterize the local MGUS cohort in depth, to determine the prevalence of albuminuria. We used the current MGRS guidelines to investigate patients who were identified as having albuminuria to determine whether screening may help to identify patients with an MGRS.

In this study at screening, 25% of the MGUS patients had evidence of albuminuria. Screening resulted in a new diagnosis of AL amyloidosis in 1 patient and led to better BP control management in the second patient. In a large population-based cohort study 6.7% subjects were found to have albuminuria; 2/3 of microalbuminuria was found in association with hypertension, 1/2 of all cases of microalbuminuria were seen in subjects with abnormal glucose metabolism and a similar percentage of all cases were found in subjects over 65 years of age [432].

Infection is common in MGUS patients and in this study more than 70% reported a prior infection. Patients with MGUS have a depressed humoral immunity and 2-fold increased risk of developing any infection [435, 436] and this supports early antibiotic use, vaccination, and referral for specialist input in recurrent infections [437].

NICE recommends a single dose of the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®) should be offered to people aged 65 years or over. In this study the coverage of pneumococcal vaccine was only 42% compared to 70.6% in all patients aged 65 and over, immunised at any time up to 31 March 2021 in England [438]. The standard set by the Department of Health and Social Care is 75% coverage [439] and this study indicates that performance standards are currently not being met. Factors affecting uptake of pneumococcal vaccine in this cohort need to be explored in future studies.

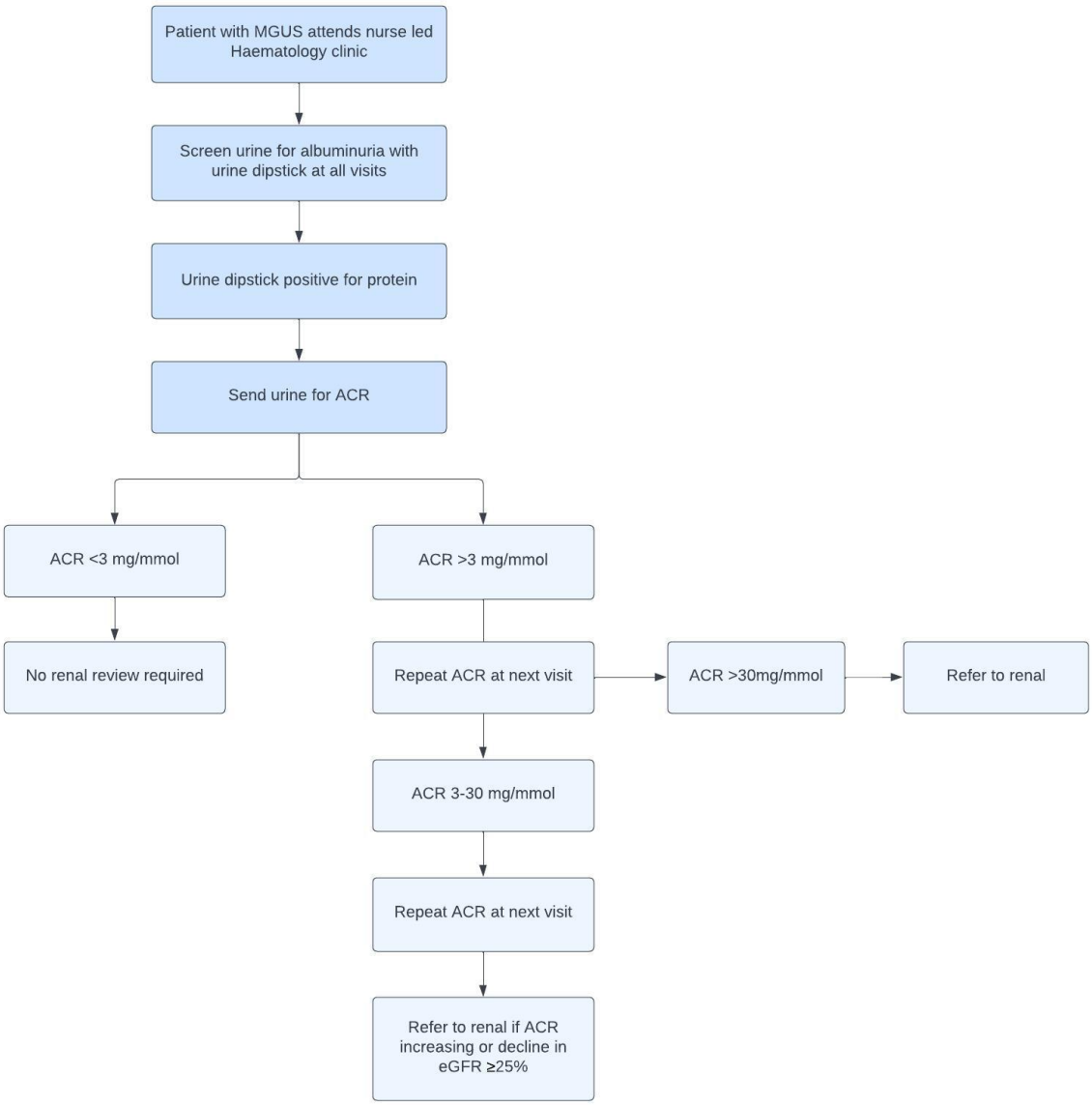
In this cohort patients with MGUS had a higher prevalence of hypertension (48 % vs 41%), diabetes (22% vs 17%), osteoporosis (13% vs 1% in males and 9% in females) and myocardial infarction (9% vs 7 % in males and 2% in females) compared to age matched general population [440] [441] [442, 443]. A Danish study comparing MGUS patients with age and sex matched controls reported a higher prevalence of hypertension (48% vs 38.5%) and diabetes (13% vs 9.3%) and an increased risk for adverse cardiovascular outcomes including venous thromboembolism in MGUS patients [444]. Disorders of the bone are all significantly increased in patients with MGUS [445] and the IMWG recommends bisphosphonate therapy in patients with proven osteoporosis [446] but whether this intervention will reduce fractures is not yet known [447].

In a recent nationwide prospective population-based screening study of nearly 70,000 individuals, no association was found between MGUS and CKD [448] and also no association was found between MGUS and autoimmune diseases [449]. 17% of patients in this study suffered with nerve problems. The vast majority of peripheral neuropathies occur in similar frequencies in patients with and without MGUS [445] except for chronic inflammatory demyelinating neuropathy and autonomic neuropathy which have confirmed associations with MGUS [445]. 9% patients in this study reported having suffered with blood clots. An increased incidence of venous thromboembolic disease (VTE) and to a lesser extent arterial thrombosis in patients with MGUS has been reported in a few studies [450, 451].

A study reported that biomarker-based (NT pro BNP for the heart, albuminuria for the kidney, and alkaline phosphatase for the liver involvement) screening for amyloid organ involvement in MGUS patients allowed pre symptomatic diagnosis of AL amyloidosis in 13 patients with excellent outcomes including those who failed to respond to upfront therapy [452]. Another study in AL amyloidosis showed that renal progression can be accurately predicted by baseline proteinuria and eGFR [453].

As a result of this project, a change to MGUS pathway has been made and all patients followed up in the nurse led MGUS clinic at the Queen Elizabeth Hospital will now have urine dipstick at every clinic visit. A flowchart of the proposed pathway to guide the hematology CNS is shown in Figure 9.3.

Figure 9.3: Flowchart to guide the Hematology CNS following screening urine for albuminuria.



Future work is required to determine, if ACR should be incorporated into risk assessment system at baseline for MGUS patients and the cost effectiveness of serial urine assessment for albuminuria at picking up undiagnosed MGRS.

9.8 Strengths and Limitations

This study is novel as it defines the prevalence of albuminuria in MGUS patients and the role for screening urine to identify MGRS lesions. A limitation of this study is that single ACR measurement is subject to substantial variability i.e., day to day variability depending on endogenous and exogenous factors and lab variability. Therefore, an abnormal ACR at screening needs follow up with serial ACR results to determine if changes in ACR reflect test variability or change in disease status [454] and this may influence diagnostic and management decisions. Another limitation is only 1/3 patients with albuminuria on screening had a follow up ACR available.

A one-off ACR screening will only identify patients with MGRS at that point and as MGUS clone can evolve to MGRS at any time, subsequent longitudinal urinalysis is required in MGUS patients to identify patients who may develop significant albuminuria and require renal review. As a result of this project, a change to MGUS pathway has been made and all patients followed up in the nurse led MGUS clinic at the Queen Elizabeth Hospital will now have urine dipstick at every clinic visit. A flowchart of the proposed pathway to guide the hematology CNS is shown in Figure 9.3.

9.9 Conclusions

In conclusion, one off screening for albuminuria in this cohort; resulted in a new diagnosis of AL amyloidosis in one patient who was subsequently treated (resulting in improvement in albuminuria and stabilization of kidney disease) and identification of 45/281 (16%) patients as having potential kidney disease who may benefit from repeat ACR and/or renal opinion.

Chapter X: General Discussion

This chapter provides a summary of the main results, draws conclusions from this research, and proposes focus for future research.

10.1 Overview of the Thesis

Monoclonal gammopathy related kidney disease is a rapidly evolving area of renal medicine with new entities such as MGRS-associated renal lesions now being defined and classified. The work presented in this thesis has looked at the following groups of patients with a monoclonal gammopathy 1) MM 2) MGRS and 3) MGUS and addresses some of the current shortfalls in evidence and understanding of these complex disorders.

In Chapter III, the timeline to diagnosis and commencement of treatment in patients presenting with AKI and new myeloma was reviewed. In chapters IV to VI data from the Myeloma XI trial was utilized to review 1) 12-month renal outcomes 2) impact of specific treatments on 12-month renal outcomes and 3) explore the relationship between albuminuria and 12-month renal outcomes in the NDMM patients with mild to moderate RI at presentation. By characterizing renal outcomes for myeloma patients this work provides important information for both, patients, and their clinicians, and can help guide counselling, risk stratification and treatment decisions. The study evaluating albuminuria and renal outcomes identifies a significant shortfall in our understanding of the relationship between albuminuria and long-term outcomes in patients with myeloma and contributes to the natural history of disease.

Chapter VII reports on a case of MPGN secondary to MGRS with excellent outcome following clone specific treatment and demonstrates that a deep clonal response is required to achieve a renal response. Chapter VIII describes post-transplant MGRS experience at a single centre and reviews the clinical, renal, overall outcomes and highlights the need to evaluate for MGRS in patients with unexplained renal dysfunction or those with risk factors for MGRS. Chapter IX reports on screening for albuminuria in the MGUS cohort, relating to evaluation of patients with monoclonal gammopathies. I show that low level albuminuria is relatively common, heavy albuminuria in this group of patients was however uncommon but did identify a patient with undiagnosed AL amyloidosis and these findings will help to inform guideline revision.

The broad themes explored in this thesis relate to diagnostics in myeloma and MGRS. Recent work continues to show delays within the diagnostic pathway for myeloma [455] and in this thesis, I have explored an approach to improving diagnostics. I have looked at urine as a biomarker for diagnosis of MGRS and how screening an MGUS cohort can identify patients with MGRS lesions. I have described renal outcomes for MM patients at 1 year post diagnosis and the impact of kidney disease. My work has contributed to the current literature and will provide important data for clinicians and people living with MM and MGRS.

The main findings from this thesis are summarized in the section below.

10.2 Summary of the key findings

10.2.1 Targeted communication in patients with AKI and de novo myeloma has the potential to improve the diagnostic pathway.

Chapter III reported on the timeline to investigations and disease specific treatment in patients presenting with AKI secondary to new myeloma and noted that delays occurred throughout the pathway with the main delay relating to referral to hematology. As a result, a simple yet effective intervention of targeted communication was developed to improve the pathway. According to a recent report 50% of myeloma patients have a delayed diagnosis and a quarter of patients wait more than a year to get the right diagnosis [455]. Patients diagnosed via non-hematology secondary care specialties have a longer diagnostic interval compared to other referral pathways with marked intra hospital delays [456]. Work from Chapter III provides unique data specifically relating to timeline of investigations in secondary care and adds to the body of work striving to improve the diagnostic pathway in myeloma [457, 458].

These findings have important implications and have led to a change in clinical practice at UHB and can be modelled by other trusts to improve their pathway.

10.2.2 12-month renal outcome in NDMM patients is favorable.

A secondary analysis of the Myeloma XI trial showed that renal prognosis for myeloma patients (presenting with eGFR> 15ml/min) at 12 months is favorable and only a few have significant decline in renal function. Recovery of renal function is more likely for patients who are younger <70 years old, male, have a lower mean GFR, higher baseline dFLC levels and deep clonal response; suggesting that most of this patient group had reversible cast nephropathy with a treatment response. Factors associated with decline in eGFR at 12 months were a dFLC response <50% and female sex.

A large French study of NDMM patients found that; age >73 years, RI, comorbidities, and non-use of drugs or ASCT were associated with excess mortality risk. Factors associated with renal response were hematologic response and previous CKD, and survival was associated with renal recovery and hematologic response [459]. These findings tie in with results from previous literature and the Myeloma XI cohort.

RI is a negative prognostic marker in myeloma and patients who recover from dialysis dependent AKI have improved long-term outcomes [142], however there is limited data relating to renal outcomes, for patients with mild to moderate RI at presentation.

The results form Myeloma XI study are significant as they are indicative of factors associated with renal outcome at 12 months in myeloma patients not requiring dialysis. The results are reassuring for both the clinicians and patients, as in the current era of novel therapies majority of patients do not experience change in their renal function at 12 months. Further studies with longer follow up time are needed to determine long term renal and overall outcomes.

10.2.3 Stem cell transplantation, hematological response and bortezomib based chemotherapy did not correlate with 12-month renal outcome.

The work presented in chapter V showed that ASCT, MIg response, or bortezomib based chemotherapy did not correlate with renal outcome at 12 months in NDMM patients from the Myeloma XI trial. These results are encouraging and show that hematological response is not affected by RI and ASCT is safe in patients with RI, consistent with previous literature.

10.2.4 Survival is longer for patients with eGFR ≥30 ml/min at baseline and 12 months.

Consistent with previous studies OS in Myeloma XI trial was significantly longer among those with an eGFR ≥30 ml/min compared to those with eGFR <30 ml/min ($P<0.001$). No advantage in OS was found for patients with improvement in renal function at 12 months and data on myeloma patients with relapse may help to extrapolate this further.

10.2.5 Regression of albuminuria at 12 months in the NDMM patients

A study by Leung et al and colleagues showed that patients with cast nephropathy have lower albumin excretion compared to other renal pathologies seen in myeloma and this may be helpful in distinguishing the various lesions in myeloma [460]. Outside myeloma albuminuria is associated with adverse outcomes but whether this holds true in myeloma is not known. Work from Chapter VI has evaluated albuminuria in NDMM patients. At 12 months most patients showed improvement in albuminuria. There was no relationship between improvement in albuminuria and improvement in eGFR at 12 months. In addition, there was no association between albuminuria at diagnosis and eGFR at 12 months. In this study, only a small number of patients had very heavy albuminuria (ACR >70 mg/mmol), and therefore may not have had sufficient patients to adequately assess the relationship between very heavy albuminuria and kidney function at 12 months.

10.2.6 Clone specific treatment improves outcome in MGRS associated MPGN.

Chapter VII reports on MGRS-associated MPGN that responded to treatment of the underlying clone with chemotherapy, resulting in improvement in renal function. As there is no standard therapy for patients with MGRS-associated MPGN and whether hematologic response improves outcomes, this work adds to the literature reporting on treatment and successful outcome in this rare condition.

10.2.7 Post-transplant MGRS.

Chapter VIII reports on a single centre experience of post-transplant MGRS and highlights important aspects for consideration i.e., 1) Screening and re screening for a MIg may be required if MGRS is suspected, as the MIg in serum and/or urine may not be easily detectable 2) Serial kidney biopsies may be required if the renal lesion is subtle 3) Challenges and complexity of treatment decisions relating to clone specific chemotherapy in elderly transplant patients on immunosuppression with comorbidities.

This work is important and contributes to the understanding and management of MGRS in transplant patients.

10.2.8 Screening for albuminuria in MGUS.

Chapter IX reports on screening for albuminuria in the MGUS cohort and has characterized this cohort in depth. By defining the prevalence of albuminuria, the project identified 1 new patient with AL amyloidosis and picked up 20% patients that may benefit from renal monitoring and/or a biopsy. A Spanish study looking at 360 patients with MGUS, reported that urine dipstick was performed in only 42% and of this dipstick albuminuria was found in 29% patients [461]. These studies support need for standardized systemic search for kidney damage in all MGUS patients. This work has led to a change in practice at UHB and all MGUS patients are screened with urine ACR at clinic follow up.

10.3 Future research

Chapter III highlights how simple measures can lead to improved timelines to diagnosis. Future work is required to determine, whether on a larger scale, faster diagnosis, and time to initial treatment results in improved renal outcomes. There remain significant issues with access to rapid diagnostic tests, including sFLC assay across the NHS. Research and collaboration are required nationally to highlight these differences, level up care delivered to patients and to highlight best practice. The Myeloma UK Early Diagnosis Programme is a national collaborative effort that aims to identify barriers preventing early diagnosis of myeloma and is working to develop future projects to tackle some of these issues. I would propose a best practice timeline and minimum standard of laboratory testing to benchmark against and determine whether units with slower turnaround times for testing have a greater proportion of patients who do not recover kidney function in those presenting with AKI stage 3.

Secondary analysis of Myeloma XI trial provides insight into 12-month renal outcome in the context of novel therapies, however longer i.e., 5-year and 10-year outcomes need to be determined. Also, outcomes for patients with more advanced RI on presentation including those on dialysis need to be defined.

In this dataset only a small proportion of patients met the IMWG criteria for renal response and as patients with severe RI and those on dialysis were excluded from the trial, conclusions regarding the proportion of patients with partial renal response could not be made. There is a need to redefine renal response criteria in patients with MM and future studies looking at relationship between renal function and long-term outcomes may contribute to this. Patients who had a decline in eGFR lost significant kidney function 26 ml/min/1.73m^2 in 12 months and it did not appear to be related to myeloma. This group warrants more study in the future. Comorbidity data in future myeloma studies may help to extrapolate factors associated with decline in eGFR and the relationship between albuminuria and long-term outcomes.

Urine ACR is a useful tool, however, is seldom performed in patients with myeloma and MGUS outside a renal setting. The role of albuminuria as a predictor of renal outcomes in myeloma patients' needs to be determined in larger prospective studies. A single centre prospective observational cohort study characterizing 1000 MGUS patients over a 10 year is planned at the UHB. This study will aim to define the phenotype of MGUS, collect albuminuria data, investigate the association of MGUS with quality of life and other associated comorbidities and determine long term hematological, renal and survival outcomes. Patients identified to have significant albuminuria will be seen in the renal monoclonal clinic at the Queen Elizabeth Hospital, Birmingham.

Following work reported in chapter IX a change to the MGUS pathway has been implemented and all MGUS patients at every clinic visit will have a urine dipstick screen for albuminuria and future study is planned to determine if longitudinal follow up with serial ACRs in MGUS patients leads to identification of undiagnosed MGRS and if this is cost effective intervention.

MGRS is a rare entity, and collaborative efforts are required to standardize clinical management for the different MGRS associated lesions, report on effect of hematologic response on outcomes, whether treatment of MGRS improves kidney outcomes and survival. MGRS is now registered as a rare disease group in National Registry of Rare Kidney Diseases (RaDaR) and multi centre recruitment of patients affected by MGRS is being prioritised.

Further, international, and interdisciplinary collaborations will enable planning high-quality research studies in patients with monoclonal gammopathy and kidney disease and improve outcomes for patients.

10.4 Final Conclusions

The work presented in this thesis provides novel insight into the diagnostic pathway of myeloma. I have evaluated renal outcomes with current therapies and examined albuminuria in patients with myeloma and MGUS. I have explored characteristics and outcomes for MGRS and post-transplant MGRS and contributed to the literature which will help in the management of patients clinically and academically. The findings from this thesis set the stage for future research projects.

Appendix 1.

SOP: Urine Albumin Measurement Using Alinity Microalbumin Reagent Kit

Purpose: The Alinity c Microalbumin assay is used for quantitative measurement of albumin in human urine on the Alinity c analyzer. Selected patients from the Myeloma XI study had their urine albumin tested at baseline and 12 months. The concentration of protein in a spot urine sample provides an estimate of the protein excretion rate but is affected by patient hydration. The ratio of protein or albumin to creatinine in a spot urine sample corrects for variations in hydration and avoids the sources of error associated with 24 hour and timed urine collection.

Principles of the procedure: The Microalbumin assay is a turbidimetric immunoassay that uses polyclonal antibodies against human albumin. When a specimen is mixed with the reagents, albumin in the specimen combines with the anti-human albumin antibody (goat) in the reagent to yield an insoluble aggregate that causes increased turbidity in the solution. The degree of turbidity is proportional to the concentration of albumin in the specimen and can be measured optically. For additional information on instrument procedure, specimen collection and preparation for analysis, procedure refer to the Alinity ci-series Operations Manual.

Caution: The Microalbumin assay uses polyclonal antibodies against human albumin that can react with closely related proteins such as bovine albumin Therefore, calibrators containing non- human protein should not be used with this assay. Therefore, control material containing non-human protein should not be used with this assay.

Expected values:

Spot collection[a] (Microalbumin: Creatinine ratio)		
	mg/g	mg/mmol
Normal	<30	< 2.5 (male) < 3.5 (female)
Microalbuminuria	30 to 299	2.5 to 29 (male) 3.5 to 29 (female)
Macro/clinical albuminuria	≥ 300	> 30 (male) > 30 (female)

a: Spot collection = microalbumin (mg/L) ÷ creatinine urine (mmol/L)

Appendix 2.

SOP: Creatinine Measurement Using Alinity Creatinine (Enzymatic) Reagent Kit

Purpose: The Alinity c Creatinine (Enzymatic) assay is used for the quantitative determination of creatinine in human serum, plasma, and urine on the Alinity c analyzer. Selected patients from the Myeloma XI study had their urine creatinine measured at baseline and 12 months.

Principles of the procedure: Creatinine in the sample is hydrolyzed by creatininase to creatine. Creatine is in turn hydrolyzed by creatinase to sarcosine and urea. Sarcosine from this reaction is oxidized by sarcosine oxidase to glycine and formaldehyde, with the concomitant production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulfopropyl-m-toluidine in the presence of peroxidase to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to the creatinine concentration in the sample. This enzymatic method is sensitive and specific for creatinine and is not affected by endogenous substances, such as ketoacids, cephalosporins, and bilirubin that interfere with the Jaffe method. The measuring interval of the Alinity c Creatinine (Enzymatic) assay is 2.50 to 400.00 mg/dL (0.22 to 35.36 mmol/L). For additional information, refer to the Alinity ci-series Operations Manual, Section

Expected values:

	Urine concentration range [a]
Adult male	5.1 to 14.2 mmol/L
Adult female	3.9 to 9.4 mmol/L

[a] Concentration is based on a daily urine output of 1.5 L.

Appendix 3.

MGUS Patient Information Sheet





Dear Patient

As a part of your MGUS clinic follow up, we are requesting you to provide a urine sample with your next set of routine blood tests. This will help us to pick up and action, any early kidney condition that may be associated with a protein in your blood, called paraprotein. We have provided you with a white top universal container and a labelled bag for the urine sample. Please remember to drop this sample, at the **Hematology centre** where you will be having a blood test. Please see the attached leaflet for further information regarding collection of urine sample.

We also request you to fill a questionnaire related to your underlying medical condition. Your decision to participate is voluntary and deciding not to participate will not affect the standard of care in any way.

Please bring the completed questionnaire and urine sample to the Hematology centre and hand it over to the nurses at the time of your blood test. Alternatively, if you have your blood tests via outreach nurses, please hand the above to the outreach nurse.

Thank you for your help.

The MGUS Clinic
Queen Elizabeth Hospital Birmingham, B15 2GW

Appendix 4.

MGUS Patient Questionnaire

The answers to the following questions will be used for research purposes only and will be confidential. They will enable us to understand your medical condition and guide future care for patients with a similar condition. Please fill out as much of this questionnaire as possible. If you cannot answer some of the questions or feel uncomfortable answering them, leave them blank. Please circle to indicate your response.

MEDICAL HISTORY

1. Have you ever had an infection? Yes/No
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago
Where was the infection? Chest Urine Other, please specify
Did you require hospital admission for this? Yes/No
Was this managed with oral antibiotics only? Yes/No
How many infections have you had in the last 3 years? One, two or more than two

2. Have you had your COVID vaccine? Yes/No
First dose Yes/No
Second dose Yes/No

3. Have you had a flu vaccine? Yes/No
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago

4. Have you ever had a pneumococcal vaccine? Yes/No
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago

5. Have you ever been diagnosed with a blood clot? Yes/No
Number of times you have had a clot: once or more than once

6. Have you ever been diagnosed with cancer? Yes/No
If yes please specify, type of cancer:
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago

7. Have you ever been diagnosed with a kidney condition? Yes/No
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago
Did you have a kidney biopsy? Yes/No

8. Have you ever been diagnosed with the following conditions?
Gout Yes/No
Diabetes Yes/No
Stroke Yes/No
Heart failure Yes/No
Angina or Heart attack Yes/No
High Blood Pressure Yes/No
Nerve problems Yes/No
Osteoporosis Yes/No
Rheumatoid Arthritis Yes/No
Sjogren's syndrome Yes/No
Any auto immune disorders Yes/No
If yes, please specify

9. Have you ever been on immunosuppressant drugs? Yes/No
If yes, current, or past
Please specify what immunosuppressants have you taken

10. Have you ever been admitted to a hospital? Yes/No
Was this in the last 1-12 months or between 13-24 months or more than 24 months ago
How many times have you been admitted to a hospital in the last 12 months? Once, twice, or more than twice

FAMILY HISTORY
1. Are there any blood disorders/cancer below that run in the family? Yes/No
If yes, please specify

Appendix 5.
Questionnaire responses in the 164 MGUS patients

Questionnaire	Response
Have you ever had an infection?	Yes 119/164 (72%) No 45/164 (27%)
<u>If yes</u>	
Last 1-12 months	46/119 (39%)
13-24 months	16/119 (13%)
More than 24 months ago	55/119 (46%)
No response	2/119 (2%)
Where was the infection?	
Chest	69/119 (58%)
Urine	23/119 (19%)
Other	23/119 (19%)
No response	2/119 (2%)
Did you require hospital admission?	
Yes	25/119 (21%)
No	90/119 (76%)
No response	4/119 (3%)
Was this managed with oral antibiotics only?	
Yes	95/119 (80%)
No	17/119 (14%)
No response	7/119 (6%)
How many infections have you had in the last 3 years?	
One	36/119 (30%)
Two	22/119 (19%)
More than 2	34/119 (29%)
No response	27/119 (23%)
Have you had your COVID vaccine?	
Yes	154/164 (94%)
No	8/164 (5%)
Missing response	2/164 (1%)
Have you had your flu vaccine?	
Yes	142/164 (87%)
No	20/164 (12%)
No response	2/164(1%)
<u>If yes</u>	
Last 12 months	123/142 (87%)
13-24 months or	11/142 (8%)
More than 24 months ago	4/142 (3%)
Missing response	4/142 (3%)

Questionnaire responses in the 164 MGUS patients (continued)

Have you ever had a pneumococcal vaccine?	
Yes	69/164 (42%)
No	79/164 (49%)
No response	16/164 (10%)
<u>If yes</u>	
Last 12 months	13/69 (19%)
13-24 months or	9/69 (13%)
More than 24 months ago	43/69 (62%)
Missing response	4/69 (6%)
Have you ever been diagnosed with blood clots	
Yes	14/164 (9%)
No	145/164 (88%)
No response	5/164 (3%)
Number of times you had a clot	
Once	9/14 (64%)
More than once	5/14 (36%)
Have you ever been diagnosed with cancer?	
Yes	38/164 (23%)
No	120/164 (73%)
Missing response	6/164 (4%)
<u>If yes</u>	
Last 12 months	4/38 (11%)
13-24 months or	4/38 (11%)
More than 24 months ago	29/38 (76%)
Missing response	1/38 (3%)
Have you ever been diagnosed with a kidney condition?	
Yes	29/164 (18%)
No	126/164 (77%)
No response	9/164 (6%)
<u>If yes</u>	
Last 12 months	6/29 (21%)
13-24 months or	4/29 (14%)
More than 24 months ago	17/29 (59%)
Missing response	2/29 (7%)
Did you have a kidney biopsy?	
Yes	5/29 (17%)

Questionnaire responses in the 164 MGUS patients (continued)

Have you ever been diagnosed with the following conditions?	
Gout	18/164 (11%)
Diabetes	36/164 (22%)
Stroke	8/164 (5%)
Heart failure	3/164 (2%)
Angina or heart attack	15/164 (9%)
High BP	78/164 (48%)
Nerve problems	28/164 (17%)
Osteoporosis	22/164 (13%)
RA	20/164 (12%)
Sjogrens	4/164 (2%)
Any AI	26/164 (16%)
Have you ever been on IS drugs?	
Yes	20/164 (12%)
Current IS	14/20 (70%)
Past IS	6/20 (30%)
Have you ever been admitted to a hospital?	
Yes	122/164 (74%)
No	35/164 (21%)
Missing response	7/164 (4%)
<u>If yes</u>	
Last 12 months	25/122 (20%)
13-24 months or	8/122 (7%)
More than 24 months ago	84/122 (69%)
Missing response	5/122 (4%)
How many times have you been admitted to a hospital in the last 12 months?	
Once	18/122 (15%)
Twice	8/122 (7%)
More than twice	3/122 (2%)
No response	93/122 (76%)
Are there any blood disorders/cancers that run-in family?	
Yes	53/164 (32%)
No	96/164 (59%)
Missing response	15/164 (9%)

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