DEVELOPMENT OF A PREDICTIVE MODEL OF THE RATE OF DISEASE PROGRESSION TO END-STAGE RENAL DISEASE IN PATIENTS WITH IDIOPATHIC IgA NEPHROPATHY

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science.
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ABSTRACT

The Development of a Predictive Model of the Rate of Disease Progression to End-Stage Renal Disease in Patients with Idiopathic IgA Nephropathy. Master of Science, 2000.
Lukasz Piotr Bartosik. Institute of Medical Science. University of Toronto.

PURPOSE To improve the ability of determining outcome in IgAN patients near presentation.

METHODS 298 IgAN patients were selected from the Toronto Glomerulonephritis Registry. Rate of decline in renal function was our outcome variable. Age, gender, creatinine clearance, blood pressure, proteinuria and the Lee, Haas and Lajoie histological classifications were parameters tested using univariate and multivariate linear regression.

RESULTS At presentation mean age was 36 years and the gender distribution was 3:2. Mean length of observation was 70 months. In total, 23% reached ESRD. The 5-year renal survival was 80%. The mean rate of renal decline was 0.4 ml/min/month. Baseline proteinuria, mean arterial pressure during follow-up, proteinuria during follow-up and degree of chronic tubulo-interstitial damage were negatively associated with outcome. Only mean arterial pressure and proteinuria during follow-up were independently associated with outcome.

CONCLUSION We did not find any variables that would independently predict the rate of disease progression at presentation.
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<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>ACEi</td>
<td>Angiotensin Converting Enzyme Inhibitor</td>
</tr>
<tr>
<td>BP</td>
<td>Blood Pressure</td>
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<tr>
<td>C3</td>
<td>Complement Number 3</td>
</tr>
<tr>
<td>C4</td>
<td>Complement Number 4</td>
</tr>
<tr>
<td>C5a</td>
<td>Complement Number 5a</td>
</tr>
<tr>
<td>C5b-9</td>
<td>Membrane Attack Complex</td>
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<tr>
<td>CFR</td>
<td>Chronic Renal Failure</td>
</tr>
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<td>Cl</td>
<td>Chlorine</td>
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<td>C-G</td>
<td>Cockcroft &amp; Gault</td>
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<td>CRCL</td>
<td>Creatinine Clearance</td>
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<td>EM</td>
<td>Electron Microscope</td>
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<td>ESRD</td>
<td>End Stage Renal Disease</td>
</tr>
<tr>
<td>ET-1</td>
<td>Endothelin Number 1</td>
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<tr>
<td>F</td>
<td>F value in statistical analysis</td>
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<tr>
<td>GFR</td>
<td>Glomerular Filtration Rate</td>
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<td>GFR_SLOPE</td>
<td>Slope of glomerular filtration rate over time, measure as creatinine clearance over time</td>
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<td>gm</td>
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gm/day - Grams per 24 hours
GN - Glomerulonephritis
Hg - Mercury
HLA - Human Major Histocompatibility Gene Complex
HP - Hydrostatic Pressure
IgA - Immuno-globulin A
IgAN - Immuno-globulin A Nephropathy
IgG - Immuno-globulin G
IgM - Immuno-globulin M
IL-1 - Interleukin Number 1
IL-6 - Interleukin Number 6
K - Potassium
kg - Kilograms
L - Liter
LDL - Low Density Lipoproteins
MAC - Membrane Attack Complex
MAP - Mean Arterial Pressure
MAP_{BASELINE} - Mean Arterial Pressure averaged over the first six months of observation
MAP_{FOLLOW-UP} - Mean Arterial Pressure averaged over the entire length of observation beyond the first six months
MCP-1 - Monocyte Chemo-attractant Protein Number 1
min. - Minute
INTRODUCTION

First described by Berger and Hinglais in 1968 (1), IgA nephropathy (IgAN) is an immune-complex mediated glomerulonephritis, characterized morphologically by IgA deposition in the glomerular mesangium. Initially thought to be a benign condition, it is now recognized that a substantial percentage of patients with IgAN will develop chronic renal failure (CRF). IgAN is now also recognized as the most common primary glomerulonephritis in the world (2), accounting for between 4-44% of patients undergoing renal biopsy (3).

Despite the severity and prevalence of this disease there are still few clinical or pathological features that are known to predict its exact progress. Some patients may retain normal renal function for more than 30 years, while others may develop renal failure soon after diagnosis. Clinically one of the most significant problems in treating this disease is the inability to identify at an early stage of their disease patients at risk of rapid progression i.e. those patients that will quickly reach end-stage renal disease (ESRD).

Indeed, there have been numerous studies that have attempted to clarify the factors that influence outcome (4,5,6,7,8,9), and although many factors have been implicated the results have not been widely accepted due to differences between the studies. In the present study we attempted to address the problems encountered in previous work and improve our ability to predict the risk of progression as an outcome variable through a more
rigorous analysis of the demographic, clinical, laboratory and pathological factors involved in this disease process.

**Background**

In order to understand the clinical, laboratory and pathological risk factors in IgAN we first need to understand the normal anatomy and physiology of the glomerulus then the effects of the disease on this organ. We also reviewed the results from previous studies on predictive modeling for this disease.

**STRUCTURE AND FUNCTION OF THE GLOMERULUS**

Grasping the structure and function of the glomerulus is paramount to understanding the disease process in IgAN. The following section briefly outlines renal blood flow, followed by a description of the anatomy and function of the glomerulus under normal physiological conditions.

**Glomerular Blood Flow**

Blood comes to the kidneys directly from the abdominal aorta through the renal arteries. Once inside the kidney the renal artery branches several times until the blood supply reaches the cortex and flows via the afferent arteriole to the site of filtration i.e. the glomerular capillary bed. The blood then flows out of the glomerulus via the efferent
arteriole, which eventually branches into a second capillary bed, made up of the peritubular capillaries.

*The Glomerulus*

The glomerulus consists of three structural elements: the glomerular capsule, the capillary tuft, and the mesangium. These structural elements segregate two spaces: the urinary space and the capillary lumina [figure 1].

The glomerular capsule is always located in the cortex of the kidney. Its inner and outer walls form the urinary space. The outer wall called the *parietal layer* is composed of simple squamous epithelial cells that have a basement membrane [figure 1]. The inner *visceral layer* is composed of specialized epithelial cells called podocytes, which surround the glomerular capillaries. The podocytes have small cell bodies, which give rise to long trabeculae. These trabeculae in turn branch to form many smaller processes called foot processes or pedicels [figure 2]. The point at which the parietal and visceral layer is continuous is called the vascular pole.

The glomerular capillary network arises by subdivision of the afferent arteriole, which enters the glomerular capsule at the vascular pole. The capillaries reunite to form the efferent arteriole, which then leaves the glomerular capsule at the vascular pole. The capillary wall consists of a thin endothelial cell membrane. Which is studded with tiny pores called fenestrations [figure 2].
The mesangium is a branching stalk of specialized connective tissue that holds together and supports the capillary tuft. It arises at the glomerular hilus, and follows the branching capillaries. The mesangium consists of mesangial cells as well as intercellular mesangial matrix. The mesangial matrix forms a branching meshwork surrounding the mesangial cells and separating them incompletely from the endothelial cells.

**Glomerular Function**

The basic function of the glomerulus is to act as a permselective barrier and ultrafilter for the movement of macromolecules and water respectively, from the capillary lumen to the urinary space via the glomerular filtration barrier.

The glomerular filtration barrier is made up of three layers. The first layer is composed of thin fenestrated endothelial cells that lay on top of the second layer called the glomerular capillary basement membrane. This second layer is composed of fibrous proteins such as Type IV collagen, heparan sulfate, proteoglycans and laminin. Finally the third layer is made up of podocyte filtration slits that are defined as the spaces between adjacent pedicals of podocyte cells [figure 2].

The movement of macromolecules is dictated by the charge and size selective properties of the glomerular filtration barrier. As opposed to smaller macromolecules such as creatinine large macromolecules such as albumin are unable to pass across the filtration
barrier based on size alone. In addition macromolecules are selected based on charge. Negatively charged macromolecules are repelled by the negative charge on the fibrous proteins that composes the glomerular capillary basement membrane while allowing positively charged macromolecules of the same size through.

As an ultrafilter the movement of water across the glomerular filtration barrier is controlled by hydrostatic (P) and oncotic pressures (Ω), collectively known as starling forces and by the physical characteristics of the filtration barrier, known as the ultrafiltration coefficient (Kf). The hydrostatic pressure throughout the glomerular capillary (Poc) of 45 mmHg is opposed by the hydrostatic pressure in the urinary space (Pu) of 10 mmHg. The resulting net transcapillary hydrostatic pressure (ΔP) is thus 35 mmHg in favor of water movement into the urinary space. The oncotic pressure at the afferent end (Ωgc) of the glomerular capillary is 20 mmHg and continuously increases along the capillary with the increasing concentration of plasma proteins as more water crosses into the urinary space. Since there are no proteins that escape into the urinary space the oncotic pressure on that side of the glomerular filtration barrier is said to be zero. Thus the net transcapillary oncotic pressure (ΔΩ) is 20 mmHg at the afferent end which favors water movement into the glomerular capillary [figure 3].

Therefore the net ultrafiltration pressure (Puf) across the glomerular filtration barrier is then [ΔP - ΔΩ] which at the afferent end is equal to 15 mmHg in favor of water movement into the urinary space. Under physiological conditions the glomerulus is said
to be in filtration dysequilibrium. That is to say that $\Delta \Pi < \Delta P$ and thus water will always be favored to move from the glomerular capillary into the urinary space [figure 3].

The ultrafiltration coefficient $K_f$ is dependent on two factors, the effective hydraulic permeability of the glomerular capillary wall ($k$) and the total surface area available for filtration ($S$).

Finally, the rate of glomerular ultrafiltration for a single nephron (SNGFR) may be expressed by:

$$SNGFR = K_f \times P_{UF}$$

In humans the quantity of glomerular filtrate formed each minute in all nephrons of both kidneys averages approximately 125 ml/min. Since the normal plasma flow through both kidneys is 650 ml/min and the normal glomerular filtration rate in both kidneys is 125 ml/min, the average filtration fraction is approximately one fifth or 19 per cent.

Once inside the urinary space the water and dissolved solutes are now called the ultrafiltrate. This ultrafiltrate continues through the proximal tubule, Henle’s loop of the nephron, distal tubule, and collecting ducts where both the fluid and electrolyte content is modified prior to the final excretory product i.e. the urine. This process results in the re-absorption of 98% of the water, the adjustment of the electrolytes (Na, K, Cl), and acid base balance of the body by re-absorption and/or secretion mechanisms. Finally, the
collecting ducts merge in the medulla of the kidney and carry the urine to the renal pelvis then via the ureter to the bladder prior to the final excretory pathway the urethra.

PATHOGENESIS OF IgAN

IgA nephropathy is a renal disease that involves primarily the glomerulus. Other renal structures such as tubules and the interstitium surrounding them may also be damaged in IgAN following the initial effects to the glomerulus. The pathogenesis of IgAN discussed below will be separated into two events: the initial damage to the structure and function of the glomerulus and the ensuing chronic renal injury.

Initial Glomerular Damage

Many aspects of the pathogenesis of IgA nephropathy have not been completely elucidated, and the exact sequence of events leading to the initial glomerular injury in IgA nephropathy has not been worked out. Numerous studies demonstrate it to be an immune complex mediated disease, where IgA is deposited in the glomerular mesangium. Evidence for this includes the identification of granular deposits of IgA and C3 by immunofluorescence and the confirmation of dense deposits in the same area by electron microscopy (EM). As well, the ability to induce in laboratory animals glomerular IgA deposits with associated histological lesions similar to those in human IgA nephropathy by both passive and active immunization has been demonstrated (10,11). The
detection of circulating IgA immune complexes in some patients also supports an immune complex mediated pathogenesis.

There are also reports of familial groupings of this disease suggesting a potential genetic predisposition \(^{(12)}\). It has been shown that some family members of IgAN patients have immunological abnormalities that include raised serum IgA levels, increased IgA production by stimulated peripheral blood mononuclear cells (PBMC), and an increased number of IgA bearing B lymphocytes \(^{(13,14,15,16)}\). Lastly, Human Major Histocompatibility Gene Complex (HLA) antigen associations with IgAN have been extensively studied although the results have been inconsistent \(^{(17,18,19,20,21)}\).

The initial event leading to glomerular damage may be dependent on the mucosal defense mechanism whereby repeated or persistent antigenic stimulation may result in the overproduction and/or accumulation of IgA in serum. This may be in part due to abnormalities in the immune regulation of this antibody, causing an overproduction of IgA \(^{(22)}\), or from impairment in the immune complex clearance by the hepatic and splenic mononuclear phagocytic system \(^{(23,24)}\). The development of immune complexes may occur either in the circulation, or in situ with circulating IgA antibodies binding directly to their corresponding antigens in the glomerulus. These glomerular antigens may be extrinsic ones that become trapped in the glomerulus, or they may be intrinsic to the glomerular structure. Possible antigen candidates may be divided into three categories: dietary antigens, antigens originating from microorganisms, and autoantigens.
implicated as the cause of human IgAN.

For simplicity, the initial damage to the structure and function of the glomerulus can be separated into three stages, activation of immunological mediators, morphological alteration of the mesangium, and glomerular structural damage (figure 4).

Stage one involves the activation of the complement cascade and the recruitment of leukocytes. It is believed that immune complex localization in the mesangium results in the activation of the complement cascade (40), including C5b-9 membrane attack complex (MAC) and C5a. Direct evidence for complement cascade activation originates from the identification of certain components at the site of immune-complex deposition by immunostaining. Indirect evidence stems from the protective effects of complement depletion experiments in animal models of glomerulonephritis (41). Many components of the complement cascade are potent stimuli for leukocyte chemotaxis (42). Alternatively, immunoglobulin has the potential to recruit leukocytes via complement independent mechanisms such as direct interaction with leukocyte Fc receptors. In vitro infiltrating immune cells have been shown to release a wide range of substances including the cytokine interleukin-1 IL-10, IL-6 (43), reactive oxygen species (ROS), tumor necrosis factor-α (TNF-α) (44), procoagulants, complement factors (45), and TGFβ to name a few.

Stage two involves changes in mesangial cell morphology. During complement activation and in the presence of macrophages it has been demonstrated that morphological changes
occur to \textit{in vitro} glomerular mesangial cells. Certain products such as IL-1 and TGFβ are known as important stimuli for the synthesis of extracellular matrix by glomerular cells. Besides extracellular matrix deposition mesangial cells also undergo cellular proliferation, and can release growth factor such as platelet-derived growth factor PDGF B-chain \textsuperscript{(46)} as well as PDGF β-receptor. It has been shown that proliferation of mesangial cells can be significantly reduced in vitro by blocking PDGF suggesting an autocrine mechanism for the maintenance of mesangial cell proliferation \textsuperscript{(47)}.

The third stage is characterized by structural alterations in the glomerulus. These structural alterations include cellular proliferation of mesangial as well as endocapillary cells, podocyte foot effacement and also sclerosis of the capillary loops. Such modifications result in the reduction of glomerular flow and the loss of selective permeability of the glomerular filtration barrier at the site of immune complex deposition which enables the passage of macromolecules i.e. plasma proteins.

\textit{Ensuing Chronic Renal Injury}

Following the initial glomerular injury, subsequent progressive scarring may be independent of immunological events. The most accepted theory proposed to explain this occurrence of events implies that the initial reduction in nephron number results in progressive injury to the remaining nephrons as a consequence of compensatory increases in glomerular pressure and flow \textsuperscript{(48,49)} . Like the initial glomerular injury this hemodynamically mediated renal injury may also be symbolized by a three stage diagrammatic representation (figure 5).
Stage one involves a unique feature of the kidney, its ability to maintain an almost constant GFR, despite the presence of severe glomerular structural damage. This compensation is hypothesized as a result of a hemodynamic adaptation, i.e. the initial decrease in glomerular mass is compensated by hyperperfusion in the remaining intact glomeruli. Experimentation in rats has shown that there is an increase in size and glomerular filtration rate in the remaining functional nephron units following uninephrectomy, renal ablation, or infarction. This is due to an increase in both glomerular transcapillary hydrostatic pressure difference, and glomerular plasma flow rate. It has been proposed however that this hemodynamic adaptation may eventually prove to be injurious to the structural and functional integrity of the remaining healthy glomeruli. Experimentally it has been demonstrated that such intrarenal hypertension and hyperperfusion lead to an increased transcapillary flux of macromolecules such as albumin. As well, there is evidence that during periods of such vasodilation non-sequestered macromolecules are transported into the mesangial area.

Stage two describes damage to the mesangium. In animal models protein deposition in the mesangial region is thought to cause changes to mesangial morphology similar to those described above i.e. cellular proliferation, and increased matrix deposition. Certain forms of low-density lipoproteins (LDL) have been identified as deposits in the mesangial area in several animal models \((50,51)\). In vitro binding of LDL with receptors on human mesangial cells stimulates production of oncogenes, \(c\)-\(fos\) and \(c\)-\(jun\). As well, LDL binding to mesangial cells causes mesangial cell proliferation \((52)\) and promotes the
production of extracellular matrix proteins, platelet derived growth factor (PDGF) and monocyte chemoattractant protein-1 (MCP)-1 \(^{(53)}\). Thus LDL promotes the recruitment of macrophages that may play an important role in propagating glomerulosclerosis \(^{(54)}\) which may ultimately result in further loss of glomeruli. This whole process may re-cycle through a positive feed back mechanism the consequence of which is the continual reduction of renal mass \(^{(55,56)}\).

Stage three deals with the escape of macromolecules into kidney tubules and their re-absorption by proximal tubular epithelial cells \(^{(57,58)}\). Proteinuric renal diseases are often associated with progressive tubulo-interstitial injury. In fact, the degree of tubulo-interstitial injury has been shown to be more-closely associated with outcome than the glomerular morphology \(^{(59)}\). Tubulo-interstitial injury caused by filtered proteins may occur through a variety of mechanisms. These include mechanical obstruction by protein casts \(^{(60)}\). Protein re-absorption by lysosomal processing leading to lysosomal swelling and rupture within the tubular cell cytoplasm. Filtered complement activation on the brush border of proximal tubular cells by the alternative pathway ultimately leading to cytolysis \(^{(61,62)}\). Lastly, the production of toxic radicals by oxidation of filtered lipoproteins that bind to and are taken up by tubular cells. In their oxidized state low density lipoproteins have been implicated in such toxic effects.

It therefore seems that regardless of the type of initial structural damage in glomerular based diseases such as IgAN the progressive deterioration in GFR may occur through a variety of mechanisms.
RESULTS FROM PREVIOUS STUDIES ON PREDICTING OUTCOME IN IgAN

We will now examine the clinical course of IgAN along with the demographic, clinical, laboratory and histological parameters previously used in predictive modeling that were found to have a significant association with renal survival. As well, we will look at some of the methodological problems found in previous studies.

Clinical Course

For patients with IgAN it has been shown that the most common clinical course is that of a slowly progressing chronic disease. The rate of progression however varies greatly. In some patient populations retrospective studies have estimated 10 year renal survival from the time of first renal abnormality at around 90% \(^ {63}\). But generally the 10 and 20 year survival rates have been estimated to be lower at 80% and 50% respectively \(^ {4,5,64}\).

Demographic, Clinical, Laboratory and Histological Parameters

Previously identified risk factors significantly related to renal survival can be divided into those found at presentation, at biopsy, or those that developed during the course of the disease.
At presentation the following clinical syndromes have been associated with a poor prognosis: microscopic hematuria \(^{(9)}\), macroscopic hematuria \(^{(6)}\), and nephrotic syndrome \(^{(8)}\). Other parameters at presentation that have been associated with renal survival include impaired renal function \(^{(3,4,7,8,65,66)}\), heavy proteinuria \(^{(4,5,6,7,8,65,66)}\), hypertension \(^{(4,8,65,66)}\), increased age \(^{(4,9)}\), male gender \(^{(6)}\), red blood cell casts \(^{(4)}\).

Clinical and laboratory parameters that either developed or change during the course of the disease process, that have also been associated with decreased renal survival include; progressive renal impairment \(^{(4)}\) \(^{(5,7)}\), as measured by changes in serum creatinine and/or creatinine clearance, hypertension \(^{(4,8,9)}\), proteinuria \(^{(4,5,7,8,9)}\), serum albumin \(^{(5)}\) and changes in serum levels of C3, and IgA \(^{(4,8)}\).

Certain features found on histological examination of renal biopsy tissue from patients with IgA nephropathy have also been associated with a poor prognosis. These include diffuse and severe mesangial proliferation \(^{(7,8,9)}\), extracapillary proliferation \(^{(8,9,63)}\), segmental and focal glomerular sclerosis \(^{(4,5,7,8,9,66,36)}\), and interstitial fibrosis \(^{(4,5,7,8,9)}\). In view of the frequency of the above features several morphological classification systems have been assembled in an attempt to grade the severity of histological damage. The most common IgAN morphological classification used by nephropathologists is an adaptation of Meadow et al.'s grading system \(^{(67)}\) by S.M.K. Lee et al. \(^{(68)}\)

Among some of the clinical parameters previously investigated at the time of diagnosis but not found to significantly affect outcome include presence of preceding respiratory
infection (4,5,6), and family history of renal disease(4). Laboratory parameters at first presentation or which developed during the course of the disease, that did not affect outcome include the number of white blood cells (WBC) (5), and presence of granular casts (5) on urinalysis. Furthermore, immunological markers such as IgG, IgM, and C4 (4) have been investigated previously and not found to be associated with renal survival.

Methodological Problems

Several methodological problems found in previous studies have prevented clinicians from reaching any agreement on the value of the individual factors. Among the issues that have contributed to this uncertainty have been the lack of sample size, and/or the short and/or incomplete clinical follow-up information. No prognostic model over the past ten years has exceeded 300 patients, with the average of 193 and a range between 121-282 (4,5,7,8,66,36,69). The largest sample population ever studied, according to the literature, includes 365 patients (9). In terms of observation these series have had a mean follow-up of 69 months with a range of 59-94 months.

Among the most comprehensive prognostication studies are those by Ibles et al (4) and Beukhof et al (64). Both studies investigated a very large number of clinical, laboratory, and histologic parameters. However, despite the fact that both studies had good long-term observational data their study populations were quite small. This resulted in algorithms that included only a few parameters that were statistically associated with poor outcome.
The lack of consistent methodology between previous studies is also a major problem. Among some of the major differences between previous studies examining factors associated with renal survival in IgAN are the variety of parameters examined and how these parameters were defined for statistical analysis. As an example, some studies examined only a few specific parameters such as the effects of hypertension on renal survival \(^{(70)}\), or the association of certain clinical features to morphological lesions \(^{(69)}\). In other cases the same parameters were used in several different studies however in these studies the parameters were treated in different ways. Heavy proteinuria for instance was defined as >3.5 gm/day \(^{(5)}\) in one study and >2.0 gm/day \(^{(64)}\) in another, both of which were found to be statistically associated with poor renal survival. Due to all of these differences comparison of results among previous work have been difficult.

The application of the histologic scoring system for prognostication has also had limitations. The extent of the association between different morphological grades and outcome has been difficult to clarify from the review of the literature. This may be because the established classification system by S.M.K. Lee et al \(^{(68)}\) considers mainly the glomerular compartment. More recent morphological classifications have been developed. Among these is Haas’ \(^{(71)}\) recent modification of S.M.K. Lee’s system. Other approaches have included a more global picture so that the glomerular, vascular, and tubular compartments are scored separately and a cumulative grade is assigned \(^{(4,8,36,69)}\). However, morphological scoring became cumbersome to use, and the total score gave little information about the exact nature of the histological lesion present. More problematic is the fact that the clinical course among IgA patients with very similar
morphological lesions are highly variable, limiting the effectiveness of the most widely used histological classification systems (7). An example of the limited value of these histologic grading systems is that some recent prognostic constructs have omitted pathology entirely (5).

Statistical methodology, particularly the use of survival analysis, has been one of the most troublesome aspects in previous prognostic modeling. The starting point of the disease process in IgAN is from a practical point usually unidentifiable since it is most commonly identified as microscopic hematuria and can only be detected in the urine when specifically testing for the presence of RBCs. The time of presentation of the clinical manifestations is not uniformly synchronous with pathophysiological damage. Thus, for practical purposes the time of first renal abnormality is impossible to measure. Researchers have used the time of biopsy as a uniform starting point, however, biopsies are usually not performed until clinical manifestations develop, and policies regarding biopsies differ around the world. This has produced a wide variation in overall rates of renal survival. Without the ability to employ an identifiable uniform starting point even the use of a uniform end point such as ESRD and/or death has produced varying renal survival data and prognostic risk factors based on such analyses have been difficult to interpret and apply.

Most importantly however, is that renal survival does not directly reflect the rate of disease progression. Two patients may have the same rate of deterioration in renal function, but if the first patient discovers his/her disease early on when their renal
function is still well preserved while in the second patient the disease is discovered near end-stage renal failure, then these two patients will have very different survival rates. Therefore, any factors that are found to be associated with renal survival between these two patients are in fact associated with disease severity and not the rate of disease progression.

The small sample sizes, short observational periods, lack of consistency between studies, confusion about the value of the histologic classification systems together with limitations in statistical methodology have produced a variety of models that are not readily used. Even agreed upon factors that confer risk such as heavy proteinuria, elevated serum creatinine, and hypertension vary greatly in terms of their individual contributions to renal survival.
The purpose of this project was to improve the ability to predict outcome in the IgAN patient population at an early stage of their disease compared to methods presently available.

Hypothesis

We will construct an algorithm from our IgA patients in the regional GN Registry using demographic, clinical, laboratory, and histologic data within six months of initial presentation that will better predict the rate of progression of IgAN than is currently available in the literature.

Objectives

1. Identify a large population base with biopsy proven IgA nephropathy.
2. Establish a uniform database of clinical and laboratory values on this population.
3. Review and score the histological damage identified on patient’s renal biopsy tissue.
4. Select the histological grading system that is best associated with outcome.
5. Select from the literature parameters to examine as potential risk factors for progression.
6. Use statistical means to test chosen parameters and histological grading system for associations with progression and their relative weight by multivariate analysis, and compare our model to previous work.
METHODS

PATIENT SELECTION

Our data was acquired through the Toronto Glomerulonephritis (GN) Registry. The Toronto GN Registry has been following patients with all histological types of glomerulonephritides including idiopathic IgA nephropathy, from several renal units in and around the greater Metropolitan Toronto area, since the mid seventies. Initial patient identification is based on renal biopsy reports. These are received in the registry from the regional nephropathologists. Demographic information on all patients who have had a renal biopsy is entered and stored on computer database in a Biopsy file. Based on information from this file, 1097 patients with idiopathic IgA nephropathy have been identified.

OBTAINING LONG-TERM OBSERVATIONAL DATA

Based on patients’ charts, the GN Registry staff fills out a standardized form recording semi-quantitative measures of clinical and laboratory parameters at the time of presentation on each patient identified as having IgA nephropathy. This information is then stored on computer database as the Initial file. The GN Registry then fills out standardized forms recording clinical and laboratory values during follow-up for each patient with an Initial form. Information on these forms is collected from the patient’s chart on average at intervals ranging from 6-12 months, and is stored on computer
database as the Follow-up file. Furthermore all of these patients have a file containing the hard copies (i.e. paper copy) of their biopsy report, Initial form, and Follow-up forms.

Five hundred and twenty three IgAN patient charts were found at the offices of Toronto area nephrologists. Thirty five presented at age <15 and were excluded since our focus was on adults. Based on the follow-up file, 299 patients had greater than 12 months of observation and the necessary clinical and laboratory variables outlined below available for analysis and are the subject of this report.

**CLINICAL PARAMETERS**

Age and gender were recorded. We also included blood pressure which, in our study, was converted to mean arterial pressure (MAP) by the mean arterial pressure formula (figure 6a).

**PATHOLOGY PARAMETERS**

Out of the 523 patients whose charts were located at the offices of Toronto area nephrologists, slides from renal biopsies were found for 224 patients at three different Toronto area hospitals, these being the Toronto General and Toronto Western Hospitals as well as St. Michael’s Hospital. The renal biopsy tissue was reanalyzed by two renal pathologists masked to the clinical data according to three grading systems. We used the S.M.K. Lee, Haas and Lajoie classifications. The latter is a newly revised grading system
proposed by our in hospital nephropathologist, Dr. Ginette Lajoie. This non-published histological classification system was developed in an attempt to improve the existing classification systems. It was based on results from recent multivariate analysis studying the effects of histology with prognosis in IgAN patients (72,73,69). All three pathological grading systems are outlined below.

LAJOIE

Grade

1. “Essentially normal glomeruli”. All the glomeruli are histologically normal or only slightly modified. [There is less than 25% glomerular sclerosis (global/segmental), and the interstitial fibrosis involves less than 25% of cortical area.]

2. “Mesangial proliferation”. Increased cellularity in the mesangial regions, defined as more than 3 cells/mesangial area in a thin light microscopic section, stained with PAS, in an area away from the vascular pole. [There is less than 25% glomerular sclerosis (global/segmental), and the interstitial fibrosis involves less than 25% of cortical area.]

3. “Diffuse or focal and segmental endocapillary proliferation”. Endocapillary proliferation involves closure of capillary loops, and may be accompanied by necrosis and crescent formation. [There is less than 25% glomerular sclerosis (global/segmental), and the interstitial fibrosis involves less than 25% of cortical area.]

4. “Glomerular sclerosis”. Any of the above category with >25% glomeruli with global and/or segmental sclerosis (+/- capsular adhesions).

5. “Interstitial fibrosis”. Any of the above categories with interstitial fibrosis involving more than 25% of cortical area, evaluated on trichrome stain.

HAAS

Grade

1. Minimal histologic lesion. The glomeruli show no more than a minimal increase in mesangial cellularity, without segmental sclerosis. No crescents. No tubular and interstitial changes.

2. Focal-segmental glomerulosclerosis-like. The glomeruli show focal and segmental sclerosis in a pattern resembling primary focal-segmental glomerulosclerosis with at most a minimal increase in mesangial cellularity. No crescents. No tubular and interstitial changes.

3. Focal proliferative glomerulonephritis. < 50% of glomeruli are hypercellular (segmental/global). The increase in cellularity may be limited to mesangial areas, or there may be obstruction of glomerular capillaries by proliferated endocapillary cells. Crescent may be present. No tubular and interstitial changes.

4. Diffuse proliferative glomerulonephritis. > 50% of glomeruli are hypercellular (segmental/global). Crescents may be present. No tubular and interstitial changes.

5. Advanced chronic glomerulonephritis. > 40% of glomeruli are globally sclerotic, or any histologic change with > 40% tubular atrophy or loss in the cortex by PAS stain.
LEE

Grade

1. Mostly normal. Occasional slight segmental mesangial thickening with or without hypercellularity. No tubular and interstitial changes.

2. < 50% of glomeruli show localized mesangial proliferation and sclerosis. Rarely, small crescents. No tubular and interstitial changes.

3. Diffuse mesangial proliferation and thickening with focal and segmental variation. Occasional small crescents and capsular adhesions. Focal interstitial oedema and infiltrate occasionally present. Tubular atrophy rare.

4. Marked diffuse mesangial proliferation and sclerosis. Crescents present in up to 45% of glomeruli. Partial or total glomerulosclerosis frequent. Tubular atrophy, interstitial inflammation, and occasional interstitial foam cells.

5. Similar to grade 4, but more severe in both glomerular and interstitial changes. Crescents present in more than 45% of glomeruli.

LABORATORY PARAMETERS

Laboratory parameters to be tested included measurements of renal function in terms of creatinine clearance (CRCL) and proteinuria (PRO). Creatinine clearance was determined using the Cockcroft & Gault formula (figure 6b) (74). This formula makes use of serum creatinine to calculate a creatinine clearance that is corrected for age, weight and gender.

STATISTICAL ANALYSIS

The statistical analysis included the following: demographic description of our total IgAN patient population, renal survival analysis, univariate regression analysis to distinguish the effects of each variable on the rate of disease progression, and multivariate regression analysis to determine the relative weights of each identified characteristic.
In order to help interpret our results we began the statistical analysis by describing our population base so comparisons could be made to previous studies. We started by using descriptive statistics on variables such as age at first presentation as well as at the time of biopsy, gender, and length of follow-up. These were followed by descriptive statistics on clinical and laboratory parameters measured at baseline and during follow-up as well as the patient population distribution among grades of all three histological classifications.

Cumulative probability of kidney survival at five and ten years was determined. This was calculated by the method of Kaplan and Meier\(^{(75)}\) where the survival function is calculated using the exact time the outcome variable occurred. The survival curves were calculated from the time of first observation and from time of biopsy. Similarly to previous studies, end-stage renal disease (ESRD) was used as the end point \(^{(4)}\) \(^{(6,7,8,9)}\). The renal survival rates for patient’s presenting in the 70’s, 80’s and 90’s was also calculated and compared using the Log Rank Test.

In the present study, the rate of progression in IgAN patients was defined as a continuous outcome variable represented by the slope of creatinine clearance over the time of observation for each patient \((\text{GFR}_{\text{SLOPE}})\). Several studies have suggested that progression of chronic renal disease can appropriately be represented as a linear decline in the inverse of serum creatinine over time \(^{(76,77)}\). We also assumed that the decline in renal function was linear. In our study, the rate of decline in renal function was determined in each patient by fitting a straight line through at least three creatinine clearance values
(calculated from the C-G formula using serum creatinine) over time using the principle of least squares, and was represented as $\text{GFR}_{\text{SLOPE}}$.

The slopes for all patients were plotted and examined visually for dramatic changes possibly representing episodes of acute renal failure during the course of follow-up. In calculating $\text{GFR}_{\text{SLOPE}}$, all creatinine clearance values as well as clinical and laboratory values during such episodes of acute renal failure, either at presentation or during the course of follow-up, defined as a rapid fall of GFR of $>40\%$ with associated systemic symptoms were censored. Descriptive statistics of $\text{GFR}_{\text{SLOPE}}$ were also carried out.

All demographic variables were tested for any associations with $\text{GFR}_{\text{SLOPE}}$, using parametric univariate linear regression analysis. Similarly, baseline clinical and laboratory parameters as well as clinical and laboratory variables measured over follow-up were also tested.

In order to use histological information obtained over time, $\text{GFR}_{\text{SLOPE}}$ was recalculated in all patients who had a biopsy performed beyond one month of initial presentation. To ascertain whether the timing of the biopsy was related to changes in the rate disease progression the $\text{GFR}_{\text{SLOPE}}$ calculated over the entire observation period was compared to the $\text{GFR}_{\text{SLOPE}}$ calculated between the date of biopsy and the last date of follow-up. Differences between $\text{GFR}_{\text{SLOPE}}$ calculated from these different time points were tested using linear regression analysis with the intercept set to zero.
As well, each histological classification system was treated as an ordinal variable and tested using univariate linear regression analysis. The classification system with the highest $R^2$ was used in multivariate analysis.

All variables that were associated with GFR$_{\text{slope}}$ using univariate techniques were included in multivariate linear regression analysis using stepwise forward selection.

We examined the $R^2$ values for all the independent variables in univariate analysis along with those of the multivariate analysis in order to determine the relative importance of each parameter.

The clinical relevance was determined for each parameter found to be independently predictive of the rate of disease progression in IgAN by examining the beta coefficient of each parameter in the multivariate model.

We also examined the effects of hypertension, hypertension control, and the class of antihypertensive drugs used (i.e. angiotensin converting enzyme inhibitor [ACEi] vs. other) on GFR$_{\text{slope}}$. In our study we defined hypertension as either an MAP of $\geq 107$ mmHg or by the use of any antihypertensive drugs. Differences between groups were tested using t-test analysis.

In order to determine the minimum length of observation necessary to accurately predict the rate of disease progression the following steps were taken. All parameters found to be
independently associated with GFR_{SLOPE} were re-examined. In each patient they were averaged starting at each year of observation up to the first ten years of follow-up. A multivariate analysis was performed for each of these ten sets of parameters. Finally, changes in adj. $R^2$ values for each of the ten multivariate analyses were observed to determine the shortest length of time of observation necessary to use these parameters to most effectively predict eventual disease progression rate.

All statistical tests were performed using SAS software version 6.12 or SPSS software version 9.0.
PATIENT POPULATION

A) Demographic Information

The average age was 36 (12.5) years at first presentation and 37 (12.8) years at the time of biopsy. The number of males versus females was 183 to 115, a ratio of approximately 3 to 2. The average length of observation from presentation was 69.9 (45.7) months. The average length of observation from the time of biopsy was 57.4 (44.3) months. The average time difference between initial presentation and biopsy was 11.9 (25.1) months. Descriptive statistics on the demographics of our patient population are outlined in table 1.

B) Baseline Clinical and Laboratory Values

Baseline parameters were defined as the averaged value of the parameter over the first six months of observation. In a few cases despite the required follow-up data, initial values were missing. Measures of MAP\textsubscript{BASELINE} were available for 255 patients where the average value was 102.2 (14.9) mmHg. Measures of CRCL\textsubscript{BASELINE} were available for 298 patients where the average value was 75.6 (34.9) ml/min. Lastly, measures of PRO\textsubscript{BASELINE} were available for 226 patients where the average value was 2.24 (2.34) gm/day. Descriptive statistics on the demographics are further outlined in table 2.
C) Clinical and Laboratory Values Averaged Over Follow-up

Average values over the entire length of observation beyond the first six months in MAP\textsubscript{FOLLOW-UP}, where measures were available for 289 patients, was 101.5 (11.3) mmHg. PRO\textsubscript{FOLLOW-UP} was 2.33 (2.14) g/day, and was based on available measures from 253 patients. [Table 3].

D) Pathology

One hundred and thirty two out of the 299 patients who were previously found to have sufficient follow-up observation had their biopsy tissue available for histological grading.

Since only 95 (32%) of patients had their biopsies performed within a month of initial presentation we first had to decide how to deal with biopsy material that was acquired beyond the first month after initial presentation. We reasoned that if GFR\textsubscript{SLOPE} calculated from the date of biopsy was the same as that calculated from initial presentation, then GFR\textsubscript{SLOPE} was the same before and after the date of biopsy. If so, we could use the information from all graded histological material regardless of when the biopsy was performed since the rate of disease progression before and after the biopsy was the same. In figure 7 we found that overall GFR\textsubscript{SLOPE} was not statistically different when calculated from presentation or biopsy.

\* ± one standard deviation
The distribution of 132 patients whose pathology was reviewed and graded is found in figure 8 for each classification that was used (the Lajoie classification, the Lee classification and the Haas classification)

PATIENT SURVIVAL

The five and ten year renal survival rates from presentation and from biopsy are 79.6% and 65.1%, 76.6% and 61.6% respectively. The cumulative probabilities of kidney survival from these time points are depicted in figure 9. Survival rates have not changed over time between groups of patients who presented over the last three decades starting in 1970 until 1997 (p=0.973) as depicted in figure 10. In total 68 patients (22.8%) reached ESRD. A summary of these results may be found in table 4.

OUTCOME VARIABLE

All 299 patients with equal to or greater than 12 months follow-up had at least 3 measures of creatinine clearance in addition to measures of MAP and PRO. The distribution of the slope appears to be normal as described in figure 11. In the calculation of GFR\text{SLOPE}, where on average 9.6 values of CRCL were used per patient, the mean correlation coefficient between creatinine clearance and time in our study was 0.76.

The assumption that disease progression in chronic renal failure can be represented as a linear decline in renal function visually appears to be appropriate in the majority of the
patients in this sample. An example of this is shown in figure 12. We examined the possibility of other non-linear types of relationships of CRCL with time i.e. inverse and logistic, using curve estimation analysis. Although non-linear decline in renal function was observed in a few cases, we found that in the majority of our patients the decline in renal function over time was most appropriately described by a linear function.

Thirteen of the 299 patients (4.3%) had one or more of their measures of creatinine clearance censored for the following reasons: 12 (4.0%) presented with acute renal failure while 1 patient (0.3%) experienced acute on chronic renal failure. Of the 299 patients, 1 (0.3%) was excluded from further analysis due to only two measures of creatinine clearance available for slope calculation after censoring. Overall, only 34 of the 3197 measures of creatinine clearance (0.1%) were censored, along with all other clinical and laboratory data at these times.

The average value of GFR_{SLOPE} as measured by CRCL over time was $-0.39 \pm 0.62$ ml/min/month [table 5]. The slopes of GFR ranged from positive values (increases in GFR), as great as 2.8 ml/min/month, to negative values (decreases in GFR), as low as $-2.3$ ml/min/month. At all levels of initial GFR with at least one year of follow-up 49 patients (16%) had slopes that were stable or improving i.e. $\geq 0$ using calculations based on least squares principle.
UNIVARIATE ANALYSIS

The following section describes the association of individual parameters with the outcome variable i.e. GFR\textsubscript{SLOPE}. A summary of the univariate analysis results for all demographic, clinical and laboratory values at baseline and during follow-up as well as pathology are listed in tables 6A and 6B.

A) Demographic Information

Age at the time of initial presentation and gender were not associated with GFR\textsubscript{SLOPE} using linear regression analysis as described in figures 13 and 14, respectively. The mean value of GFR\textsubscript{SLOPE} for males and females was -0.396 (0.659) and -0.406 (0.567) ml/min/month, respectively.

B) Baseline Clinical and Laboratory Values

At baseline, a negative relationship between PRO\textsubscript{BASELINE} and GFR\textsubscript{SLOPE} was found to be highly significant (p<0.001) [figure 15]. MAP\textsubscript{BASELINE} and CRCL\textsubscript{BASELINE} had no association with GFR\textsubscript{SLOPE} (p<0.09) and (p<0.40), respectively [figure 16 and 17, respectively]
C) Clinical and Laboratory Values Averaged Over Follow-up

Over follow-up, both MAP\textsubscript{FOLLOW-UP} and PRO\textsubscript{FOLLOW-UP} were negatively associated with GFR\textsubscript{SLOPE} (p<0.001 for both) [figure 18 and 19, respectively].

D) Pathology

Overall, only the Lajoie and Lee classifications were found to have at least one grade associated with GFR\textsubscript{SLOPE} (overall p<0.003 and p<0.025 respectively). Whereas the Haas classification had no association (overall p<0.12), Grade 5 in the Lajoie and the Lee histologic classification had a significant negative association with GFR\textsubscript{SLOPE} (p<0.001 for both). Graphical representation of the association of all three classifications with GFR\textsubscript{SLOPE} can be found in figures 20, 21 and 22 respectively.

MULTIVARIATE ANALYSIS

The variables that survived univariate tests were used in the multivariate analysis. These were PRO\textsubscript{BASELINE}, MAP\textsubscript{FOLLOW-UP}, PRO\textsubscript{FOLLOW-UP} and Grade 5 of the Lajoie histologic classification. Because of the smaller sample size available with graded histology our initial multivariate analysis was performed using only clinical and laboratory parameters (i.e. PRO\textsubscript{BASELINE}, MAP\textsubscript{FOLLOW-UP}, PRO\textsubscript{FOLLOW-UP}) via linear regression with stepwise forward selection. The selection criteria used a probability of F <0.05 for entry and >0.1 for removal. Out of our original sample of 298, 190 patients had measures for all three
variables included in this multivariate test. The only variables that were found to independently predict $GFR_{SLOPE}$ were $MAP_{FOLLOW-UP}$ and $PRO_{FOLLOW-UP}$. The results of this multivariate analysis are found in table 7.

In order to assess the impact of histological classifications we performed another multivariate linear regression, which included all the clinical, and laboratory parameters as well as Grade 5 of the Lajoie classification. All of the above mentioned variables, with the exception of histology, were found to independently predict $GFR_{SLOPE}$. The results of this multivariate analysis are found in table 8. Out of our original sample of 298 only 80 patients had measures of all four of the above variables.

**OBSERVATIONAL PERIOD NEEDED FOR PREDICTIONS**

Because $MAP_{FOLLOW-UP}$ and $PRO_{FOLLOW-UP}$ were averaged over the entire length of observation beyond the first six months of initial presentation, we investigated the minimum necessary period of observation over which these variables could be averaged in order to be used as predictors. Figure 23 describes the change in $R^2$ associated with these variables over the entire range of ten years of observation. The $R^2$ value approaches its maximum within the first two to three years after initial observation and does not change significantly over the remaining seven to eight years. This suggests that using $MAP$ and $PRO$ to predict the rate of renal function requires only two to three years of observation.
DISCUSSION

IgAN is an immune-complex mediated glomerulonephropathy that is now recognized as the most common primary glomerulonephritis in the world. A substantial percentage of patients with IgAN will develop chronic renal failure and will require renal replacement therapy. Despite the severity and prevalence of this disease there are still few demographic, clinical, laboratory or pathological features known to accurately predict a patient’s renal survival and there are fewer factors still that can be used to predict this disease’s exact rate of progression.

In this detailed retrospective univariate and multivariate analysis in adult patients with IgAN we examined the impact of several factors in order to develop a predictive model of progression rate in this disease at/near the time of presentation. Using the slope of GFR as an outcome variable, as measured by creatinine clearance, we found that only MAPFOLLOW-UP and PROFOLLOW-UP are independent predictors of the rate of disease progression. Even though these two parameters only explain one third of the variability in the variance of GFRSLOPE, they can be useful predictive tools in a clinical setting.

INCEPTION COHORT

The results of all studies that attempt to either describe the natural history of IgAN or identify potential risk factors associated with progression are often compromised due to
several types of methodological problems. One of the biggest problems in these types of studies is due to the mode of disease identification.

In order to ensure that the diagnosis of IgAN is correct a renal biopsy is absolutely necessary. Since a large number of patients present asymptomatically it is likely that there are many more in this category of IgAN patients that are never identified. Thus, these patients are never biopsied and confirmed to have IgAN. In addition, biopsy policies around the world differ greatly. In some countries a very mild manifestation such as microscopic hematuria will trigger a renal biopsy, whereas in other countries a renal biopsy will not be performed until specific clinical manifestations develop eg. significant proteinuria and/or renal insufficiency. It is likely that these regional differences in renal biopsy policy result in local IgAN patient populations representative of different stages of disease severity and potentially with different rates of disease progression. Presumably, mild cases of IgAN will represent a greater percent of the total cases in those patient populations that are reported from countries where very liberal biopsy policies exist. Conversely, more severe cases of IgAN may compose a greater percentage of the IgAN patient populations in countries where the requirement for renal biopsy demands a more severe clinical picture.

Based on the local screening process it is probable that our inception cohort does not represent the total IgAN patient population i.e. many cases of microscopic hematuria are never identified. In addition, Canadian nephrologists’ biopsy policy will produce a further selection bias since in most cases more than simple microscopic hematuria is
required before a renal biopsy is performed. Thus, our inception cohort will be different then other IgAN sample populations. This will iminge on the generalizability of our results.

Our inception cohort may not even represent the IgAN patient population within the greater Toronto area. The Registry was unable to find over half of the 1097 biopsied IgAN patients that it had identified from the 1974-1995 period. Perhaps the reason why these patients were never found is because their clinical manifestations were very mild or disappeared completely and no follow-up was performed. In addition, the results of our multivariate analysis are based on 190 out of 298 (64%) patient included in our original sample. This reduction occurred because many patients did not have measures of all parameters necessary for multivariate testing. This sub-set sampling could have further biased our results.

One of the drawbacks of using retrospective data was that not all data was collected over constant time intervals. Thus, a few patients had data missing for some or all of their parameters at baseline or over follow-up. To accurately detect associations between our dependent and independent variables in univariate testing, patients with data missing in some of their parameters were not excluded from univariate analysis. Since fewer patients had complete data for each parameter, a smaller sub-sample was available for multivariate analysis. For example, 289 patients had available data to calculate MAP\textsubscript{FOLLOW-UP} that was used in univariate testing. However, only 190 patients had complete data to calculate PRO\textsubscript{BASELINE}, MAP\textsubscript{FOLLOW-UP} and PRO\textsubscript{FOLLOW-UP} which were
used in multivariate testing. The multivariate sub-sample of the final model may have introduced selection bias since it could have been different from the populations used in univariate testing. However, both the partial regression coefficients and beta coefficients of both \( \text{MAP}_{\text{FOLLOW-UP}} \) and \( \text{PRO}_{\text{FOLLOW-UP}} \) that were included in the final model are very similar to their coefficients from univariate tests. These observations suggest that the sub-sample used in our final multivariate model was similar to those populations used in univariate testing.

PATIENT POPULATION COMPARED TO OTHER STUDIES

A) Demographic Parameters

With a mean age of 36 years at initial presentation and 37 at biopsy our sample population is the oldest so far reported. The mean age at onset and biopsy in other IgAN populations vary greatly from around the world from as low as 22 \(^{(63)}\) to as high as 32 \(^{(78)}\) years and 26 \(^{(7)}\) to 39 \(^{(65)}\) years respectively.

With a distribution of male : female gender of 3:2, our study population is similar to that reported in Korea. Like age gender distributions also vary greatly throughout the world. \(^{(5,65,70,79)}\). They range from the most even distribution of 3:2 \(^{(7)}\) in Korea to the most unbalanced distributions of 3:1 to 4:1 \(^{(6)}\) in The Netherlands, France and England \(^{(8,64,66,69)}\). In the middle of this range are distributions found in North America, Australia and Sweden that have reported ratios very close to 2:1 \(^{(4)}\).
B) Length Of Observation

Compared to other studies whose reported observation periods range from 59 to 107 months, our mean length of observation of 70 months is in the middle.

C) Baseline Clinical And Laboratory Parameters

We have treated all of our clinical and laboratory parameters as continuous variables. For this reason, direct comparison with previous work where these parameters were categorized differently is difficult.

For purposes of comparison we used the common definition of hypertension as a blood pressure measure of $\geq 140$ mmHg systolic or $\geq 90$ diastolic or on antihypertensive medications. The frequency of hypertension at initial presentation has been previously reported as high as 53% (70). In our population, hypertension (defined as a mean arterial pressure greater than 107 mm Hg, the equivalent of 140 over 90 mm Hg, and/or any patient on anti-hypertensive treatment regardless of their MAP) at presentation was found in 37%.

The frequency of patients with impaired renal function at presentation, commonly defined as a serum creatinine $>120 \mu$mol/L, has been suggested to be greater than 30% in several studies (4,65). In our group of patients the frequency of impaired renal function at initial presentation was higher at 43%. However, a better measure of renal function is
creatinine clearance. In two previous prognostic studies in this disease renal function impairment at presentation was classified as a creatinine clearance < 80 ml/min \(^{(4,5)}\). In both studies the frequency of patients with an initial creatinine clearance of < 80 ml/min was over 35% and 33% respectively. Again, in our study the frequency of renal impairment defined this way was higher at 46%. However, our method of calculation of creatinine clearance was not the same as that used in the above studies. Thus, we cannot directly compare our population.

Renal function at initial presentation in IgAN patients varies greatly because a large proportion of these patients discover their disease by chance i.e. discovery occurs during routine medical examinations. Thus, as previously mentioned, a patient whose disease is discovered early on will present with preserved renal function as opposed to a patient with advanced disease who presents with moderate or severe renal impairment. Therefore, the higher frequency of renal impairment at presentation in our sample, using C-G calculated creatinine clearances, suggests that our patient population may consist of patients with more advanced disease. Additional evidence of our population having more advanced disease at presentation comes from renal survival data. Both of the studies, which reported the lower frequencies of initial renal impairment, were from IgAN patients in Australia. Their reported renal survival rates are much higher than in North America \(^{(6)}\). Since renal survival in patients with IgAN is dependent on how advanced their disease is at presentation, we could say that our lower survival rates together with our higher frequencies of initial renal impairment reflect our patients having more advanced disease.
Lastly, if >1 gm/day is used to define moderate urinary protein excretion, previous work has shown that as many as 74% of patients with IgAN present with this level of proteinuria. In our population that number was found to be similar at 70%.

The lack of available data in the literature on parameters such as MAP and PRO measured over follow-up, together with inconstancies in the use of pathology makes comparisons between the remaining parameters in the present analysis and previous studies impossible.

C) Renal Survival Analysis

At 62% the ten-year renal survival measured from the time of biopsy in our study population is the lowest reported to date. This low renal survival rate is consistent with that observed elsewhere in North America i.e. 67% in the United States (65) but lower than others reported such as 94% in France (8).

Overall, the increased age at initial presentation, the higher incidence of impaired renal function at presentation together with the lower cumulative renal survival at ten years after biopsy all suggest that our IgAN patients sample may not be exactly the same as those IgAN patients reported elsewhere. On the other hand our decreased renal survival and increased incidence of impaired renal function at presentation is similar to those in IgAN studies reported elsewhere in North America (65). These similarities may be a result
of similar renal biopsy requirements on this continent. In summary, our Toronto sample population of IgAN patients appear to be at the more severe end of the clinical spectrum compared to IgAN patients in other geographical locations likely based on these bias rather than a unique IgAN mode of disease.

OUTCOME VARIABLE

Since the mid-eighties there have been 11 multivariate analyses, both retrospective and prospective, that examined demographic, clinical, laboratory and histological factors as potential prognostic indicators in IgA nephropathy (4,5,6,7,8,9,64,65,66,69,70). Even though in present study the statistical theory and the parameters examined are similar to that of previous work, our approach in developing a predictive model of the rate of progression in this disease has many unique aspects.

Probably the most unique feature of this study was the choice of our dependent variable. In order to test which variables are predictive indicators of progression we chose the rate of deterioration in renal function as a continuous dependent variable. The rate of disease progression was defined as the slope of creatinine clearance over time. All previous attempts to identify prognostic factors have been carried out using dichotomous outcomes (4,5,6,7,8,9,64,65,66,69,70). The dichotomous variable mainly used has been the presence/absence of ESRD. In the past, multivariate associations of potential prognostic factors with renal survival were tested using either the Cox proportional hazards model (80) or logistic regression. In the Cox’s proportional hazards model the potential
prognostic variables are tested for any association with time to ESRD, whereas in logistic regression parameters are tested for their association with the ESRD group. In previous studies the most common prognostic variable that remained after multivariate analysis was the initial measure of renal function \((5,6,7,64,65,66,69)\).

For a clinician, initial renal function at presentation may be useful in determining the probability of a patient reaching ESRD within a given period of time. However, it does not inform the clinician of how much time the patient has until they reach ESRD because initial renal function does not indicate the rate at which IgAN progresses.

To illustrate this point, consider the three patients in figure 24a. The slope used in this figure was the average slope found in our study \((-5 \text{ ml/min/year})\). All three patients present with different initial values of renal function and all three patients reach ESRD at different times. Thus, the probability of reaching ESRD within 10 years for example is in this case dependent on each patient’s initial renal function. Yet all of these patients have the same disease severity as demonstrated by the same rate of decline in renal function i.e. same rate of progression.

In our study, in order to ascertain whether patients with the same rate of renal decline reach ESRD at different times 223 patients were examined whose slopes were less than \(-1.0 \text{ ml/min/yr}\). These patients were segregated into five groups, according to an initial creatinine clearance of less than 30, 60, 90, 120 and > 120 ml/min, respectively. The five groups did not differ in their mean rate of disease progression (i.e. \(\text{GFR}_{\text{SLOPE}}\)) despite
differences in baseline creatinine clearance. It was found that the lower the baseline creatinine clearance was, the less time was required to bring each patient’s initial creatinine clearance down to 0 ml/min (p-value<0.001), see figure 25. With the same rate of disease progression in these five groups, the more impaired the renal function was at presentation the shorter was the time to ESRD and thus the higher the probability was of ESRD at any given time interval. In this example ESRD was defined as a creatinine clearance projected to zero ml/min.

The real difficulty for clinician is their inability to predict time to ESRD given a patient’s initial renal function. In clinical practice we know that some patients with well preserved renal function at presentation progress to ESRD within a couple of years whereas others presenting with impaired renal function do not reach ESRD as quickly as predicted. The reason for the above observation as demonstrated in figure 24b, is that regardless of the initial renal function the rates of renal deterioration across patients may vary. Therefore, without knowing the rate of disease progression the predicted probability of ESRD for patient A in figure 24b would be very high if it was based only on that patient’s initial renal function, and the exact time to ESRD could not be predicted. However, given a patient’s rate of disease progression together with their initial renal function, clinicians could accurately predict the time to ESRD in each individual patient.

Knowing the rate of disease progression is most helpful for those patients who present with preserved renal function because affecting the rate of decline in their renal function
through clinical intervention will have the greatest impact on prolonging their renal survival.

On average $GFR_{SLOPE}$ was $-4.8 \text{ ml/min/year}$. This rate of renal decline is higher than that of $-1.4 \text{ ml/min/year}$ (N=153) reported from Sweden \cite{81} and is lower than that of $-7.1 \text{ ml/min/year}$ (N=51) \cite{82} reported in the United States. However, it is difficult to make direct comparisons on the rate of decline in renal function between our population and the two mentioned above given the differences already discussed regarding the inception cohort plus the fact that in the present study the GFR was determined by different method.

Since a biopsy is required to diagnose IgAN it is possible that the average rate of renal decline in the total IgAN patient population is much less than that reported in the literature and in this study. In addition, regional differences in biopsy policy may result in IgAN patient samples with different mean rates of progression due to different proportions in each quartile of the rates i.e. in North America suspected IgAN patients that rapidly lose renal function are perhaps biopsied more frequently. This could overestimate the average rate of renal decline in our Toronto area patients compared to those IgAN sample populations reported elsewhere, such as Sweden.

It is generally believed that patients with renal insufficiency, regardless of the cause, continue to lose renal function over time. This generalization has lead to hypotheses that there might be a common final pathogenic pathway leading to renal failure. However,
this concept of universal progression has been challenged in at least three studies in addition to this one where 16-22% of patients with renal insufficiency were reported to have stable renal function over periods of years\(^{(83,84,85)}\). In our study at all levels of baseline GFR, 49 patients (16%) had at least one year of follow-up over which renal function was stable or improving i.e. GFR_{SLOPE} was calculated to be \(\geq 0\). In fact some patients exhibited stable or improving GFR for periods of up to and over ten years. This small group of patients had a lower MAP_{BASELINE} (p<0.017) and a lower MAP_{FOLLOW-UP} (p<0.02). This may suggest that blood pressure control is a critical factor in stabilizing the rate of IgAN disease progression.

There were possible selection bias introduced in our study by using GFR_{SLOPE} as an outcome variable. Firstly, in calculating GFR_{SLOPE} in our patient sample, periods of acute renal function were censored. Since periods of acute renal failure would increase the variability of our dependent variable (GFR_{SLOPE}), all measures of creatinine clearance, and accompanying clinical and laboratory values, were censored. Censoring of such data could have introduced bias in our analysis but, based on the observation that only 0.1% of all data points were in this category, it would be very small.

Secondly, it is possible that there is bias introduced by the entry of those patients with more complete clinical data since patients that have more severe clinical manifestations tend to be followed-up more closely. The effect on the results of less complete data on milder patients would impair the likelihood of detecting true differences between mild and severe disease. Furthermore, those parameters that were found to be significant may
in fact explain more of the variability in GFR$_{\text{slope}}$ if more data on milder patients were available.

Lastly, the used of retrospective data does not allow us to study the directionality of events. That is to say that treatment effects may have altered the renal function and GFR$_{\text{slope}}$ in our IgAN sample population. If our dependent variable is bias to treatment then this would further decrease our ability to detect true associations between potential risk factors of disease progression and GFR$_{\text{slope}}$.

CLINICAL RELEVANCE

Based on the partial regression coefficients from table 7 the clinical implications of the results from the first multivariate analysis are as follows. For a patient with a mean arterial pressure of 90 mmHg and a mean urinary protein excretion of 0.25 g/day the predicted renal function would be improving by 0.9 ml/min/yr [table 9]. However, if the same patient exhibited a mean urinary protein excretion of 1 or 3 g/day the predicted renal function would now decline by 0.3 or 3.5 ml/min/yr, respectively. Likewise for a patient with a mean urinary protein excretion of 0.25 g/day and a mean arterial pressure of 100 mmHg or 110 mmHg, the predicted renal function would decline by 1.1 and 3 ml/min/yr [table 9].

We have pointed out that our inception cohort may not be representative of IgAN patients described in other geographical areas, and that perhaps it is even different than the local
Toronto area IgAN population. These observations do not necessarily mean that our results have no clinical relevance in our IgAN population and those in other countries. It has become a cultural norm to say that when the adjusted $R^2$ of a significant correlation reaches around 0.5, that correlation is considered to be clinically important \(^{(86)}\). We have already discussed the increased difficulty of finding real associations between our dependent and independent variables given the lack of more available data on milder patients. Thus, it is more probable that we have missed possible associations with disease progression rather than having found false ones, and that perhaps the variables included in our final model really explain more variability in GFR\textsubscript{SLOPE} than we estimated. It is therefore very likely that mean arterial pressure and proteinuria are clinically relevant in other IgAN populations, and that these two parameters can be used to predict (with limitations) the rate of IgAN progression in our patient population as well as those in other geographical areas.

**HYPERTENSION**

Since MAP\textsubscript{FOLLOW-UP} was found to be independently associated with GFR\textsubscript{SLOPE} we examined the presence of hypertension over time. In our study hypertension was defined as either a MAP of $\geq 107 \text{ mmHg}$, or by the use of antihypertensive drugs. We found that within the first two to four years of observation there was a great increase in the number of patients who became hypertensive [figure 26]. There was a closely matched increase in the number of cases where hypertension was controlled (about half of the patients). At four years of observation there was a significant difference in the rate of disease
progression between those patients who did and those who did not develop hypertension [figure 27]. More interestingly, within four years those patients who developed and controlled their hypertension had a less severe rate of disease progression than those patients whose hypertension was not controlled. Lastly we examined the class of anti-hypertensive drugs used in all patients who experienced hypertension. It was found that in those patients who controlled their hypertension the most effective type of anti-hypertensive drug used were those of the ACE inhibitor class. Patients who controlled their systemic hypertension with other antihypertensive drugs demonstrated significantly more rapid disease progression [figure 27].

In our entire population the incidence of hypertension increased most rapidly within the first two to four years. This increase was matched by an increase in the number of patients who controlled their hypertension with anti-hypertensive drugs. Blood pressure control greatly decreased the rate of disease progression. However, not all anti-hypertensive drugs were equally effective. As shown in previous studies, (87) ACE inhibitors seemed to have the greatest impact on improving and/or stabilizing the rate of disease progression in hypertensive IgAN patients.
UNIVARIATE ANALYSIS

A) Demographic Parameters

Age at initial presentation was not associated with the rate of renal decline in our
analysis. However, previous work has shown increased age to be an indicator of poor
prognosis \(^{4,9,65,66}\). In humans there is a decrease in renal function with age. As we grow
older, the natural increase in global glomerulosclerosis reduces the filtration capacity of
the kidney. In the present study, we have corrected for age by utilizing the C-G formula
\(^{74}\), which takes age into consideration when determining creatinine clearance. When we
adjusted CRCL\_BASELINE and GFR\_SLOPE, age was not a significant factor. Perhaps the
reason why age had been previously found to be an indicator of poor prognosis is because
with increased age there would also be a decreased creatinine clearance at presentation.
And as has previously been shown in this study, a decrease in initial renal function may
result in an increase in the probability of ESRD.

In the present study gender was not associated with any statistical difference in
GFR\_SLOPE. There have been reports that males may progress more rapidly than females
\(^{6}\). However, similarly to age, gender is also indirectly associated with renal function.
Creatinine is a metabolic waste product of muscular contraction. Since males on average
tend to have greater muscle mass than females with the same GFR, they on average also
tend to have higher levels of serum creatinine. However, GFR is most commonly
estimated using serum creatinine values. Therefore, the higher serum creatinine values in
males could be falsely interpreted as being associated with initial lower GFR compared to females.

B) Baseline Clinical And Laboratory Parameters

Even thought the univariate association of PROBASELINE with GFRSLOPE was statistically significant, it did not explain much of the variability of the dependant variable given that the adjusted $R^2$ for this association was 0.06. Therefore, the degree of proteinuria at initial presentation alone is not a very accurate predictor of subsequent GFRSLOPE.

C) Clinical And Laboratory Parameters Averaged Over Follow-Up

Both MAPFOLLOW-UP and PROFOLLOW-UP were strongly associated with GFRSLOPE. With an adjusted $R^2$ of 0.11, and 0.17 respectively, although statistically these two parameters still explained only part of the variance in GFRSLOPE.

It is worth noting that in this study we did not look at changes in MAP and PRO from baseline. We are therefore uncertain of whether the magnitude of the difference had the same effect over the entire range of values. i.e. whether the association of a 2 g/day decrease in proteinuria was the same for a patient whose baseline urinary excretion was 2 g/day versus a patient with an excretion of 5 gm/day.
D) Pathological Parameters

All of the multivariate prognostication studies in IgAN examined histology using different classifications. Although this has lead to similar general conclusions the exact associations of different grades of severity of histological damage with time to ESRD is not clear.

In an attempt to clarify the issue of pathology we decided to examine and compare three histological classification systems in IgAN using disease progression rather than renal survival as an outcome.

In the present study, two renal pathologists graded the renal biopsy material. Each observer was blinded to the clinical data, and grades were assigned independently by both observers. Any disagreement resulted in a re-analysis of that particular biopsy. Although no formal testing of inter observer variability was done, disagreement occurred within one grade in only 14 out of the 224 (6.3%) graded biopsies. This is unlikely to produce any effect on our results. Bias could have resulted since the accuracy of renal biopsy grading may have improved with practice over time. We assumed that regardless of where in time the biopsy was performed along the course of the disease in IgAN patients, the impact of histology on GFRsLOPE was constant. That is to say that for example a grade 3 of the Lee classification has the same impact on GFRsLOPE regardless of whether the biopsy was performed within a month or within twelve months of initial presentation.
This assumption was based on our finding that \( \text{GFR}_{\text{slope}} \) was the same regardless of whether it was calculated from initial presentation or from the time biopsy.

The assumption that each increase in grade in the histological classifications represent increasing severity of the disease process does not seem to hold in our study. Two points illustrate this. Firstly, as ordinal variables in univariate analysis, only the Lajoie and Lee classifications had any association with \( \text{GFR}_{\text{slope}} \). Secondly, even in these classifications only Grade 5 related to \( \text{GFR}_{\text{slope}} \) i.e. severe chronic tubulo-interstitial damage and glomerulosclerosis. Based on our univariate results, none of the other four grades had any significant associations with slope suggesting perhaps only two grades (1 to 4) and 5 are necessary.

It may be a bit surprising that the Haas classification did not prove to be significant since grade 5 is similar in definition in all three classifications. There were several biopsy specimens that did not nicely fit into one particular grade in each classification. It is possible that some cases were therefore misclassified. In addition, the tubulo-interstitial damage in the Haas classification was only represented by grade 5, as opposed to a progressive increase in damage represented by grades 4 and 5 in the other two classification systems. Grade 4 representing milder forms of tubulo-interstitial damage was not negatively associated with \( \text{GFR}_{\text{slope}} \) in the Lajoie and Lee classifications. Thus, the lack of association in grade 5 of the Haas classification could be attributed to the fact that this grade contained cases with both mild and severe forms of chronic tubulo-interstitial damage and glomerular sclerosis.
The observation that histological grades representing severe chronic tubulo-interstitial damage and glomerulosclerosis are most strongly associated with poor outcome is in keeping with previous studies which found significant associations between these pathological features and poor survival. It has generally been shown that when looking at a single point in a patient’s disease the most severe chronic changes, including glomerulosclerosis, and tubulo-interstitial damage are the most accurate indicators of a poor renal survival and rapid progression.

However, the degree of histological damage alone does not serve as a statistically accurate predictive variable of GFRSLOPE based on its $R^2$ value of 0.09.

MULTIVARIATE ANALYSIS

When all clinical and laboratory variables were examined together using multivariate techniques the best model for predicting the rate of decline in renal function included only the variables MAPFOLLOW-UP and PROFOLLOW-UP and excluded PROBASELINE, based on a study of 190 patients. The two variables together explained 32% of the variability observed in the rate of decline in renal function.

When we investigated histological classifications we found that all three clinical and laboratory parameters survived, however, histology did not. Unfortunately, out of 298 patients only 80 were used for this second multivariate analysis due mainly to the lack of
patients with graded histology. Therefore the validity of our results based on such a small sample was possibly unsound. In fact we found a positive association between PROBASE with GFRSLOPE in the second multivariate analysis. However, after partial correlation coefficient analysis selection bias was assumed to be responsible for this positive association. Thus, it was not possible in the present study to assess the impact of severe chronic tubulo-interstitial damage and glomerulosclerosis together with MAPFOLLOW-UP and PROFOLLOW-UP due mainly to a lack of sample size.

PREDICTING THE RATE OF DISEASE PROGRESSION

Since MAPFOLLOW-UP and PROFOLLOW-UP were found to be associated with GFRSLOPE these two variables could be used to make some predictions of the rate of disease progression. In our study however, these parameters were averaged over the entire length of a patient’s time of observation. When we examined the changes in $R^2$ for these two variables measured over different time intervals we found that two to three years of observation are needed in order to make accurate predictions of the rate of disease progression in our patient population.

It is important to remember that the results of the multivariate analysis do not necessarily mean that by themselves other parameters found associated with GFRSLOPE in univariate analysis are not significant predictive variables. Based on the results of our univariate tests, we can say that for newly biopsied IgA patients in the Toronto area, factors such as gender, age at presentation, initial GFR and baseline MAP will have no impact on how
the disease in these patients will progress. The fact that both initial urine protein excretion and severe chronic tubulo-interstitial damage and glomerulosclerosis all drop out of the multivariate analysis may imply that the information contained within these two variables is almost entirely accounted for by measures of mean arterial pressure and urine protein excretion measured during follow-up.

LIMITATIONS

There are limitations in all retrospective studies that attempt to describe the natural history of a disease and to identify potential predictors of progression rate. Among the limitations in the present study that have already been discussed are patient population selection and renal biopsy policy bias. In addition, selection bias due to the decreasing sample sizes available for analysis, resulting from a lack of complete data, may compromises the generalizability. This may make the comparison of our results to IgAN patient reported elsewhere difficult. Another limitation may be measurement bias. It is probable that the more complete clinical and laboratory data in our sample belong to patients that are followed-up more closely due to more severe disease. The data collectors may have also compromised the reliability of our data, where with practice both chart review and pathological grading of renal biopsy tissue may have increased in accuracy over time. Also, the validity of using $GFR_{SLOPE}$ as a linear function may be questioned in some patients. Non-linear decline in renal function would decrease the validity of the associations found using linear regression analysis. Another limitation in our study was that we did not consider any treatment effects. It is likely that treatment with
antihypertensive drugs, such as ACE inhibitors and/or anti-inflammatory agents such as prednisone or fish oils may alter the clinical status of a patient. Such treatment would decrease our ability to detect true associations of potential risk factors with disease progression. Lastly, we must keep in mind that predicting the exact rate of disease progression will be limited by factors that were not considered in the present study such as polymorphism in certain genes (88), or other as yet unknown aspects regarding the pathogenesis involved in IgAN.

POTENTIAL FUTURE WORK

There are two main areas that could be explored in order to improve our current model. Firstly, it seems that the greatest limitation is the lack of available data on patients representing milder forms of IgAN. Such data could be collected by concentrating our efforts on searching for those patients that were originally identified by the registry but not found at the offices of Toronto area nephrologists. Also, by searching out those patients identified and found by the registry staff but who were lost to follow-up during the course of their observation, presumably because they got better and stopped visiting their nephrologists. Another question that could be addressed is the validity of our current model. This model could be validated by several methods. As new IgAN patients are identified in the Toronto area an internal prospective validation using our model to predict the rate of disease progression for these new patients could be carried out. The predictions could be compared to the actual observed rates of decline in renal function.
Similarly, a retrospective or prospective external validation could also be attempted using patients from another geographical region.
CONCLUSION

We did not find any clinical, laboratory or histological variables within the first six months of presentation that would independently predict the rate of disease progression near the time of initial presentation based on this patient population. This may be explained by the current limitations of our study or by the fact that there are other factors that are more important in determining the rate of disease progression than those considered in this study. In addition, since patients present at different stages of their disease trying to determine which parameters are predictive of disease progression near the time of initial presentation may be impractical. In examining 298 cases of IgAN we found that only two parameters mean arterial pressure and urinary protein excretion on follow-up are found to be statistically associated with the rate of disease progression. Although these two parameters only explain a third of the variance in the rate of disease progression they can be useful in clinical predictions if used over the first two to three years of observation.
Figure 1 – The Glomerulus

[Note: the mesangium is not shown in this diagram, but is usually found within the empty spaces of the capillary tuft]
Figure 2 – The Filtration Barrier
Figure 3 - Profile of transcapillary hydrostatic and oncotic pressure differences
Figure 4 – Possible sequence of events leading to initial glomerular injury in IgA nephropathy.
Figure 5 – Hemodynamically mediated renal injury
\[
\text{MAP} = \frac{[\text{systolicBP} - \text{diastolicBP}]}{3} + \text{diastolicBP}
\]

**Figure 6a – Mean Arterial Pressure Formula**

\[
\text{Creatinine Clearance (mL/min)} = \left[\frac{\left(140 - \text{AGE}(\text{yrs})\right) \times \text{WEIGHT (kg)}}{\text{SERUM CREATININE (µmol/L)}}\right] \times 1.2 \times 0.85(\text{FEMALE})
\]

**Figure 6b – Cockcroft & Gault Creatinine Clearance Formula**
Rate Of Disease Progression At Presentation vs Biopsy

Linear Regression Through Origin

R-Squared = 0.63
p-value < 0.001

Figure 7
Figure 8 – Distribution Of Patients By Grade In All Three Histological Classifications
Figure 9 – Renal Survival From Presentation And Biopsy

Figure 10 - Comparison of Renal Survival Rates Over Time (p-value<0.973, Log Rank)
Figure 11 – Distribution Of \( \text{GFR}_{\text{SLOPE}} \) (\( N = 298 \))

Figure 12 – Example of Linear Decrease in Renal Function Over Time
Figure 13

GFRslope vs AGE

Linear Regression

Adj. R-Squared = 0
Slope = -0.003
Beta Coefficient = -0.062
p-value<0.298
Mean = 36.26 (years)
N = 298
Association Between Gender And GFRSLOPE

Figure 14

GFRSLOPE vs PROBASELINE

Figure 15
Figure 16

GFR slope vs MAP baseline

Linear Regression

Adjusted R-Squared = 0.086
Slope = -0.005
Beta Coefficient = -0.108
p-value < 0.006
Mean = 102.26 (mmHg)
N = 255

Figure 17

GFR slope vs CrCl baseline

Linear Regression

Adjusted R-Squared = 0.001
Slope = 0.0008
Beta Coefficient = 0.049
p-value = 0.397
Mean = 75.59 (ml/min/month)
N = 298
Figure 18

Linear Regression
Adj. R-Squared = 0.111
Slope = -0.019
Beta Coefficient = -0.337
p-value < 0.001
Mean = 101.49 (mmHg)
N = 269

Figure 19

Linear Regression
Adj. R-Squared = 0.166
Slope = -0.117
Beta Coefficient = -0.411
p-value < 0.001
Mean = 2.33 (gm/day)
N = 253
Figure 20

GFR SLOPE vs The Lajoie Classification

Linear Regression
Adj. R-Squared = 0.001
Overall p-value = 0.003
Grade 5 PARAMETER ESTIMATE = -0.446
p-value<0.001
N = 132

Figure 21

GFR SLOPE vs The Lee Classification

Linear Regression
Adj. R-Squared = 0.055
Overall p-value = 0.025
Grade 2 PARAMETER ESTIMATE = 0.424
p-value<0.027
Grade 5 PARAMETER ESTIMATE = -0.638
p-value<0.001
N = 132
Figure 22

Adjust R-Square Values Associated With Models Computed Using Clinical And Laboratory Parameters Averaged Over Varying Lengths Of Time

Figure 23 – Performance of Multivariate Model Based On Parameters Measured Over Different Time Intervals
Figure 24a – Example Of The Difficulties In Predicting Rate Of Progression Using The Cox Proportional Hazards Model Or Logistic Regression

Figure 24b – Examples Of Different Disease Severity
Time To ESRD vs Creatinine Clearance At Presentation

**Linear Regression**

- **p value** <0.001
- **Time To GFR 0 = 0.206 X CrCl/BASELINE**
- **Adj R-Squared = 0.22**

**Figure 25**

Presence and Control of Hypertension Over Time

**Figure 26**
Figure 27 - Effects of Hypertension, Hypertension Control and Type of Antihypertensive Drugs Used On GFR_{SLOPE} Over First Four Years After Presentation
Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>St.Dev.</th>
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<th>Max</th>
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<td>(years)</td>
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<td>298</td>
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<td>at biopsy</td>
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<td>Gender</td>
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<tr>
<td>FEMALE</td>
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<tr>
<td>Observation time</td>
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<td>(months)</td>
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<td>from presentation</td>
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<td>69.83</td>
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<td>57.41</td>
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Descriptive Statistics on the Demographics Of Our Patient Population
N = 298

Table 2

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<th>Mean</th>
<th>St.Dev.</th>
<th>Min</th>
<th>Max</th>
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</thead>
<tbody>
<tr>
<td>MAPB remodel (mmHg)</td>
<td>255</td>
<td>102.28</td>
<td>14.94</td>
<td>70</td>
<td>161.67</td>
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<td>CRLBASELINE (mL/min)</td>
<td>298</td>
<td>75.59</td>
<td>34.95</td>
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<td>PROBASELINE (g/day)</td>
<td>226</td>
<td>2.24</td>
<td>2.34</td>
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Descriptive Statistics for Baseline Clinical and Laboratory Parameters

Table 3

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<th>Parameter</th>
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<th>Mean</th>
<th>St.Dev.</th>
<th>Min</th>
<th>Max</th>
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<td>PROFOLLOW-UP (g/day)</td>
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Descriptive Statistics for Clinical and Laboratory Parameters Averaged Over Follow-up
Table 4

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<tr>
<td>non-ESRD</td>
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<td>Rate of survival</td>
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<td>from biopsy</td>
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<td>at 5 years</td>
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<tr>
<td>at 10 years</td>
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<td>61.6</td>
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Summary Of Survival Data

Table 5

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<tr>
<th>Variable</th>
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<th>Mean</th>
<th>St.Dev.</th>
<th>Min</th>
<th>Max</th>
<th>Skew</th>
<th>Kurtosis</th>
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</thead>
<tbody>
<tr>
<td>GFR slope (mL/min/month)</td>
<td>298</td>
<td>-0.399</td>
<td>0.624</td>
<td>-2.342</td>
<td>2.801</td>
<td>0.137</td>
<td>3.323</td>
</tr>
<tr>
<td># GFR Values Slope Based On</td>
<td>298</td>
<td>9.63</td>
<td>7.26</td>
<td>3</td>
<td>56</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>R-Square</td>
<td>298</td>
<td>0.584</td>
<td>0.328</td>
<td>0.0001</td>
<td>0.969</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Descriptive Statistics of the Slopes of Creatinine Clearance Over Time N = 298
### Table 6A

Summary of Univariate Regression Analysis of All Significant Variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient</th>
<th>Beta Coefficients</th>
<th>R-Squared</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROMAT (mm/day)</td>
<td>-0.0971</td>
<td>-0.289</td>
<td>0.083</td>
<td>0.001</td>
</tr>
<tr>
<td>MAPFOLLOW-UP (mmHg)</td>
<td>-0.01859</td>
<td>-0.337</td>
<td>0.114</td>
<td>0.001</td>
</tr>
<tr>
<td>PROFOLLOW-UP (mm/day)</td>
<td>-0.117</td>
<td>-0.411</td>
<td>0.169</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Lejoi1 Classification OVERALL</strong></td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0.238</td>
<td>0.119</td>
<td></td>
<td>0.249</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.253</td>
<td>0.195</td>
<td></td>
<td>0.113</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0.159</td>
<td>0.077</td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Grade 4</td>
<td>-0.241</td>
<td>-0.18</td>
<td></td>
<td>0.138</td>
</tr>
<tr>
<td>Grade 5</td>
<td>-0.448</td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Lee Classification OVERALL</strong></td>
<td></td>
<td></td>
<td>0.055</td>
<td>0.025</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0.43</td>
<td>0.215</td>
<td></td>
<td>0.065</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.424</td>
<td>0.327</td>
<td></td>
<td>0.027</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0.201</td>
<td>0.146</td>
<td></td>
<td>0.302</td>
</tr>
<tr>
<td>Grade 4</td>
<td>-0.001</td>
<td>-0.001</td>
<td></td>
<td>0.995</td>
</tr>
<tr>
<td>Grade 5</td>
<td>0.538</td>
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<td>0.001</td>
</tr>
</tbody>
</table>

### Table 6B

Summary of Univariate Regression Analysis of All Non-significant Variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression Coefficient</th>
<th>Beta Coefficients</th>
<th>R-Squared</th>
<th>Prob&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.00305</td>
<td>-0.062</td>
<td>0.004</td>
<td>0.288</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>-0.0105</td>
<td>-0.006</td>
<td>0</td>
<td>0.887</td>
</tr>
<tr>
<td>MAPBASELINE (mmHg)</td>
<td>-0.00456</td>
<td>-0.108</td>
<td>0.012</td>
<td>0.086</td>
</tr>
<tr>
<td>GFRBASELINE (mL/min)</td>
<td>0.0008</td>
<td>0.049</td>
<td>0.002</td>
<td>0.397</td>
</tr>
<tr>
<td><strong>Miss Classification OVERALL</strong></td>
<td></td>
<td></td>
<td>0.026</td>
<td>0.118</td>
</tr>
<tr>
<td>Grade 1</td>
<td>0.485</td>
<td>0.226</td>
<td></td>
<td>0.024</td>
</tr>
<tr>
<td>Grade 2</td>
<td>0.12</td>
<td>0.044</td>
<td></td>
<td>0.634</td>
</tr>
<tr>
<td>Grade 3</td>
<td>0.246</td>
<td>0.19</td>
<td></td>
<td>0.099</td>
</tr>
<tr>
<td>Grade 4</td>
<td>0.049</td>
<td>0.037</td>
<td></td>
<td>0.746</td>
</tr>
<tr>
<td>Grade 5</td>
<td>-0.541</td>
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<td>0.001</td>
</tr>
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</table>
Table 7

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Remaining</th>
<th>Variables Removed in Order</th>
<th>Adj. R-Square</th>
<th>N</th>
<th>F-Value</th>
<th>Model Sig.</th>
<th>Partial Reg. Coefficient</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Beta Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Constant</td>
<td></td>
<td>0.322</td>
<td>180</td>
<td>45.84</td>
<td>0.001</td>
<td>1.576</td>
<td>0.362</td>
<td>0.001</td>
<td>-0.272</td>
</tr>
<tr>
<td></td>
<td>MAP/FOLLOW-UP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.346</td>
<td>0.026</td>
<td>0.001</td>
<td>-0.378</td>
</tr>
<tr>
<td></td>
<td>PRO/Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.133</td>
<td>0.018</td>
<td>0.001</td>
<td>-0.444</td>
</tr>
</tbody>
</table>

Multivariate Analysis Without Histology

Table 8

<table>
<thead>
<tr>
<th>Model</th>
<th>Variables Remaining</th>
<th>Variables Removed in Order</th>
<th>Adj. R-Square</th>
<th>N</th>
<th>F-Value</th>
<th>Model Sig.</th>
<th>Partial Reg. Coefficient</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>Beta Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Constant</td>
<td></td>
<td>0.42</td>
<td>40</td>
<td>20.05</td>
<td>0.001</td>
<td>0.089</td>
<td>0.468</td>
<td>0.001</td>
<td>0.338</td>
</tr>
<tr>
<td></td>
<td>MAP/FOLLOW-UP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.024</td>
<td>0.026</td>
<td>0.011</td>
<td>-0.248</td>
</tr>
<tr>
<td></td>
<td>PRO/Baseline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.018</td>
<td>0.026</td>
<td>0.016</td>
<td>0.349</td>
</tr>
<tr>
<td></td>
<td>PRO/FOLLOW-UP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.256</td>
<td>0.029</td>
<td>0.001</td>
<td>-0.474</td>
</tr>
</tbody>
</table>

Multivariate Analysis Including Histology.

N.B. Using Grades 2 and 5 of the Lee Classification gave the same results.

Table 9

<table>
<thead>
<tr>
<th>MAP (mmHg)</th>
<th>PRO (gms/day)</th>
<th>GFR Slope (ml/min/yr)</th>
<th>More Rapid Decline (ml/min/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>0.25</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>0.25</td>
<td>-1.1</td>
<td>2</td>
</tr>
<tr>
<td>110</td>
<td>0.25</td>
<td>-3</td>
<td>3.9</td>
</tr>
<tr>
<td>90</td>
<td>0.25</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>110</td>
<td>4</td>
<td>-9</td>
<td>9.9</td>
</tr>
</tbody>
</table>

Comparison Of Decline In Renal Function At Different Levels Of PRO And MAP By Themselves And Together.
Reference List


