APPLICATIONS OF FAILURE-TIME ANALYSIS

IN A LONG-TERM FOLLOW-UP STUDY

OF PATIENTS WITH GLOMERULAR DISEASE

A thesis submitted to the

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by

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'Seek simplicity

and mistrust it'

Attributed to R.A. Fisher

ABSTRACT

The aim of this study was to explore the application of failure-time methods in a search for markers of prognosis in a long-term follow-up study of patients with biopsy-proven glomerular disease with significant proteinuria.

From 1972, all such patients referred to the Manchester Royal Infirmary were followed at approximate four-monthly intervals, and clinical, biochemical and haemotological data were obtained. Between 1981 and 1984 these data, together with other demographic and histopathological data, were transferred to a University main-frame computer, for statistical analysis. Retrospective data were available for some patients biopsied between 1960 and 1971 and where possible these too were included. A total of 444 patients were analysed.

The end-point for most of the analyses presented was end-stage renal failure (ESRF). The onset was easier to define for the 246 patients whose disease first manifest as a nephrotic syndrome. For these, a series of univariate and multivariate analysis were undertaken. Cox 'proportional hazards' regression analysis of data at one year (including plasma creatinine, urinary protein loss, blood pressure, age and sex) showed that the best predictors were increased plasma creatinine ($P<0.001$) and proteinuria equivalent to more than 5 g/day ($P=0.049$) at one year. Urinary protein loss, blood pressure and haemoglobin concentration were considered as time-dependent covariates (Gail, 1981), relating prevailing values with risk of ESRF up to two years subsequently.

The hazard rate for this group of patients showed a 'peak' at about 3-4 years from onset. Both the 'lognormal' and the 'log-logistic' models fitted the data well despite depending on different assumptions.

A more restricted series of analysis were undertaken for the patients whose first manifestations of disease was asymptomatic proteinuria. This group should be considered separately.

From 1972 new patients had a more extensive series of investigations carried out at the time of the biopsy, and this was repeated on an ad hoc basis at 2-4 year intervals. A selection of these variables were examined. These included the selectivity ratio, total serum immunoglobulins (IgM, IgG, IgA), serum complement components, total cholesterol, lipoprotein 'scores' and plasma fibrinogen. Kay's method was used to relate these individual variables to ESRF, (Kay, 1986). This method assumes a Markov model. The correlations between the variables of prognostic importance were studied.

The limitations of this study are discussed and some suggestions are made as to how a new prospective study could be analysed statistically.

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Dr. Mallick is the consultant physician in charge of the Renal Unit at the Manchester Royal Infirmary. This follow-up study was initiated by him, and undertaken with the support of other clinical staff in the Unit, particularly Dr. E.J. Acheson and Dr. C. Short. Particular thanks must go to Dr. Short, since he painstakingly abstracted all the data from the case histories ready for computation. None of the work described would have been possible without this mammoth undertaking! Thanks also to Mrs. Drucker who assisted in this, and to all the above persons for helpful discussions and comments.

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Finally, I must thank the friends and colleagues who have given me moral support, especially Dr. I. Burgess who encouraged me to begin this piece of work.

Some of the work presented here has been published already (Hunt, Short and Mallick, 1988). Tables V, VI, VIII, IX, X, XIV and Figures IV, VII, VIII are reproduced from **Kidney International** with permission.

BIOGRAPHY OF AUTHOR

Linda Hunt obtained a B.Sc. Honours degree in Mathematics (with Physics) at Bristol University in 1972. She worked as trainee Actuary at the Refuge Assurance for one year and then worked for two years as an Assistant Medical Statistician at the Christie Hospital and Holt Radium Institute in Manchester. Whilst at the Christie Hospital she studied and passed the Final Examinations for Membership of the Institute of Statisticians.

In 1975 she went to Bolton College of Education (Technical) and obtained a Certificate in Education. This was followed by two years as a Lecturer I, North Trafford College.

Since 1978 she has been employed by the University of Manchester, first as a Research Associate and then, in 1980, as a Lecturer in the Faculty of Medicine Computational Group.

She is the head of a subsection of this Unit based in Central District (Manchester Royal Infirmary, St. Mary's Hospital and the Royal Eye Hospital). Here her role has been to give advice and assistance to research workers throughout Central District in all aspects of the design of their projects and the computerisation and statistical analysis of their data. This has led to collaboration in a variety of research projects, particularly for Gastroenterology and the Renal Unit.

The study described here is perhaps the largest study in which she has been involved. She submitted an exploratory study of the first 100 cases as an M.Sc. thesis in 1982.

DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

LIST OF ABBREVIATIONS

ESRF	End-stage renal failure
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
HDL	High density lipoprotein
HLA	Human leucocyte antigens
HSP	Henoch Schönlein purpura
LDL	Low density lipoprotein
MCN	Minimal change nephropathy
MRI	Manchester Royal Infirmary
NLMC	No light microscopic change
NS	Not significant
P	Probability
SLE	Systemic lupus erythematosus
Sf	Svedberg floatation index

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CHAPTER ONE

INTRODUCTION

1.1 Background

1.1.1 The kidney and the glomerulus

The main function of the kidneys is to regulate the volume and composition of body fluid by the production of urine. Each human kidney contains approximately one million nephrons through which urine is produced and collected. Each nephron commences in the cortex of the kidney, where the blind but permeable end tissue is closely apposed to a network of capillaries and is called the glomerulus.

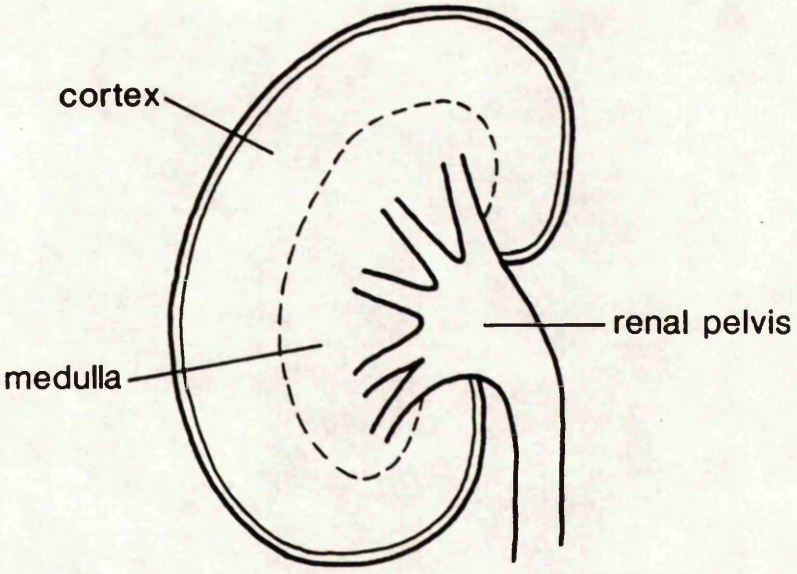
The glomerulus can be regarded as a molecular filter which removes selectively substances from the blood to be excreted as urine. The interface for this process is the glomerular basement membrane (GBM). From the glomerulus, the nephron 'tubule' loops into the medulla of the kidney, back into the cortex and again into the medulla where the urine is collected into a duct (see Figure I). During this process the composition of the urine is altered gradually.

Urine is an aqueous solution of such waste products from metabolic processes as urea, uric acid and creatinine. It also contains salts and hydrogen ions excess to body requirements. The kidneys re-absorb glucose and amino-acids and these are returned to the blood.

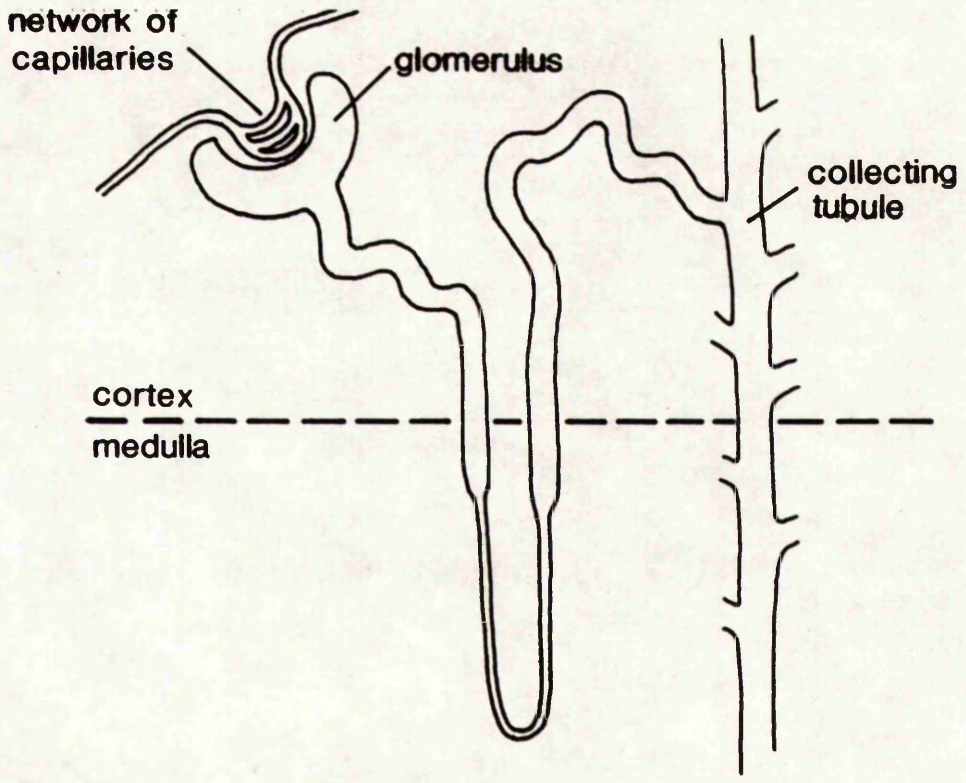
The kidneys also produce hormones, for example, renin and angiotensin, which play a part in regulating blood volume and pressure, and erythropoietin, which stimulates the production of red blood cells by the bone marrow.

FIG. 1

Diagrams of a kidney and a nephron showing the glomerulus



A Kidney



A Nephron

1.1.2 Glomerular diseases

Disorders of the kidneys include damage to the glomeruli and/or the tubules. This study is concerned only with patients with glomerular disease. From the early 1960s, with the development of the percutaneous renal biopsy, it has been possible to confirm glomerular abnormalities in a functioning kidney. The nature of the abnormalities observed under light microscopy have led to the definition of a number of histopathological groups (vide infra). Some of these groups have been subdivided further according to deposition of immunoglobulins or complements observed using electron microscopy and immunofluorescence. In one group of patients, no abnormality can be detected on light microscopy (the so-called 'No Light Microscopic Change' or NLMC group); only on electron microscopy can abnormalities of the epithelial cells along the GBM be detected. Sclerosis observed on light microscopy is associated with nephron death, but some degree of sclerosis occurs with normal ageing.

With glomerular disease there may be continuous or intermittent periods of proteinuria. This probably signifies disease activity, but may also reflect previous glomerular damage. Some proteins normally retained in the blood by the glomerular barrier, especially albumin, are excreted in the urine. The nature of the protein is determined by molecular size, shape and charge (see Pesce and First, 1979). With heavy protein losses, the plasma albumin concentration falls, the liver may be unable to synthesise enough albumin to compensate for that which is lost and fluid retention occurs. This is a 'nephrotic syndrome'. For some patients, the

disease first manifests in this way. Some patients have significant but asymptomatic proteinuria detected at a routine work or insurance medical examination. In other patients, renal damage may present with haematuria, oliguria or impaired renal function. Red and white cells are leaked in small numbers by the normal kidney, but a damaged kidney may leak significantly more and the red cells may colour the urine (haematuria). Impaired renal function becomes apparent when the measured glomerular filtration rate (GFR) is found to be low (vide infra). In some patients there may be a severe acute illness, a 'nephritic syndrome', characterised by macroscopic (visible) haematuria and proteinuria, and often by hypertension, oedema and an impaired glomerular filtration rate. While many of these patients recover completely, for others this is the start of long-term renal disease.

The majority of glomerular diseases are likely to have arisen from immunological damage. Either circulating (soluble) antigen-antibody complexes lodge in the kidney, thus causing inflammation, or antibodies react with antigens inherent to or lodged in the GBM itself and form in-situ complexes. Evidence for both of these processes has come from experimental work and from renal biopsy studies, especially using immunofluorescent markers to label specific immune reactants. There the former has been observed more commonly in human disease (Couser and Salent, 1980; Cameron, 1982).

Whilst most glomerular disease appears to be idiopathic, some occurs in association with other diseases. These include diabetes, systemic diseases (for example systemic lupus erythematosus (SLE), Henoch Schönlein purpura (HSP),

amyloidosis, malignancies (for example bronchial carcinoma or Hodgkin's disease) and bacterial infections such as malaria and syphilis. Infection with particular, 'nephritogenic' strains of the Group A streptococcus is associated with the acute nephritic syndrome. There may be a genetic predisposition also; a nephritis with associated defects in the ocular lens and deafness (Alport's syndrome) is inherited by a dominant trait with variable sex-associated penetrance. Associations have been reported with Class I and Class II human leucocyte antigens (HLA); for example, HLA B8-DR3 were found much more frequently in Caucasians with membranous nephropathy, than in a control series (Klouda, Manos, Acheson, Dyer, Goldby, Harris, Lawler, Mallick and Williams, 1979).

Glomerular disease therefore is a heterogeneous group of disorders and within any of its subgroups there is a diversity of outcome. Proteinuria may remit, suggesting that the underlying lesion has healed, or following a relapsing and remitting course or may persist. In a proportion of patients, at some stage, renal function will decline irreversibly and end-stage renal failure (ESRF) will occur. Levels of urea, creatinine, and other waste materials increase in the blood and these patients die unless they are dialysed or are given a renal transplant. The patient in severe renal failure also becomes severely anaemic, principally because of the loss of erythropoietin production in the damaged kidneys, which leads to a decrease of red cell haemoglobin production.

As yet there is no treatment which will halt the progression to renal failure. A subgroup of the NLMC patients have been found to be steroid responsive, in the sense that

adequate doses of prednisolone will eliminate heavy protein losses (Black, 1970). This is known as 'Minimal change Nephropathy' (MCN) and is regarded as being a more benign disease; no patient from this group progresses to renal failure. Cyclophosphamide has also been used to treat these patients. Earlier studies have suggested that only patients with MCN are steroid responsive; more recently patients with membranous nephropathy have been found similarly to respond to 'pulse' prednisolone therapy (Short, Soloman, Gokal and Mallick, 1987).

Anti-hypertensive drugs are given as necessary to control blood pressure, which tends to rise when renal function is impaired. As well as increasing the risk of cardiovascular disease, hypertension itself may cause hyperfusion and further damage to the kidney; therefore it must be controlled. Diuretics may be given for the fluid retention of the nephrotic syndrome, but these do not affect the underlying biochemical processes. A high protein caloric diet is given to compensate for the heavy protein losses. Conversely, in chronic renal failure low protein diets may be advocated to reduce urea and creatinine levels in the blood. It has been suggested that a high protein intake may 'overload' the kidney and the ensuing hyperfiltration may exacerbate the renal damage (Brenner, Meyer and Hostetter, 1982; Brenner, 1983). Animals fed ad libitum have shown accelerated age-associated sclerosis, and the process is slower in female animals, who eat less. Despite intensive study, to date there is, however, no good evidence for this in humans.

1.2 Natural History Studies

The development of the renal biopsy heralded a shift of emphasis of natural history studies, away from the clinical classification (Ellis' 'Type I' and 'Type II' nephritis, see Ellis, 1942) to a histopathological one. Since the early 1970s studies have tended to focus on single histopathological groups (see a few of the examples: Cameron, Ogg, White and Glasgow, 1973; Cameron, Turner, Ogg, Sharpstone and Brown, 1974; Brown, Upadhyaya, Hayslett, Kashgarian and Siegel, 1979; Ramzy, Cameron, Turner, Neild, Ogg, Hicks, 1981; Nicholls, Fairley, Dowling and Kincaid-Smith, 1984 and Pei, Cattran, Delmore, Katz, Lang and Rance, 1987) and, occasionally, 'secondary' glomerular diseases, such as SLE, were selected for special study (see for example, Leaker, Fairley, Dowling and Kincaid-Smith, 1987). It was believed that for certain histological patterns each could be identified with a consistent clinical evolution (Cameron, 1979), even though the histopathological findings did not represent different glomerular diseases. Some evidence for the latter statement was that animal studies showed that the same antigen, in different individuals of the same species, could induce most of the different patterns of histopathology seen in man (see Cameron, 1982 for a review of this work).

Differences are apparent between some of main histopathological groups (for example, minimal change nephropathy follows a benign course), but there is still a diversity of outcome within each of the histopathological groups. All patients within any single group do not proceed inevitably to ESRF nor do all fully recover. There was a need,

therefore, for studies to address the whole spectrum of pathology, and to examine clinical features as well. In such studies, comparisons can be made between groups without the 'confounding' effects of other factors which influence outcome, for example such differences in inclusion criteria as the inclusion with the nephrotic syndrome of all other manifestations, definitions, patterns of referral and follow-up periods, (see for example Brahm, Balslov, Brun, Gerstoft, Jorgensen, Jorgensen, Larsen, Larsen, Lorenzen, Lober and Thomsen, 1985; Blainey, Brewer and Hardwicke, 1986). Relationships between clinical variables and histopathology may be studied also. This has led to attempts to predict general histological groups from clinical features. Tomura, Tsutani, Sakuma and Takeuchi (1985) claimed to be able to distinguish some types on the basis of 10 clinical variables.

An important part of a natural history study is the determination of long term outcome, to enable accurate prognoses to be made and to help assess the benefits of new treatment regimens. A knowledge of significant markers of prognosis may help with understanding the evolution of the disease, and may, hopefully, lead to new treatment regimens. 'High risk' patients, those most likely to proceed to ESRF, can be ear-marked for special study. An appraisal of the statistical approaches used in contemporary natural history studies, their draw-backs and some suggestions for improvements, have been reported by this author (Hunt, 1982). The methods which are of special value in determining prognosis, and markers of prognosis, in particular with regard

to ESRF, are those of failure-time analysis. This study will be concerned primarily with the latter.

1.3 Statistical Failure-time Modelling and its Application in Renal Studies

Failure-time analysis is concerned with modelling the time to the occurence of some specific event, usually a failure or a death. There are many industrial applications, concerned with the life-testing of components and fatigue failure of structures (see for example Parzen, 1959), but a major field of application is in medicine. Indeed applications in the long-term study of chronic disease, notably cancer, and in graft survival after transplantation themselves have stimulated further statistical research (see Kalbfleisch and Prentice, 1980).

The analysis requires clear definitions of start and end-points. In cancer studies 'start' may be defined as the date of diagnosis or the date of treatment and 'end-point' may be death. Other end-points are possible, such as recurrence or occurence of distant metastatic disease. In studies of chronic disease, it may be that some patients have not reached their end-point by the date of analysis. These are regarded as 'censored'. Other instances commonly regarded as censored are deaths form intercurrent disease or accident and being lost to follow-up. Statistical methods have evolved and adapted to incorporate the use of as much information as possible from the censored patients, that is we know that their survival time exceeded a certain time, but after that no more information is available.

The earliest failure-time methods used in medicine were actuarial life-tables; percentages of survivors simply were calculated at fixed intervals of time from the start and plotted. The survival experience of sub-groups of patients could thus be summarised and compared (Cutler and Ederer, 1958). Life-tables were recommended for long-term studies of survival in glomerulonephritis (Cameron, 1979) and are still being used (see Nicholls, Fairley, Dowling, Kincaid-Smith, 1984; Blainey, Brewer and Hardwicke, 1986).

In glomerulonephritis, the start and end-points are difficult to define. For example, is it reasonable to regard the date when asymptomatic proteinuria is detected as a valid 'start' date? Should the end-point be renal deaths only, or should any death be considered on the basis that they are probably renally related and the distinction is too difficult to make (Cameron, 1979).

The product-limit, or Kaplan-Meier, survival curve estimates incorporate individual patients survival time, rather than using status at fixed points in time. In cancer studies, the logrank test became the standard method to compare the survival curves of subgroups of patients (see Peto, Pike, Armitage, Breslow, Cox, Howard, Mantel, McPherson, Peto and Smith, 1977). Correspondingly the method was adopted in follow-up studies of glomerulonephritis. Patients could be subdivided according to the values of a potential prognostic variable and the survival of the subgroups compared, thus simple univariate comparisons could be made (Magil, Ballon, Chan, Lirenman, Rae and Sutton, 1984; D'Amico, Minetti, Ponticelli, Fellin, Ferrario, Barbiano di Belgioioso,

Imbasciati, Ragni, Bertoli, Fogazzi and Duca, 1986; Magil and Ballin, 1987). It was possible to consider two or more prognostic variables simultaneously, but only by further subdivision of the data base, so large data bases would be needed at the outset.

A multivariate approach would enable several prognostic variables to be assessed concurrently, each adjusting for the effects of the others. Cox formulated a regression solution (Cox, 1972) based on proportional changes in the hazard rate (see Methods section 2.3.4 and Appendix II). The 'hazard rate' (equivalent to the 'force of mortality' in actuarial studies) is at any point in time the rate at which failures/'deaths' occur in the surviving group of patients. If the prognostic variables can be assumed to affect the hazard rate proportionally, then their effects can be quantified without the need for the hazard rate to be evaluated. This is therefore a 'semi-parametric' approach. The method has been used widely in cancer studies and more recently in kidney studies. It has been used to discern factors associated with survival on different treatments for end-stage renal failure, for example on dialysis or after transplantation (Hutchinson, Thomas and MacGibbon, 1982; Gore, 1983; Gokal, Jakubowski, King, Hunt, Bogle, Baillod, Marsh, Ogg, Oliver, Ward and Wilkinson, 1987; Gokal, Bogle, Hunt, Jakubowski, King, Baillad, Marsh, Oliver, Ogg, Ward and Wilkinson, 1989). There have been recent applications in glomeronephritis, especially in the study of 'IgA nephropathy'. (Diffuse mesangial proliferation with IgA deposits is probably the most common pathology worldwide). Beukhof and his co-workers included a stratification for

patients with insufficiently controlled hypertension (Beukhof, Kardaun, Schaafsma, Poortema, Donker, Hoedemaeker and Van der Hem, 1986); stratification allows subgroups of patients to have different underlying (baseline) hazard rates. Stratification also can aid the checking of the proportional hazards assumption (see Methods section). D'Amico and his co-workers (see above) included fine biopsy detail, for example glomerular obsolescence and segmental glomerular sclerosis, as potential prognosticators in the Cox model in addition to clinical variables. This author was involved in a parallel study of 'IgM nephropathy' (O'Donoghue, Lawler, Hunt, Acheson, Gokal and Mallick, in preparation). Similar approaches have been adopted for other histopathological groups (Heilman, Oxford, Holley and Velosa, 1987).

If the shape of the hazard function is known, then this knowledge can be incorporated into the analysis (a 'parametric' approach). In a study of lupus nephritis (Austin, Muenz, Joyce, Antonovych, Kullick, Klippel, Decker, Balow, 1983), a Weibull model was used. The Weibull model is an example of an 'accelerated failure-time' model. This is a class of models in which the prognostic variables, or covariates, increase or decrease the rate at which a patient proceeds along his or her time course. In the Weibull model, however, the hazard rate is assumed to increase or decrease monotonically with time. (The exponential model, where the hazard rate is constant, is a special case.) The authors did not justify their assumption in this case.

The Weibull model is the only accelerated failure-time model where the assumption of proportional hazard holds. It is, however, worth considering other accelerated failure-time

models in glomerular disease. They have an intuitive appeal, since the prognostic variables may accelerate or decelerate the time to ESRF. The choice of model is governed by the shape of the underlying hazard rate, and this first must be ascertained. There are two models in which the hazard rate is 'peaked'; it increases to a maximum and decays slowly. These are log-normal and log-logistic models (Bennett, 1983; Aitken, Anderson, Francis and Hinde, 1989). Covariates in the model cause the peaks to occur earlier (and higher) or later (and lower). The use of such models has been explored in studies of cancer (Gore, Pocock and Kerr, 1984), but not in glomerular disease, and consideration is given to this approach in this present study.

In the log-logistic model, the hazard rates converge as time proceeds for all covariate values; the effects of fixed covariates matter less. Gore (Gore, 1983; Gore, Pocock and Kerr, 1984) also considered 'step-functional' proportional hazards models, in which the proportional effects of fixed covariates were allowed to vary between distinct epochs. Their justification was that information measured at the start may be less relevant later on, or, conversely may emerge as being more relevant in the long term.

It is difficult to compare studies of glomerular disease between centres because of the different definitions adopted. Some studies have considered 'start' as the date of first clinical presentation; others the date of first biopsy, which may be some time later; likewise the 'end-point' may be actual death or renal death. In the applications of Cox's method described above, prognostic variables are measured at the 'start' time ('fixed covariates').

The collaborative study from four hospitals in Copenhagen (Gerstoft, Balslov, Brahm, Brun, Jorgensen, Jorgensen, Larsen, Larsen, Lorenzen, Lober and Thomsen, 1986) which incorporated patients from across the histopathological spectrum, included the findings from light microscopy and clinical variables in a search for prognosticators. Clinical variables were obtained at first presentation (first plasma creatinine level); biopsy was within one year (median 11 days). Plasma creatinine was measured sufficiently often throughout the follow-up to define the current renal status (less than 2, 2-7, more than 7 mg/100ml), and an elaborate series of applications of the Cox regression model was used to model transitions between the states and to death.

The authors did not remeasure the predictor variables at the start of the second or subsequent transitions; it is likely that some clinical variables, notably 24 hour urinary protein loss, and therefore its effect, may have changed with time. They claimed that models in which variables change with time were not available. In fact this is not the case, as the Cox analysis does allow incorporation of 'time-dependent' prognostic variables. Cox himself, however, considered only covariates which were mathematical functions of the time from onset (Cox, 1972). Breslow, on the other hand, discussed the case where the covariates were not mathematical functions of the time from onset but represented changes in treatment or prognostic status (Breslow, 1975). A patient moves in time from one covariate state to another and the risk of death is determined solely by the current state and not his past history.

The hazard rate is assumed to be altered proportionally

by the prevailing covariate status rather than, say, the status at onset. In practice, one also can define a 'current state' according to past history; for example, by using the value obtained six months earlier, or even the mean or maximum value during the previous six months. Breslow assumed that the baseline hazard rate was constant between failure-times. The covariate states needed to be known at each of the failure-times for all of the patients.

Gail (Gail, 1981) developed Breslow's approach and described a method to evaluate serially measured cancer markers. He applied this to patients with colorectal cancer, and found that high levels of carcinoembryonic antigen were significantly associated with increased risk of death. His method is equally applicable to glomerular disease to relate the prevailing covariate state with the risk of ESRF.

Gail's method depends on regular, sequential and extended follow-up, since the covariate value must be known precisely at the failure-times, or be estimated reasonably accurately by interpolation. The study which is about to be described offered a unique opportunity to explore this analysis in detail for a select group of variables.

Instances where covariates have been measured infrequently need a different approach; a method based on a Markov model was proposed by Kay (Kay, 1986). The method was developed initially to investigate the usefulness of alpha-fetoprotein as a marker of prognosis in hepatocellular carcinoma. Levels of alpha-fetoprotein were measured at infrequent intervals during follow-up and the changing levels were related to death. The method seems equally applicable to the study of glomerular disease, to relate sparsely collected

covariates with ESRF. The use of this method has been explored in this present study.

1.4 The Manchester Study

This study began prospectively in 1972. Netar Mallick, together with colleagues at the Manchester Royal Infirmary (MRI), decided to review and document all patients referred to the hospital with biopsy-proven glomerular disease and significant proteinuria, and to follow them on a regular out-patient basis.

Patients were to be seen at four monthly intervals, when clinical, biochemical and haematological data were obtained. For new patients, a more extensive series of investigations, the 'renal screen', was carried out at the time of the biopsy. The screen was to be repeated at intervals of 2-4 years if the proteinuria persisted.

A list of the variables collected throughout most of the study period is given in Appendix I. Between 1981 and 1984 the data was transferred to a computer for statistical analysis. The criteria for inclusion are given below (see Patients and Methods section 2.1.1). Retrospective data were available for some patients biopsied between 1960 and 1971; where possible these too were included.

The data for this study were transferred to a University main-frame computer. Computer compatible documents were designed by this author and a renal physician, Colin Short, who then encoded the information. Separate files were created on the computer to accommodate the 'basic' data, that is information collected once only (such as details of the onset, past history, histopathology and outcome), and the 'follow-up'

data, that is the information from the variable number of clinic attendances for each patient, which totalled over 17,000. The existing file structures are described briefly in Appendix I.

The computer documents, validation of the data and an early analysis of the first 100 cases have been described elsewhere (Hunt, 1982). Principal clinically-associated findings on all 444 patients in the study have been reported (Mallick, Short and Hunt, 1987; Short, 1990).

The data files were large and, although some of the earlier data were incomplete, substantial sequential information was available for a number of variables. A series of analyses were undertaken in an attempt to assess the relative value of these variables in prognosis, and the relationships between the variables. These analyses form the basis of the present study. Part of this work has been published already (Hunt, Short and Mallick, 1988).

1.5 The Present Study

The primary aim of this study was to explore the application of failure-time methods to search for markers of prognosis in a long-term follow-up study of patients from across the histopathological spectrum. There was, for these patients, a comparatively regular sequential follow-up, and this gave the opportunity to explore more in-depth analyses involving time-varying, as well as fixed, covariates. A large number of variables were measured routinely, and it was hoped that the analyses might high-light the variables that would yield the most fruitful study.

Initially, analysis was focussed on early information regarding urinary protein loss, hypertension and plasma creatinine (see Methods section 2.2.1). Plasma albumin and haemoglobin were considered later. The role of these variables in the natural history of glomerular disease has been discussed (sections 1.1.1 and 1.1.2).

Hypertension was usually treated as soon as it was confirmed. Two studies of 'IgA nephropathy' have suggested that hypertension which is controllable carried a better prognosis than that which is not (Beukhof, Kardaun, Schaafsma, Poortema, Donker, Hoedemaeker and Van der Hem, 1986; Payton, McLay and Boulton Jones, 1988).

Plasma creatinine was used as a marker for prognosis rather than to define ESRF; for some patients the concentrations rose to high levels (over 0.2 mmol/l) and then fell, and we were unable to define a level above which ESRF was inevitable. Creatinine clearance was not used in this study even though it was regularly measured. Inaccurate urine collections often rendered the creatinine output rate inaccurate (see earlier work, Hunt 1982), and attempts to estimate this from age, sex and weight (see Appendix to Mawer, 1976) were not very successful as fluid retention caused the weight to fluctuate. More importantly, the reliability of creatinine clearance in the measurement of glomerular filtration rate has been questioned. It has been suggested that in fasting subjects the reciprocal of the plasma creatinine level may be better (Payne, 1986). In normal adults this is constant with age but may differ between the sexes.

Analyses of the above variables was extended to use time-dependent variables. Gail's method (Gail, 1981) was used rather than that of Aitkin (Aitkin, Anderson, Francis and Hinde, 1989). The reasons were firstly that the data set was very large and secondly that, with Gail's method, if a patients marker state could not be estimated at a point in time, the patient could be temporarily dropped from the data set. (Although the data were collected sequentially, there were a number of instances when results could not be obtained by interpolation).

A number of the screen variables were selected for study. Typically, these were measured two to three times per patient, and at variable times form the disease onset. A brief background is given below for each:-

(a) Selectivity index

In this study, the selectivity index is the ratio of the clearance of a large molecule (IgG, of molecular weight 150 KDa) to the clearance of a small molecule (transferrin, 88 KDa) (Hardwicke, Cameron, Harrison, Hulme and Soothill, 1970).

When the selectivity index is high, the kidney allows the leakage of higher molecular weight proteins to the urine; such proteinuria is regarded as 'poorly selected' (Cameron, 1968). Low selectivity indices occur commonly in minimal change nephropathy, and Hardwicke and his co-workers suggested that there is little chance of steroid response in patients with selectively indices of more than 0.3.

(b) Serum immunoglobulins

Immunoglobulins are antibodies, that is, proteins which are produced as part of an immunological response to an antigen. Antibodies can recognise the same type of antigen in subsequent encounters. Immunoglobulins also activate complement pathways (vide infra).

The molecule of each antibody is made up of four chains, two light and two heavy chains, and has a number of antigen binding sites. The composition of the heavy chains determine the class of antibody and its physiological function, and, within each class, antibodies exist with different binding sites.

The three main classes of immunoglobulins are IgM, IgG and IgA. IgM has a large molecule (800 KDa) and thus poor tissue penetration. Within the vascular system, however, it has an important role in the neutralisation of organisms, especially viruses. The molecule has five complement binding sites and therefore excellent complement activation. IgG has a smaller molecule (150 KDa) which can penetrate tissue easily and also can activate complement. Dimeric IgA (320 KDa) is the major mucosal immunoglobulin. It neutralises antigens that enter the body via mucosal routes, for example via intestinal or bronchial secretions.

Antibodies are produced by B cells. B cells can switch production from IgM to IgD and later to IgG, IgA and IgE as they mature. Typically, in response to an antigen, the serum level of IgM rises first then falls as the serum IgG rises (see for example Chapel and Haeney, 1986).

In immune complex glomerular diseases, the subclass and size of the immunoglobulin may determine where in the glomerulus the complexes will be deposited, for example complexes formed from the large molecule IgM are more likely to be deposited in the mesangium.

(c) Serum complement components

The complement 'cascade' comprises a series of serum proteins activated in sequence to assist in antigen removal. Most are made in the liver.

Each complement component is split into two fragments. The major fragment has binding sites for cell membranes but also acts as an enzyme to split the next component. Complement activation begins with the splitting of the complement C3. This is achieved in two ways, the 'classical' and the 'alternate' pathway.

In the 'classical' pathway, the component C1q is activated by antigen-antibody complexes formed from IgM (or, less easily, from IgG); this in turn splits both C4 and C2. The two major fragments from C4 and C2 form a complex (C3 convertase) which then splits C3. A complex formed from all three major fragments splits C5, and then C6, C7, C8 and C9 in sequence. The components C5-C9 form the membrane attack complex which lyses the cell.

In the 'alternate' pathway, C3 is activated by bacterial cell walls and endotoxin. Although the major fragment of C3 decays quickly, if it binds to a suitable surface it can utilise Factor B and Factor D to produce more C3. From C3 the pathway is the same as the classical pathway, that is C5 to C9.

Some complement component levels increase within a few days of infection; levels of others may fall as they are utilised. A knowledge of which complements are low may indicate the pathway involved. For example, C1 and C4 are involved in the classical pathway, Factor B is involved in the alternate pathway, whilst C3 is common to both. In some diseases such as SLE rapid complement consumption occurs so that there may be a reduction of complement activity.

Sequential serum complement levels over the period 1971-1976 were studied by Dr. Mallick and colleagues in relation to glomerular disease (Mallick, Eyres, Acheson, Golby, Jeacock, Lawler, Lucas, Taylor and Williams, 1981). Connective tissue disorders were excluded. Persisting abnormalities were found in all histopathological groups, and more commonly in mesangiocapillary (low CH50 and C3) and diffuse proliferative disease, although did not appear to be related to the natural history over a five year period. Intermittent abnormalities, however, were sometimes associated with relapse or exacerbation of proteinuria. This suggested involvement of the complement pathway in idiopathic immune-complex disease, although lower complement levels also may indicate that production is impaired. There was a significant negative correlation between serum C3 and glomerular deposition, and for both mesangiocapillary and diffuse proliferative disease the deposition was found to be related to increase in plasma creatinine. In fact, initial absolute levels of C3 and

Factor B were found to be significant predictors of a rise in plasma creatinine during the study period.

(d) Lipids and lipoproteins

Lipids are insoluble in water but in the plasma they are kept in solution as complexes with protein molecules, the lipoproteins.

Lipoproteins can be separated into groups according to their hydrated density by ultracentrifugation (see Varley, Gowenlock and Bell, 1980 for details). Firstly high density liproprotein (HDL) is separated from the remainder. This has a density greater than 1.063g/ml. The ligher fraction is further subdivided according to the Svedberg floatation index (Sf) into low density lipoprotein (LDL), very low density lipoprotein (VLDL) and chylomicrons. Particles with greatest density have the lowest Sf index.

Chylomicrons and triglycerides are circulating particles formed in the gut from exogenous fat. VLDL is synthesised predominantly in the liver rather than the gut. Its triglyceride component is synthesised from fatty acids arriving in the circulation or, de novo, from carbohydrate. LDL is largely formed in the circulation from VLDL as triglyceride is removed, and is rich in cholesterol.

Levels of total serum cholesterol, LDL and VLDL are frequently high in patients with the nephrotic syndrome, probably because of increased synthesis by the liver in response to a fall in albumin. Increased serum cholesterol is correlated with a fall in albumin, rather than renal pathology or steroid resistence (Chopra,

Mallick and Stone, 1973; Querfield, Gnosso, Haberbosch, Augustin and Scharer, 1988).

(e) Plasma fibrinogen

Fibrinogen is a plasma protein probably made in the liver which is utilised in the clotting of blood. An enzyme, thrombin, converts fibrinogen to a fibrous gel, fibrin, which is the basis of a blood clot.

Plasma fibrinogen concentration may be slightly raised in early pregnancy or in menstruation, but is raised markedly in the nephrotic syndrome. The formation of epithelial crescents may be stimulated by products of local fibrin deposition.

CHAPTER TWO

PATIENTS AND METHODS

2.1 A Profile of the Patients in the Study

2.1.1 The criteria for inclusion

The criteria were as follows:

(a) The patients had a renal biopsy performed at the MRI to confirm glomerular disease, and had significant urinary protein loss (more than 1g/24h) observed on at least one occasion.

(b) The apparent onset of disease was before 1st January 1980, thus all patients had a minimum potential follow-up of one year.

(c) Patients requiring dialysis within one month of onset were excluded.

(d) Patients with diabetic nephropathy and pre-existing diabetes mellitus were excluded.

2.1.2 The patients

444 (293 males and 151 females) patients met the above criteria. The majority (95%) were white; 4% were asian/oriental and 1% african/afro-carribean.

A number of patients had connective tissue or other systemic disorders (SLE (31); amyloidosis (17); HSP (13); polyarteritis nodosa (4) and Wegener's granulomatosis (2). These have been included but, in some instances, they have been omitted or treated separately from the remainder.

The 'onset' of disease was taken as the date first manifested. The year of onset ranged from 1945 and 1979, and, for the majority of patients (92%) occurred from 1961 onwards. Table I shows how glomerular disease first

TABLE I

FIRST MANIFESTATION OF DISEASE

Fluid retention	246
Asymtomatic proteinuria	145
Nephritic syndrome	7
Oligoanuria	2
Macroscopic haematuria	25
Microscopic haematuria	4
'Impaired renal function'	14
Not known	1

manifested in these patients. Most of this study was concerned with the two largest groups: those in whom the 'onset' of disease was a nephrotic syndrome (246) and those with asymptomatic proteinuria (145). These were considered separately.

Since the MRI is a tertiary referral centre and the 'onset' was frequently documented at the outlying hospital this meant that the data sets at the time of onset were often incomplete. Most patients (89%), however, had some quantitative assessment within four months of onset. The total follow-up ranged from two months to 33 years (median six years).

2.1.3 The histopathological groups

The biopsy was often performed some considerable length of time after the first manifestation of illness (median seven months, range 0 weeks to 29 years). The numbers of patients in the main histopathological groups are shown in Table II. The groups corresponded with the current WHO classification and are described briefly as follows:-

(a) No light microscopic change (NLMC): No abnormality was detected on light microscopy. On electron microscopy some of epithelial cell 'foot processes' are flattened (fused) along the GBM.

(b) Minimal change nephropathy (MCN): This was a subgroup of patients with NLMC lesions who showed a prompt and complete remission of heavy proteinuria after steroid therapy (see 'Treatment').

TABLE II

HISTOPATHOLOGICAL GROUPS

Minimal change nephropathy		33
No light change (not MCN)		48
Membranous nephropathy		101
Mesangial proliferative		
IF IgA predom.	23	
IgM predom.	34	143
Other	22	
Not done	64	
Focal segmental proliferative		20
Diffuse endothelial proliferative		13
Mesangiocapillary		40
Others		46

(c) Membranous nephropathy: On light microscopy, the GBM appeared thickened uniformly. Immunofluorescence studies show immune complexes are incorporated into its structure.

(d) Mesangial proliferative: There was an increase in the number of cells in the mesangium. This may progress to a collapse of the glomerular 'tuft' and the formation of epithelial crescents. Immunofluorescence may show deposits of IgA or IgM in the mesangium.

(e) Focal segmental proliferative: This was a mesangial proliferation but involved only some glomeruli, and in a segmental fashion.

(f) Diffuse endothelial proliferative: There was a proliferation of endothelial cells as well as mesangial cells.

(g) Mesangiocapillary (Membranoproliferative): There was mesangial proliferation **AND** thickening of the GBM. The GBM had a double contour appearance due to a layer of tissue formed beneath it. Electron microscopy can distinguish two types, I (immune complex) and II (dense deposit disease).

(h) Others: These included a small number of patients with Focal Segmental Sclerosis (11 patients), as well as patients not classified into one of the above groups ('crescents' 19 patients; 'amyloid' 12 patients and 'vasculitis' 4 patients).

2.1.4 Treatment

Diuretics and antihypertensive agents were prescribed to the patients when clinically indicated. Hypertension was treated as soon as it was confirmed. Thiazides were used as a first line treatment if diuretics had not been used previously; antihypertensive agents (methyldopa and beta-blockers) were used subsequently.

From 1971, immunosuppressive agents and steroid therapy were reserved for patients with potentially responsive lesions, namely NLMC (vide infra). In the earliest years, a number of other patients received immunosuppressives and/or steroids. Preliminary results did not suggest the presence of any unsuspected cohort whose disease remitted on what was considered to be adequate therapy. The exception may be membranous nephropathy where some patients had benefitted from 'pulse' therapy (Short, Soloman, Gokal and Mallick, 1987).

2.2 The Variables Used and the Preparatory Statistical Analysis

2.2.1 The four-monthly follow-up data

Preliminary study was focussed on the following variables which were measured normally at four-monthly intervals: urinary protein loss (g/24h), diastolic blood pressure (mm Hg) and plasma creatinine (mmol/l). A ratio of the urinary protein loss to the urinary creatinine output (g/g over a 24h period) was calculated to minimise errors caused by inaccurate urine collection, since creatinine output is independent of urinary flow rate. (Since creatinine output is proportional to body weight, this may adjust for body size as well (Barratt,

1983)). Plasma albumin and haemoglobin concentrations (g/l and g/dl respectively) were added for some later analyses.

Plasma creatinine and albumin concentrations were measured by the hospital service laboratories using standard auto-analyser methods and urinary protein was measured by a turbidometric method using salicylsulphonic acid. Haemoglobin was measured using a standard Coulter counter.

The reference ranges for adults were as follows:

Plasma creatinine	up to 0.11 mmol/l
Plasma albumin	38-48 g/l
Haemoglobin males:	13-18 g/dl
females:	11.5-16.5 g/dl

In some of the analyses to be described the patients were grouped according to the above variables. Previous work had suggested urinary protein losses over 5 g/24h had significance (Mallick, Short and Hunt, 1987), therefore cut-offs of 5 and 10g were chosen for this variable. Cut-offs used for the urine protein/creatinine ratio were 4 and 8; these 'corresponded' to 5 and 10g in the sense that they were the same percentiles for all available measurements in the study.

A diastolic blood pressure of 110 mm Hg or more irrespective of age and sex was chosen to indicate 'moderate' hypertension. Such levels are associated with increased risk of cardiovascular disease. Treatment had often been initiated at lower levels (vide supra). For some analyses 'mild' hypertension was defined according to the normotensive limit for the patient's age and sex (Documenta Geigy, 1970).

Prior to statistical failure-time analysis, a separate computer data file was prepared which contained measurements

of these variables at the 'onset' (first manifestation of disease) and at each anniversary up to the fifth. Only measurements obtained within eight weeks from the relevant date were used. Other information such as sex, age (in years), histopathological group, period of follow-up and final outcome (see note in Appendix I) were included. Inter-relationships between the variables at the onset anniversaries were studied using standard statistical procedures available on the SPSS-X statistical package (SPSS 1988) on a University mainframe computer. A 5% level of significance was used throughout.

2.2.2 The 'renal screen'

The 'renal screen' was a battery of biochemical and haematological measurements tests carried out at infrequent intervals in addition to the four-monthly follow-up. From 1971, each new patient was screened at first biopsy, and thereafter at two to four-year intervals if proteinuria persisted. Typically each patient had between one and three renal screens, which were at variable times from the onset of renal disease.

From the screen variables measured in the laboratories, the following were chosen for statistical analysis:-

(a) Selectivity index:

The selectivity index was the ratio of the clearance of IgG (a large molecule, of molecular weight 150 KDa) to the clearance of transferrin (a small molecule, 88 KDa) (Hardwicke, Cameron, Harrison, Hulme and Soothill, 1970).

(b) Serum immunoglobulins:

Serum levels of IgM, IgG and IgA were measured by radial immunodiffusion. The laboratory reference ranges were:

IgM 0.5-2.0 g/l
IgG 7.0-16.0 g/l
IgA 1.0-4.3 g/l

(c) Serum complement components:

The total classical pathway haemolytic complement activity (CH50) was measured, and also serum levels of C1q, C4, C3 and Factor B.

For some components, the laboratory methods have changed and given rise to different reference ranges. The ranges for the period of this study were:

CH50	45-85 units	
C1q	50-150%	
C4	60-200%	(to mid-1981)
C3	100-200 mg/dl	(1971 to mid-1981)
Factor B	60-200%	(to mid-1981)

(d) Lipids and lipoproteins

The total fasting serum cholesterol concentration was measured, and also the S (small), M (medium) and L (large) particle 'scores'. S particles are LDLs (density 1.063-1.006 g/ml, Sf 0-20), M particles are VLDLs (density 1.006-0.96 g/ml, Sf 20-400) and L particles are chylomicrons (density <.96 g/ml, Sf >400). The laboratory converted the particle concentration to a 'score' which brought the lower limits of significant abnormality to approximately 4 on a scale 0 to 10 (Stone, Thorpe, Mills and Dick, 1971). The S score was a calculated quantity which was derived partly from the M

score but predominantly from the serum cholesterol concentration, with which it therefore would be expected to be highly correlated. The scoring system incorporated a transformation to remove skewness for M and L particles, but not for S.

The reference range for total serum cholesterol in the laboratory was 3.6-7.2 mmol/1. Normal fasting lipoprotein ranges were those given by Stone et al and specifically excluded nephrotic syndrome patients. Ranges for M particles differed for men and women. In addition, at high M concentrations, the M and L particle scores were correlated. The 95th percentiles were:

S score		3.6
M score	men	3.4
	women	2.3
L score		3.3

(e) Plasma fibrinogen:

Plasma fibrinogen was measured by the Folin-Coilcalteu method. The reference range was 1.5-4 g/1.

In a series of preparatory exercises, the relationships between each of the above variables (or set of variables) and plasma creatinine, 24 hour urinary protein loss and histopathological group were examined. Only one result, the first, was used per patient, so that the results would be statistically independent. These were transferred to separate computer data files, together with the plasma creatinine and urinary protein levels measured at the same time, and other details including the patient's sex and the time interval that has elapsed from the first manifestation of disease. Relationships were examined using standard statistical procedures available on SPSS/PC+ (SPSS/PC+ 1988) on an IBM compatible microcomputer.

2.3 Statistical Techniques for Failure-time Modelling

2.3.1 Start and end-points

Time was measured from the disease 'onset', where 'onset' was defined as the first recorded manifestation of disease.

The end-point for most of the analyses presented was ESRF (dialysis, transplantation without prior dialysis or death due to renal failure), and patients who died from other causes were censored. Plasma creatinine concentration was not used to define end-point because we were unable to define a level of plasma creatinine above which ESRF was inevitable.

In some separate analyses deaths not from renal failure but from causes which may have been related to the consequences of prolonged renal disease, such as ischaemic heart disease or cerebro-vascular disease, were examined.

The total number of deaths was compared with the expected number of deaths from all causes (the ICD 'F' codes). This was calculated by totalling the contributions made by each individual (see for example Hill, Laplanche and Rezvani, 1985), for each year of the study. The program written for this analysis is given in Appendix III. The number of observed and expected deaths can be compared assuming a Poisson distribution (see for example Pocock, Gore and Kerr, 1982).

The mortality rates used in the calculations were the death rates documented in the OPCS Registrar General's Statistical Review of England and Wales (see References) for the 'North West' region up to 1962. Between 1963 and 1973, rates for 'Manchester Hospitals Region' were available and these were used instead. They were slightly higher than the

'North West' figures and probably represented the study population more closely. Similar rates for the 'North West Regional Health Authority' were obtained from the OPCS Mortality Statistics by Area (see References) from 1974. All these rates include a small number of deaths from ESRF, but their contribution is so small that it can be ignored.

2.3.2 Univariate analyses

Patients were grouped according to values of the main variables (covariates) at onset and at the first anniversary (see section 2.2.1). The data at one year was more complete, since onset was frequently documented at an outlying hospital.

Kaplan-Meier survival estimates were calculated for the subgroups and compared by the Mantel-Cox test, which was implemented on the BMDP statistical package (BMDP 1983) and is equivalent to the logrank test.

Kaplan-Meier estimates are estimates of the survivor function S(t) (see Appendix II).

2.3.3 The hazard rate

The hazard rate at a point in time is the rate at which failures/deaths are occurring in the patients who have survived up to that time (see Appendix II).

To gain an initial impression of how the hazard rate changed with time, two functions were plotted: a 'piecewise-constant' event rate and a 'conditional' event rate (Kay and Schumacher 1983). For the former, the number of failures occurring in each twelve-month interval were divided by the

total number of patient-years at risk. For the latter, the proportional change in the survivor function S(t) over a twelve-month period was calculated at quarterly intervals.

Hazard rates were plotted for the subgroups of patients defined by the main covariates. This gave a crude indication as to whether the covariates changed the hazard rates proportionally (see also Appendix II).

2.3.4 Cox 'proportional hazards' regression analysis

This multivariate method was used to assess the independent effects of age, sex, plasmas creatinine concentration, moderate hypertension and urinary protein loss, measured at onset or at one year, on progression to ESRF (Cox 1972; Breslow 1974; BMDP 1983). Only patients with complete data sets could be analysed . The year of onset (minus 1900) was included to see if there was an underlying trend of improvement in survival over the study period.

The statistical significance of each variable was assessed by deleting the variable and using the likelihood-ratio test (BMDP 1983).

Plasma albumin and haemoglobin concentration and mild hypertension, defined according to the patients age and sex (see 2.2.1) were added in later analyses.

Once the most important prognostic variables were established, these were compared with the histopathological groups in the Cox analyses. A series of 'dummy' variables were included (no=0; yes=1) for each group; the baseline group for the analysis was membranous nephropathy. Dummy variables were also used to assess the effect of systematic disease on survival.

At this stage the model was checked. Proportionality of hazards was checked more carefully by stratification. Each of the significant covariates was chosen in turn to subdivide the patients into strata. Survivor functions S(t) were estimated for each stratum using the means of the remaining covariates: if the stratifying covariate changes the hazard proportionally, then plots of the ln (- ln(S(t))) against time for each should show constant vertical separation between strata (BMDP, 1983). Interactions between covariates also were considered. There were too few failures/deaths to consider the effects of fixed covariates changing with time (see Introduction section 1.3).

Finally, the Cox 'proportional hazards' model was refitted using the statistical package GLIM 3.77, available on a departmental IBM compatible microcomputer (GLIM 1986). The method was to fit a 'piecewise exponential' model, with the hazard rate assumed to be constant between successive failure-times (Aitkin, Anderson, Francis and Hinde 1989). In this method the individual exponential hazard rates were estimated after adjustment for the influence of significant covariates. They then were plotted to show the shape of the 'baseline' hazard function, since this may suggest an appropriate parametric model. The analysis included a test for equality of the individual exponential hazard rates; if they are equal, an exponential model can be fitted. If the rates increase or decrease monotonically, then a Weibull model can be fitted (see Aitkin et al for details of these models). In either case, the Cox 'proportional hazards' method would be appropriate, but may be less efficient since the hazard rate is left as an unknown entity.

2.3.5 Accelerated failure-time models

The use of accelerated failure-time models was explored. Both the log-normal and the log-logistic models were fitted, since these were suggested by the shape of the hazard function (see Results). The models were fitted using GLIM 3.77.

In the log-normal model, the logarithms of the failure-times are assumed to have a Gaussian or 'normal' distribution. The mean is modelled by a weighted linear composite of the covariates (see Appendix II); a positive weighting coefficient for a covariate indicates a slower progression to failure, and a negative coefficient indicates that the progression is more rapid. In the absence of censoring, the logarithms of the failure-time are modelled using ordinary least-squares regression analysis. In this instance, where there was censoring, the modelling was less straightforward; Aitkin's method, based on the Expectation-Maximisation algorithm, was used (Aitkin, Anderson, Francis and Hinde, 1989). The statistical significance of the covariates was obtained by dropping each covariate in turn and performing the likelihood-ratio test. Diagnostic plots can indicate whether the model is a good fit. Standardised residuals of the logarithmically transformed survival times (x-axis) were plotted against the times which correspond to the same survival estimates assuming a 'normal' distribution (y-axis); a straight line indicated a good fit.

In the log-logistic model, the distribution of failure-times is skewed and, therefore, similar to that of the log-normal. A different type of proportionality is assumed, however: that of 'odds on failure/death before time t' (see

Appendix II). If the derived weighting coefficient for a covariate is positive, then the odds-on failure is increased for increased values of the covariate, and vice versa. The hazard rates will eventually converge, irrespective of the initial covariate values; thus, after sufficient time has elapsed from onset, the covariates cease to affect the hazard rate.

As a preliminary check to see whether the log-logistic model was appropriate, estimates of the log-odds were calculated from the Kaplan-Meier survivor estimates. These, when plotted against the logarithms of the times, should yield a straight line; furthermore, lines for subgroups of patients defined by their covariate values should be parallel.

Roger and Peacock's method was used to fit the log-logistical model (Roger and Peacock 1983). Two diagnostic plots were used to check the fit of the model (Bennett and Whitefield 1981). First, the observed log-odds of death were plotted against the fitted values. Secondly, the estimates of the survivor function were plotted against their fitted values, both after arc-sine transformation. Both plots should give a straight line with a slope of unity if the fit is good.

Finally, using coefficients derived from the log-logistic model, it was possible to estimate, for certain covariate values, the expected time when the hazard rate would be maximum, and the maximum hazard attained.

2.3.6 Time-dependent covariates

The dependency of the hazard rate on covariates which changed with time was incorporated into the Cox regression analysis. This approach could only be used for covariates that were measured regularly. There were two areas of application, and these are described below.

In patients presenting with the nephrotic syndrome, the method was used to test whether there was a significant change in risk of ESRF associated with the occurrence of a complete, spontaneous remission of proteinuria. Patients with minimal change nephropathy were excluded for this analysis.

Lists of patient results were scanned manually to see whether a remission occurred; 'remission' was defined as a 24-hour urinary protein level below 0.3g, validated by a second result of less than 0.5g. The dates of remission were put onto a computer file, together with details of onset and outcome, and analysed using the BMDP program. The main covariate was a 'dummy' variable, defined for each patient, which took the value '0' before remission and '1' afterwards. The reciprocal of the plasma creatinine was also used to take into account renal function. If urinary protein measurements were not available for an extended period, then the patients survival was censored.

Further step changes in proteinuria could be incorporated using this approach, but with increasing difficulty of interpretation. Instead a more general approach, that described by Gail (Gail 1981), was used by us to see if there were differences in risk of ESRF associated with prevailing high or low covariate values. Computer programs were written in Fortran to carry out each stage of this analysis.

Marker states were defined according to the covariate status; for 24-hour urinary protein loss, for example, three states were defined, 0-4.99g, 5-9g and 10+g. One state was chosen as a baseline and 'relative risks' were calculated for the others. The significance of differences between the states overall was assessed using the likelihood-ratio test. Approximate 95% confidence intervals were derived assuming asymptotic normality.

In order to carry out Gail's analysis, it was necessary to determine the marker states for all patients at the time of each discrete ESRF event. An interpolation convention was needed to estimate covariate values at dates between the four-monthly attendances. A number were tried and finally the 'nearest' result within eight weeks was used. This was equivalent to assuming that the covariate changed in a stepwise fashion half way between attendances. Linear interpolation gave very similar results but the data are not shown. If the marker state could not be estimated, the patient was temporarily excluded. This gave the method an advantage over the method used for remission, above.

A computer program was written to extract the 'numbers at risk' at each discrete ESRF, the corresponding marker states, and the marker states of the ESRF patient. A second computer program was written to substitute the information into the log partial-likelihood equation and to maximise this equation using a library subroutine (Numerical Algorithms Group 1977). Details of both programs are given in Appendix IV. The relative risks were calculated from the marker state coefficients, which were assumed to be constant over time.

For the ESRF patient, proteinuria may be reduced because of the fall in GFR. The risk of ESRF may be better predicted by the marker state some time previously. Time lags of 4, 8, 12 and 24 months therefore were introduced into the analysis to explore this.

A further refinement was the incorporation of the prevailing level of plasma creatinine into the definition of marker state: whether, for example, the concentration was 0.2 mmol/l or more.

2.3.7 Markov models

The models outlined in the previous sections could not be used for the variables which were measured only at a renal screen. There were relatively few observations (usually between one and three per patient), and these were obtained at variable times from the onset of renal disease. It was impossible to analyse data at the onset or at the onset anniversary, since there were so few measurements for analysis, and interpolation to estimate between-screen values was impossible. Therefore, Cox proportional hazards regression models, using either fixed or time-dependent covariates, could not be applied.

The method developed by Kay (Kay, 1986), therefore, was used to relate levels of the screen variables with risk of ESRF. The method is univariate; two marker states were defined, depending on the value of the prevailing covariate. The two states were defined according to whether the level was above or below a certain cut-off point. The rates of transition from each of these marker states, both to other

states and to the death/failure state, were estimated (for definitions, see Appendix V). The rates were assumed to be the same for all patients, and constant in time.

Kay showed how to formulate the probability of moving between any two states i and j (or the death state) in a given time interval, in terms of these transition rates, providing that process is Markov. The Markov assumption is that the sojourn time is independent of the disease history prior to entering state i; the process thus 'forgets' any transition the patient has made before. He constructed a likelihood function from the probabilities calculated for the time intervals for each transition observed in the data set. The transition rates between states and to the death state were estimated by maximum likelihood.

The transitions from each marker state to the death state were compared using a Chi-squared test, so determining whether an increased or decreased level of the marker variable was associated with a worse outcome. Transition rates between the states also were examined.

A Fortran program to carry out the analysis for two marker states was kindly made available by its author Richard Kay. The program used subroutines E04EBF and F01AAF of the NAG subroutine library (Numerical Algorithms Group 1977); E0FEBF recently has been superceded by E04LAF. Minor modifications were made to the program to accommodate this, and to change the data input format.

A program was written by this author to abstract and compile, for any screen variable, the data in a form suitable for input to Kay's program. Details are given in Appendix

V. Briefly, for each patient, the number of data values had to be specified, together with the coded values of the variable ('1' for state 1 and '2' for state 2) and the associated time intervals from onset when the variable was measured. ESRF, if it occurred, was coded as '3', and again a time was specified.

A series of cut-points were explored for each variable which spanned the range of observed values.

In practice, it was difficult to verify whether the process was Markov. The checks suggested by Kay were followed wherever possible; transitions from state 1 to the death state were compared between those patients who had previously moved from state 1 to state 2 and those had not. Likewise, transitions from state 2 to the death state were compared between patients who had previously moved from state 2 to state 1 and those who had not. (In each case, Kaplan-Meier survival estimates were calculated and compared using the Mantel-Cox test).

2.4 Statistical Methods Used to study the Interrelationships Between Prognostic Variables

The preceding analyses established the variables most significantly related to survival. The study was concluded with an examination of the correlation structure between the variables, but without regard to the subsequent outcome.

One set of measurements were used per patient so that the measurement were statistically independent; this was the first complete set of measurements, and was usually the first renal screen. The data were analysed separately for males and

females since the reference ranges for some of the variables were different. Variable transformation were used as necessary to render the distributions approximately Gaussian.

A correlation matrix was calculated for these variables and studied. A Factor Analysis was carried using the Maximum Likelihood method available in SPSS/PC+ (SPSS/PC+ 1988).

The matrix of partial correlations was calculated from the correlation matrix using a computer package for mixed-interaction modelling (Edwards, 1987; Edwards, 1989). The aim was to find groups of variables which were highly correlated with each other. Each partial correlation coefficient represented the relationship between two variables eliminating the effects of all the others; the matrix showed which pairs of variables were unconditionally correlated with each other, and which appeared to correlate only because they were themselves related to others (Whittaker, 1990). MIM allowed non-significant interactions to be omitted to try to simplify the correlation structure.

CHAPTER THREE

RESULTS

Overview

The first section (3.1) of this chapter describes the mortality of the whole group of patients.

Results of a detailed study of the patients with a nephrotic syndrome onset are presented in the second section (3.2); the onset was more clearly defined in these patients. The variables which were measured four-monthly were analysed statistically as possible markers of prognosis. Results of analyses using 'fixed' covariates and time-varying covariates are presented.

In the third section (3.3), findings from the patients first presenting with asymptomatic proteinuria are compared and contrasted with the nephrotic syndrome patients. The same variables were used as markers of prognosis.

The more sparsely collected 'renal screen' variables were analysed for all the patients in the study and the results are given in the fourth section (3.4). The aim was to assess each variable as a marker of prognosis, but consideration also was given to possible correlations with the other variables.

The concluding section (3.5) describes in detail the correlations between the variables which had emerged as being the most significant indicators of prognosis.

3.1 Overall Survival

Table III shows the final outcome for all the patients in the study. The outcome depends on the follow-up time, therefore the survival experience is better described by the Kaplan-Meier curves shown in Fig. II. Eleven patients were

TABLE III

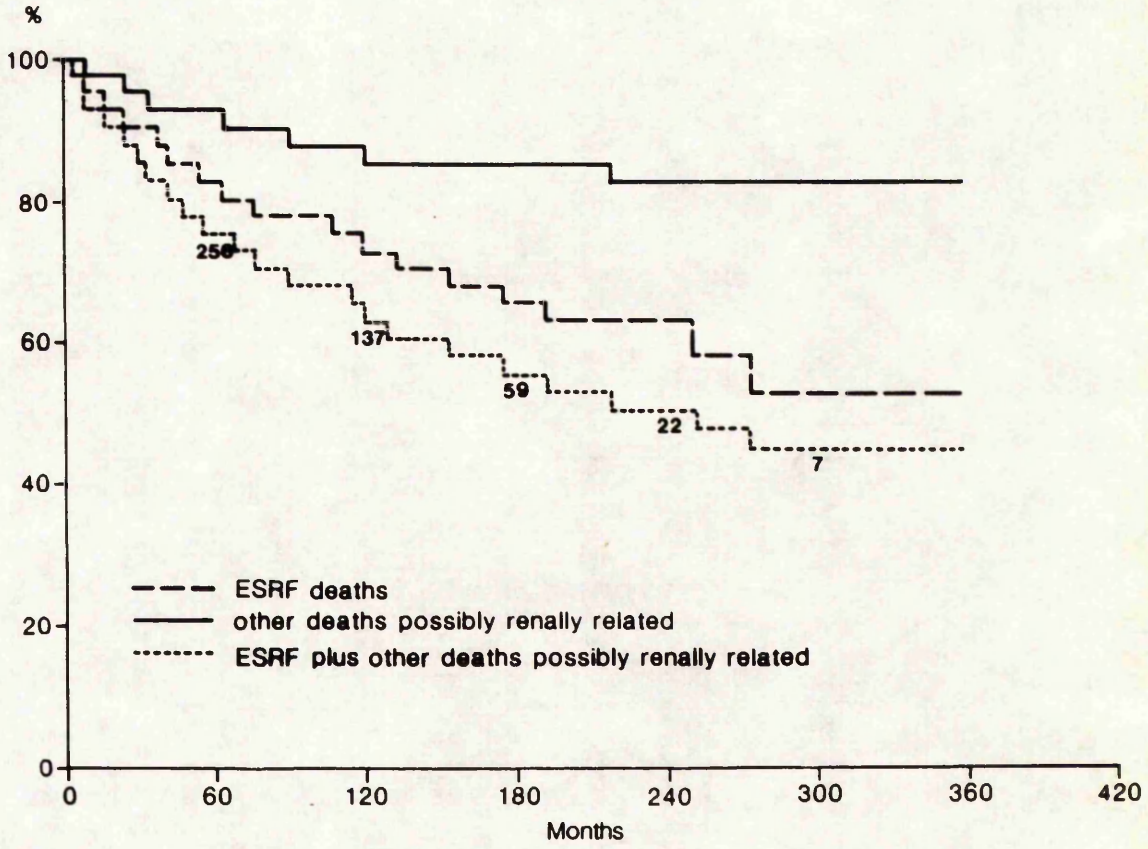
Final Outcome for All Patients in the Study

Alive with own functioning kidneys	271	61%	
Renal failure (dialysis/transplant)	75	17%	*
Death due to:-			
Renal failure	34	8%	*
Ischaemic heart disease	15	3%	
Hypertension/cardio-vascular accident	7	2%	
Malignancy	6	1%	
Other (possibly renally related)	17	4%	
Other (not renally related)	5	1%	
Cause not known	14	3%	

*These two groups combined = ESRF

FIG. II

Kaplan-Meier survival estimates for all the patients in the study



excluded because the month of onset was not known. The 'onset' was the first disease manifestation. The three curves shown correspond to three definitions of end-point, namely:-

(a) ESRF, censoring for other deaths and for patients alive and with their own functioning kidneys

(b) deaths which may have been related to the consequences of renal disease, such as ischaemic heart disease or cerebro-vascular disease, but censoring for ESRF, for deaths not believed to be related to renal disease or of cause unknown, and for patient alive and with their own functioning kidneys, and,

(c) either of these, whilst censoring for unrelated deaths and for patients alive with their own functioning kidneys.

The curves were plotted for a series of age subgroups and initial findings suggested that risk of ESRF deaths was unrelated to age (this was further explored in sections 3.2 and 3.3). There was, however, a trend with increased age of an increased mortality from other deaths, and this probably reflected normal ageing. The number of expected deaths was calculated for the whole study group, based on figures for the North-West region and taking into account the varying age and sex composition. These were compared with the observed numbers of deaths, including and excluding ESRFs, for a series of five-year follow-up periods, and are shown in Table IV.

The number of deaths for causes other than ESRF was significantly greater than the total number expected (Chisquared = 82.59, df=1, $P<0.001$). The ratio of observed to expected deaths however appeared to decrease over the years of the study, and the changes did not appear to coincide with the

TABLE IV

Comparison of Observed and Expected Mortality by year of the study

Years	ESRF	Death other than for ESRF (O)	Expected deaths from all causes (E)	Ratio O/E
1945-1959	0	0	0.166	(0)
1960-1964	3	6	0.715	8.4
1965-1969	26	14	2.914	4.8
1970-1974	25	21	6.156	3.4
1975-1979	41	19	8.970	2.1
1980-1984	14	4	2.762	1.4
All years	109	64	21.683	2.95

changes in rates used for the calculations. They may reflect a decrease in the deaths occurring in association with complications of renal disease, such as those associated with prolonged hypertension. This needs further study but was felt to be outside the scope of this work.

Figure III shows the Kaplan-Meier estimates of renal survival according to the first manifestation. The three largest groups only have been included, nephrotic syndrome, asymptomatic proteinuria and macroscopic haematuria; group sizes for the others were too small. There are differences in survival: in particular, patients with asymptomatic proteinuria, where 'onset' is the date when proteinuria was first apparent, appear to have a worse prognosis. The two larger groups are considered separately below. The macroscopic haematuria group proved to be too small to analyse in detail.

3.2 Patients with a Nephrotic Syndrome Onset

3.2.1 Analyses using fixed covariates

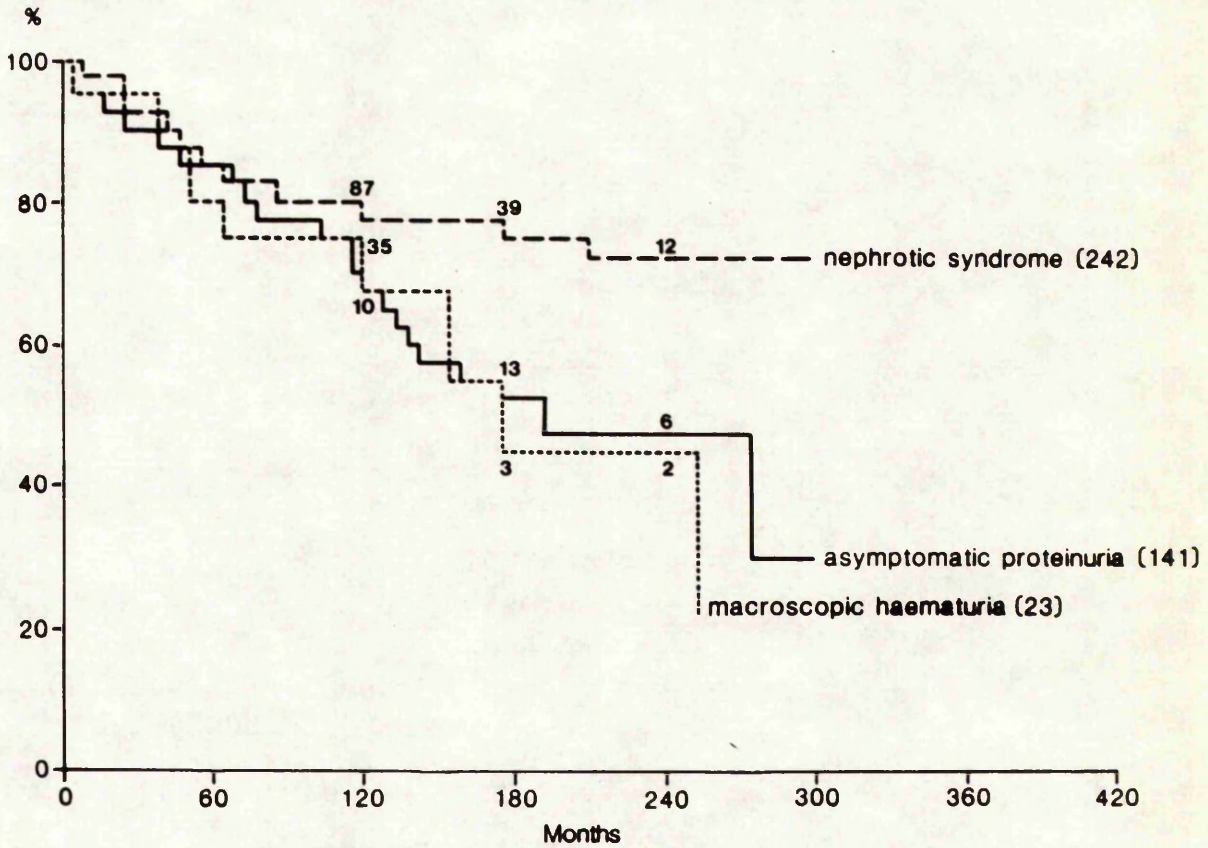
3.2.1.1 Preliminary findings

Of the total of 246 patients in this group, 162 (66%) were alive with their own functioning kidneys when last seen, 44 (18%) progressed to ESRF, and the remainder died from other causes (ischaemic heart disease 8, hypertension/cerebro-vascular disease 4, malignancy 5, others possibly renally related 9, others 3 and cause unknown 11). Thirty-one patients with systemic disease were included (systemic lupus erythematosis 15, amyloidosis 11, Henoch Schonlein purpura 3 and polyarteritis nodosa 2).

Kaplan-Meier survival estimates for ESRF for these patients have been shown previously (Fig. III). Four patients

FIG. III

Renal survival according to first manifestation



were excluded because the precise month of onset was not known.

Plots of the estimates of the hazard rates suggested a 'peak' risk of ESRF 3-4 years from onset (Fig. IV), but no peak was detected for the other deaths thought possibly related to renal disease. Only the piecewise-constant event rates are shown, since the two methods described (see Methods section 2.3.3) produced similar results.

The patients were subdivided according to variables of most probable prognostic importance (Table V); data at onset and at one year were considered. The mean age at onset was 38 (SD 19) for men and 36 (SD 18) for women. The combined age distribution was bimodal, with peaks occurring for age groups 10-19 and 50-59 (data not shown).

Comparisons between these groups with regard to renal survival (end-point ESRF) are summarised in Table VI, and a selection of the analyses are illustrated in Figs. V(a) to (f). Only two patients progressed to ESRF during the first year.

An elevated plasma creatinine, either at onset or at one year, and heavy urinary protein loss persisting at one year were the factors most significantly related to eventual ESRF. Figures V(b) and V(c) show a trend of worsening prognosis with increased plasma creatinine. Other factors associated with a worse outcome were male sex and a diastolic blood pressure of 110 mm Hg or more at onset.

Blood pressure levels of 90 and 100 mm Hg were also considered as cut-offs (data not shown). Patients with levels of 90 mm Hg or more at onset appeared to have a slightly higher risk but did not differ significantly from those with

FIG. IV

Nephrotic syndrome onset -
Piecewise-constant event rates for ESRF and
other possibly renally-associated deaths

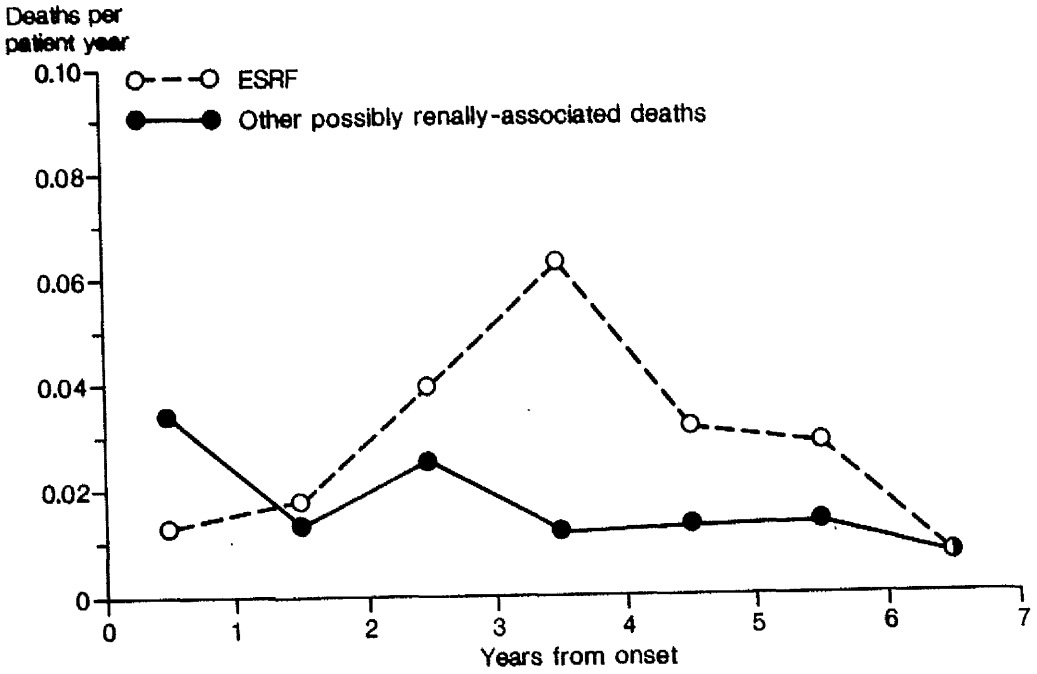


TABLE V
Nephrotic syndrome onset - groupings of variables showing numbers of patients for whom data were available

Sex:	Male	156	(63%)
	Female	90	(37%)
Age at onset (years):	Less than 20	67	(27%)
	20 to 34	40	(16%)
	35 to 49	61	(25%)
	50 or more	78	(32%)

	At onset:		At one year:	
Plasma creatinine (mmol/l):				
less than 0.12	41	(53%)	77	(55%)
0.12 to 0.19	22	(29%)	39	(28%)
0.20 or more	14	(18%)	23	(17%)
Total	77		139	
Diastolic blood pressure (mm Hg):				
less than 110	114	(83%)	155	(92%)
110 or more	24	(17%)	13	(8%)
Total	138		168	
24 hour urinary protein (g):				
less than 1	0	(0%)	29	(19%)
1 to 4.99	19	(21%)	31	(21%)
5 to 9.99	26	(29%)	24	(16%)
10 or more	45	(50%)	67	(44%)
Total	90		151	
Urinary protein/creatinine ration (g/g):				
less than 4	7	(12%)	42	(34%)
4 to 7.99	23	(40%)	29	(24%)
8 or more	28	(48%)	52	(42%)
Total	58		123	

TABLE VI
Nephrotic syndrome onset - comparison of renal survival in subgroups of patients

Variable	Groups Compared	Significance *	Comments:
Sex	male v female	NS (P=0.53)	worse survival for males
Age (years)	see table 1	NS	slight trend towards better survival in younger patients
Plasma creatinine (mmol/l)	less than 0.2 v 0.2 or more	at onset: P=0.037 at one year: P<0.001	} trend towards } worse } survival with } increased
	less than 0.12 v 0.12 to 0.19	at onset: NS at one year: P<0.001	} creatinine }
Diastolic blood pressure (mms Hg)	less than 110 v 110 or more	at onset: P=0.037 at one year: NS	worse survival with increased blood pressure at onset only
Urinary protein (g/24hr)	less than 5 v 5 or more	at onset: NS at one year: P = 0.005	worse survival with higher levels at one year only
	less than 1 v 1 - 4.99	at one year: NS	
	5 - 9.99 v 10 or more	at one year: NS	
Urinary protein/ creatinine ratio (g/g)	less than 4 v 4 or more	at onset: NS (P=0.075) at one year: P = 0.011	worse survival with increased levels at one year
	4 - 7.99 v 8 or more	at one year: NS	

*Significance by the Mantel-Cox test.

NS = Not significant

FIG. V(a)

Nephrotic syndrome onset -

Renal survival according to:

(a) Sex

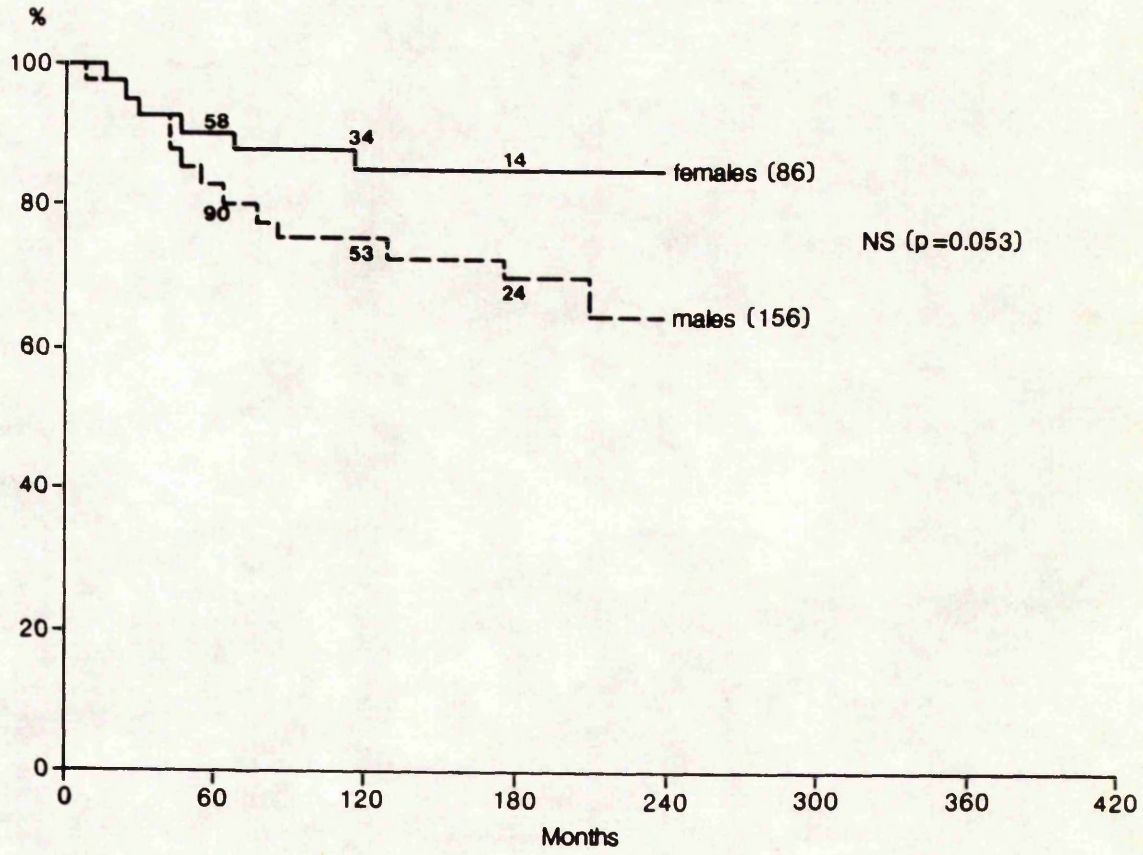


FIG. V(b)

Nephrotic syndrome onset -

Renal survival according to:

(b) Plasma creatinine at onset

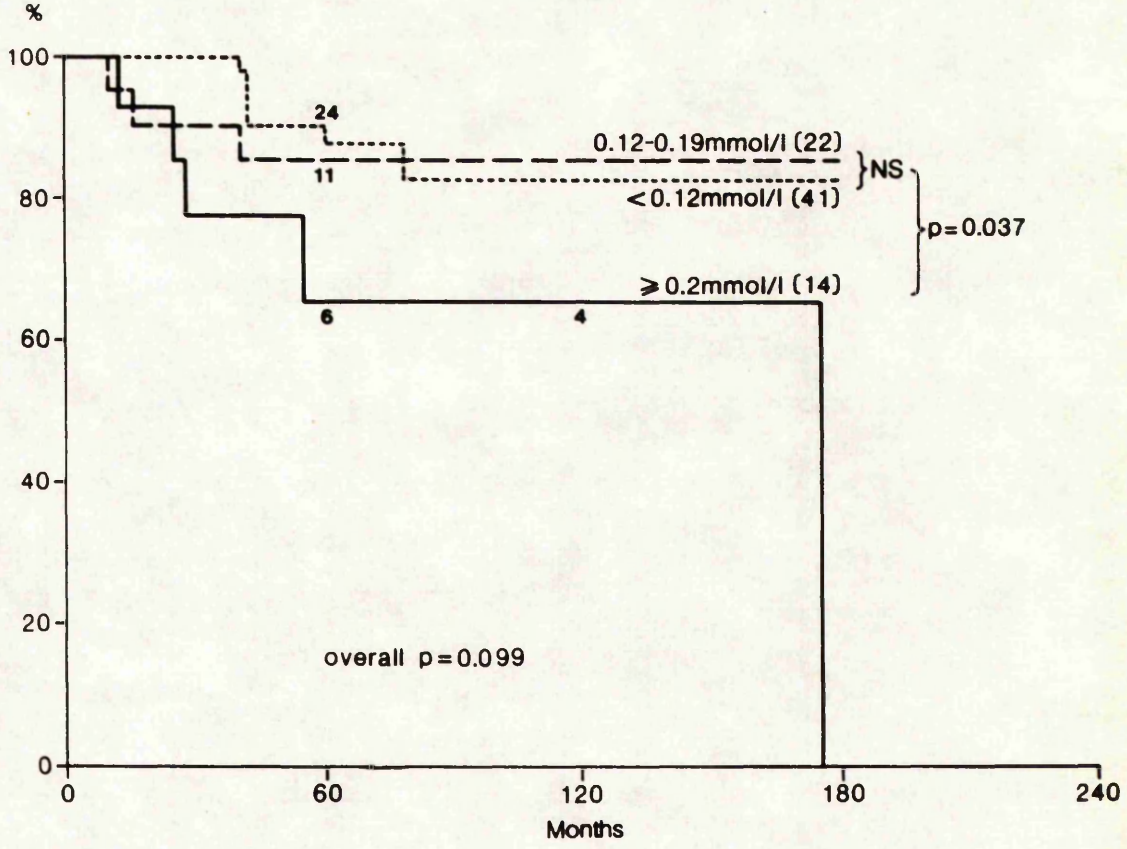


FIG. V(c)

Nephrotic syndrome onset -

Renal survival according to:

(c) plasma creatinine at one year

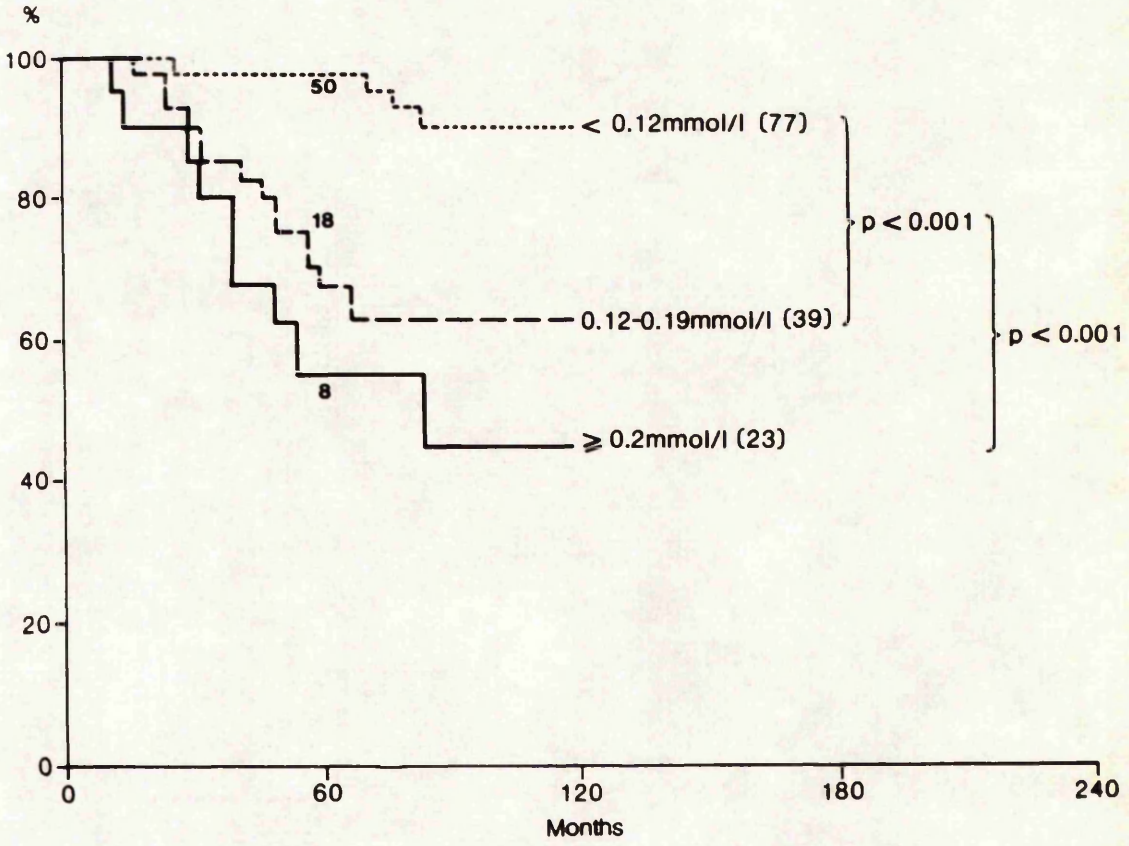


FIG. V(d)

Nephrotic syndrome onset -

Renal survival according to:

(d) diastolic blood pressure at onset

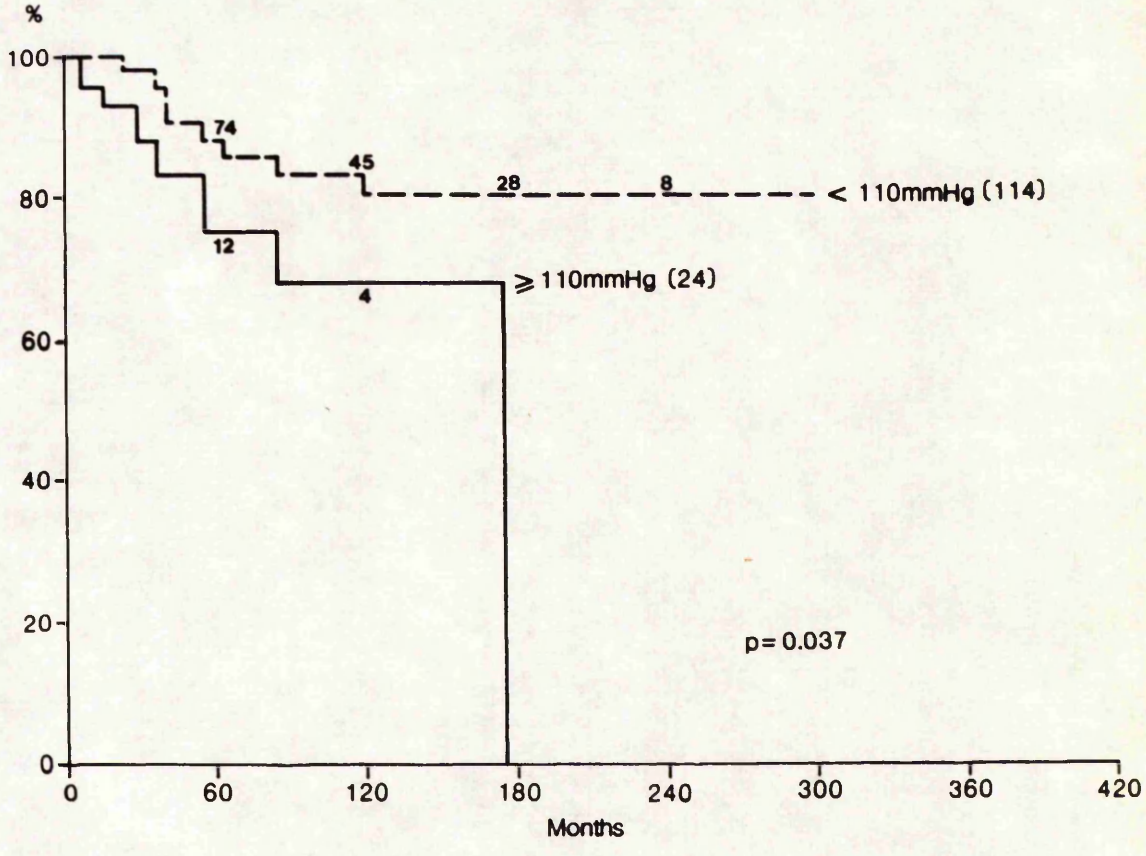


FIG. V(e)

Nephrotic syndrome onset -

Renal survival according to:

(e) 24h urinary protein loss at one year

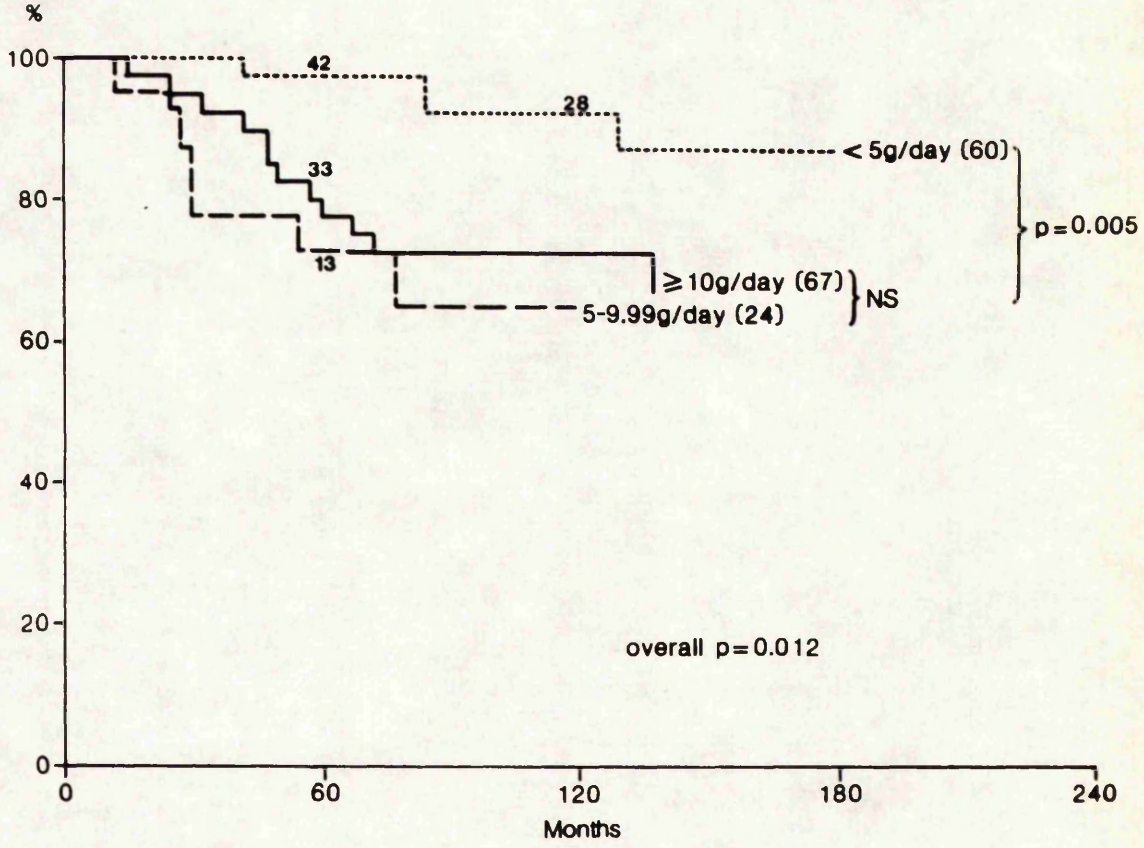
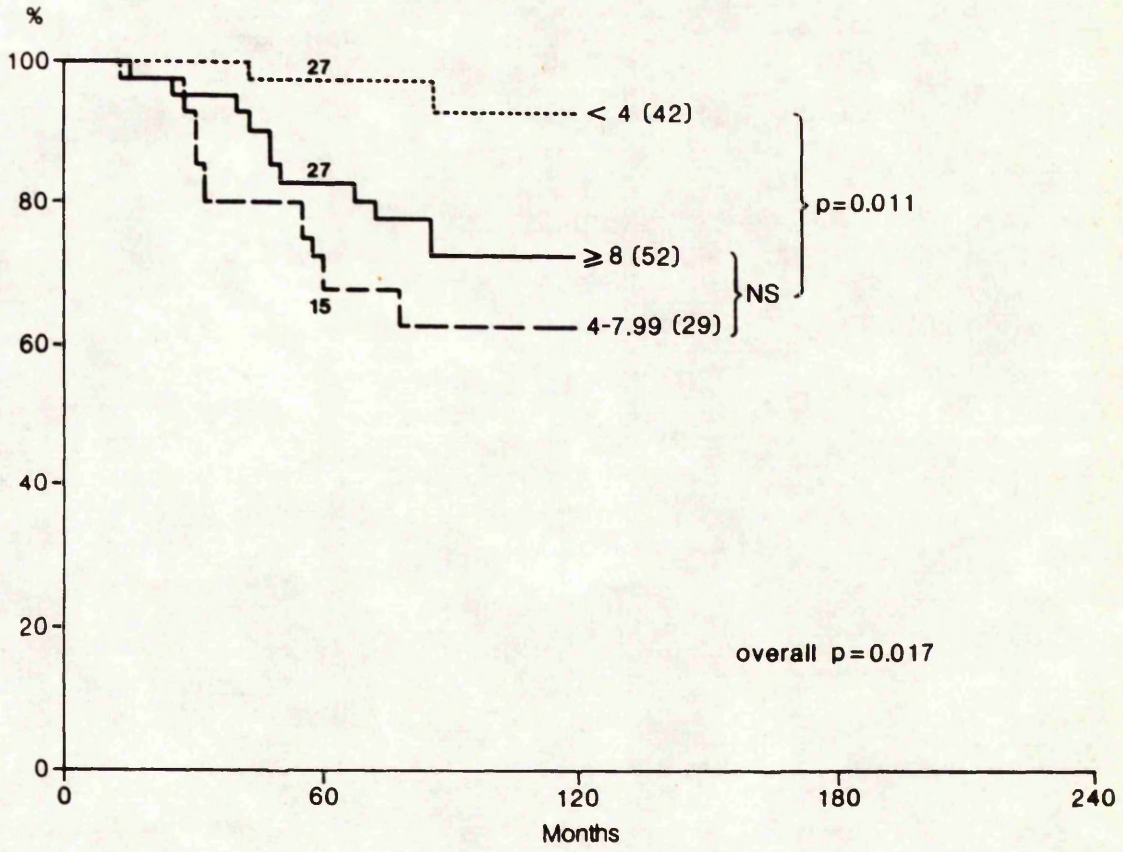


FIG. V(f)

Nephrotic syndrome onset -

Renal survival according to:

(d) urinary protein/creatinine ratio at one year



levels below 90 (P>0.10). Patients with levels of 100 mm Hg or more observed at onset had a poorer prognosis than the remainder, but this difference also was not statistically significant (P=0.086).

Hypertension was difficult to assess prognostically, since it was usually treated promptly and rarely persisted. Of the 24 patients hypertensive (diastolic pressure 110 mm Hg or more) at onset, 'control' if defined by two successive levels below 100 mm Hg, was achieved in all but two patients after a median of 11 weeks. Levels fell spontaneously for five patients and, for the remainder, occurred after a median of 6 weeks therapy. The cohort with hypertension at one year were previously untreated; almost all had been normotensive at onset, and their hypertension was not associated with prognosis.

Two thirds (62%) of patients were hypertensive at some time in their follow-up, but the levels did not remain high. For example, they had fallen below 110 (on two successive occasions) within four months in 83% of patients and within eight months in 91%. Levels fell spontaneously in 46% of patients and were associated with therapy in the remainder (antihypertensives +/- thiazides 37%, thiazides only 17%). Half of the patients went on to have further periods of hypertension which lasted rather longer; levels had fallen in only 70% by four months, but in 89% by eight months. Spontaneous falls were less common (28%).

Mild hypertension, as defined by the Ciba-Geigy limits, which took into account age and sex, was also considered as a prognostic variable. 25% of patients were hypertensive at onset and 14% of patients at one year. Survival in this group

of patients did not differ significantly from the remainder, although the patients hypertensive at one year showed a trend towards worse survival.

Plasma albumin and haemoglobin concentrations were analysed in a similar way (Table VII). The cut-off points used for haemoglobin were the lower limits of the reference ranges, separately defined for males and females.

There was some evidence that albumin levels below 30 g/l at one year were associated with a worse prognosis (see Fig. VI(a)), but this was not statistically significant. The inverse relationship between plasma albumin and urinary protein loss was shown previously in this series (Hunt, 1982), and there was a rise in mean level over the first year which corresponded to the fall in proteinuria (see below).

In Table VII it can be seen that fewer women than men were anaemic; these differences were statistically significant (Chi-squared test: at onset and at one year $P<0.001$). The patients who were anaemic at one year had a worse prognosis, but the differences were not statistically significant (see, for example, Fig. VI(b)). Haemoglobin concentration was positively correlated with the reciprocal of the plasma creatinine concentration; haemoglobin concentration were lowest for patients with lowest GFR. This correlation, however, was statistically significant only for males, whose average results were higher (for example, at one year males $r=0.45$ two-tailed $P<0.001$, females $r=0.23$ NS; the results at onset were similar).

If the deaths for causes other than renal failure were actually renally related, one might expect an increased risk of such deaths in those patients with high plasma creatinine

FIG. VI(a)

Nephrotic syndrome onset -

Renal survival according to:

(a) plasma albumin at one year

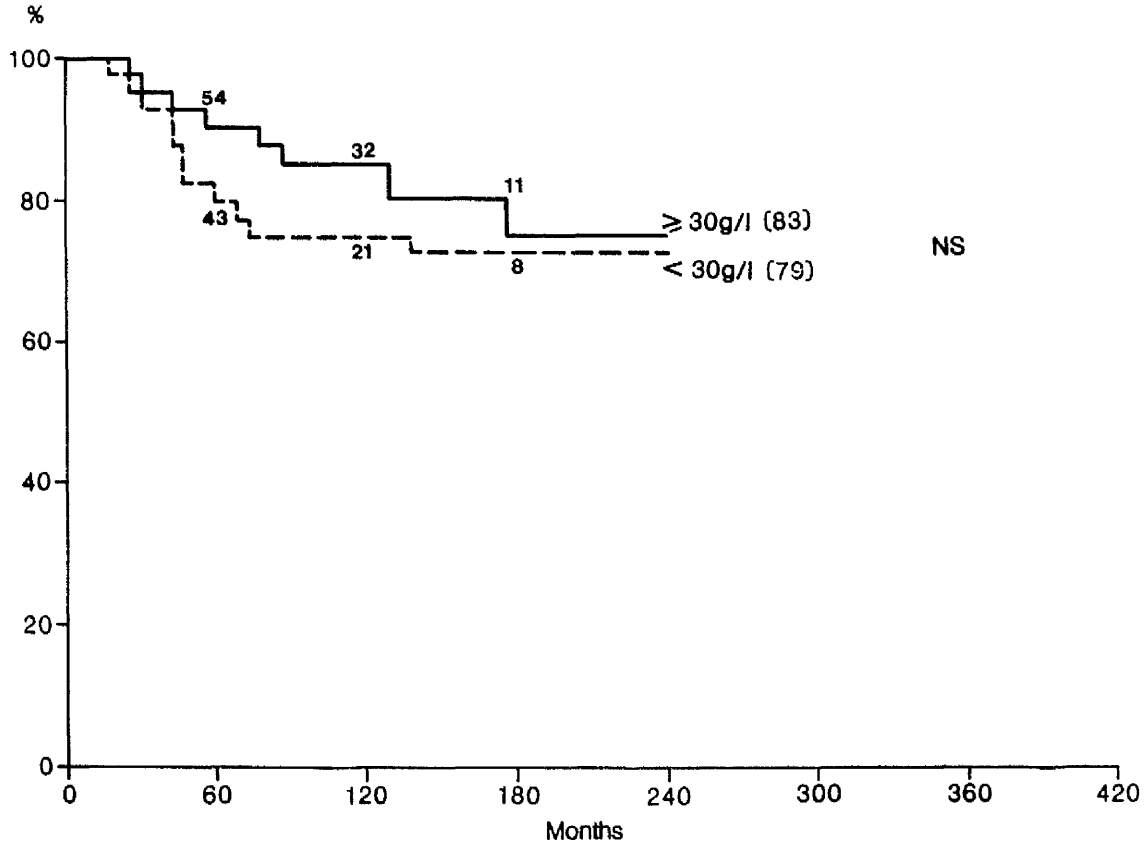


TABLE VII
Nephrotic syndrome onset - further variable groupings and comparisons of renal survival

Plasma albumin (g/l)

at onset:			
less than 20	53	(43%)	}
20 to 29	57	(47%)	} NS
30 or more	12	(10%)	}
Total	122		
at one year:			
less than 20	15	(9%)	}
20 to 29	64	(40%)	} NS but 30+ group
30 or more	83	(51%)	} better
Total	162		

Haemoglobin (g/dl)

for males:

at onset:			
less than 13	22	(28%)	} NS
13 or more	57	(72%)	}
Total	79		
at one year:			
less than 13	26	(33%)	} NS but below 13
13 or more	54	(67%)	} worse
Total	80		

for females:

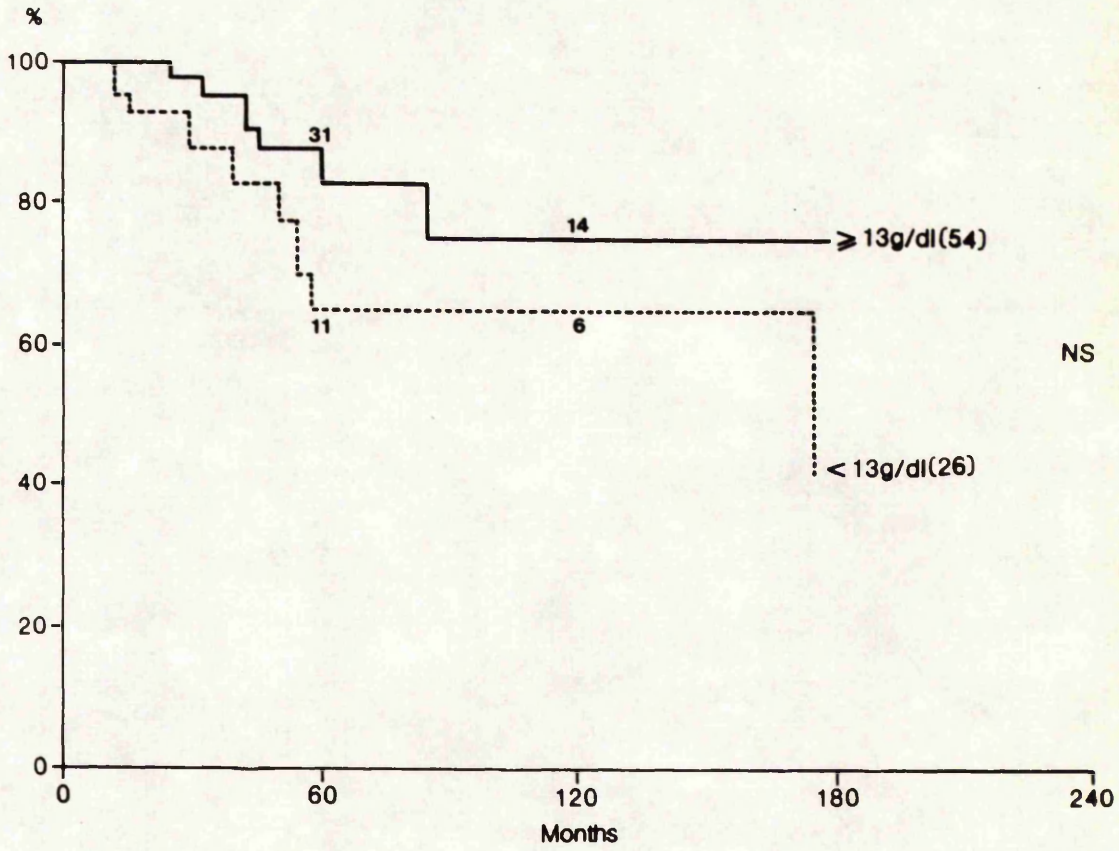
at onset:			
less than 11.5	6	(14%)	} Too few to test
11.5 or more	36	(86%)	} (only one ESRF)
Total	42		
at one year:			
less than 11.5	10	(17%)	} NS but below 11.5
11.5 or more	49	(83%)	} worse
Total	59		

FIG. VI(b)

Nephrotic syndrome onset -

Renal survival according to:

(b) haemoglobin at one year for males only



(0.2 mmol/l or over at one year), when ESRF and unrelated deaths are censored. (Plasma creatinine was strongest indicator of prognosis above). The prognosis in this group of patients, however, did not differ significantly from the remainder (P>0.1).

It was not possible to relate the concentration of plasma creatinine with the development of, rather than mortality from, the other related diseases; 32 patients, for example, were known to have developed angina or myocardial infarction over the course of their follow-up, but the dates for these were not available.

3.2.1.2 Interrelationships between variables longitudinally

Figures VII(a) and VII(b) show that the percentage of patients with heavy proteinuria fell with time, and that there was a significant difference in urinary protein loss between the sexes at onset and at one year (Chi-squared test P<0.025 and P<0.05). This may be a reflection of body size, but at one year, differences between the urinary protein/creatinine ratios were also significant (P<0.025). This correction for urinary creatinine output may adjust for body size, since, unlike urinary protein output, the ratio did not correlate significantly with weight and surface area. The fall in urinary protein loss with time was not attributable exclusively to the death (and so loss from further analysis) of patients with heavy losses, and a similar trend was observed within the five-year survivors, suggesting overall that the underlying lesions in surviving patients healed gradually.

While overall plasma creatinine levels remained steady

FIG. VII(a)

Nephrotic syndrome onset -

Distribution of:

(a) 24h urinary protein loss for males and females, at onset and first and fifth onset anniversaries.

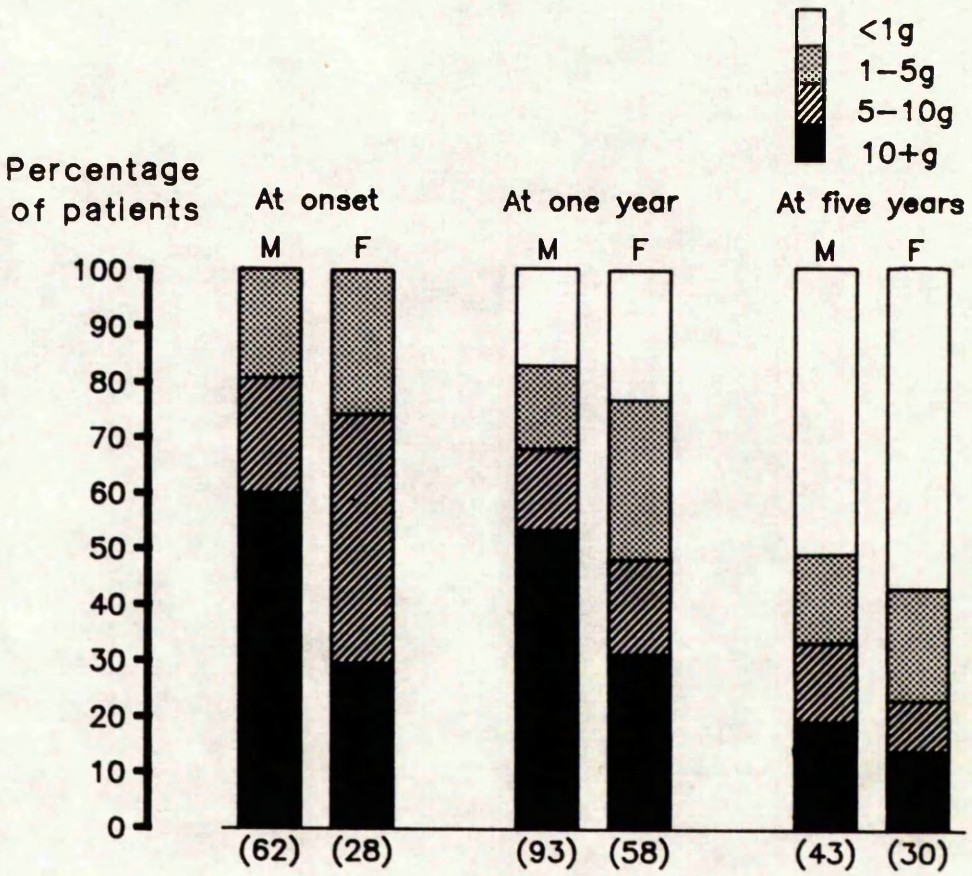
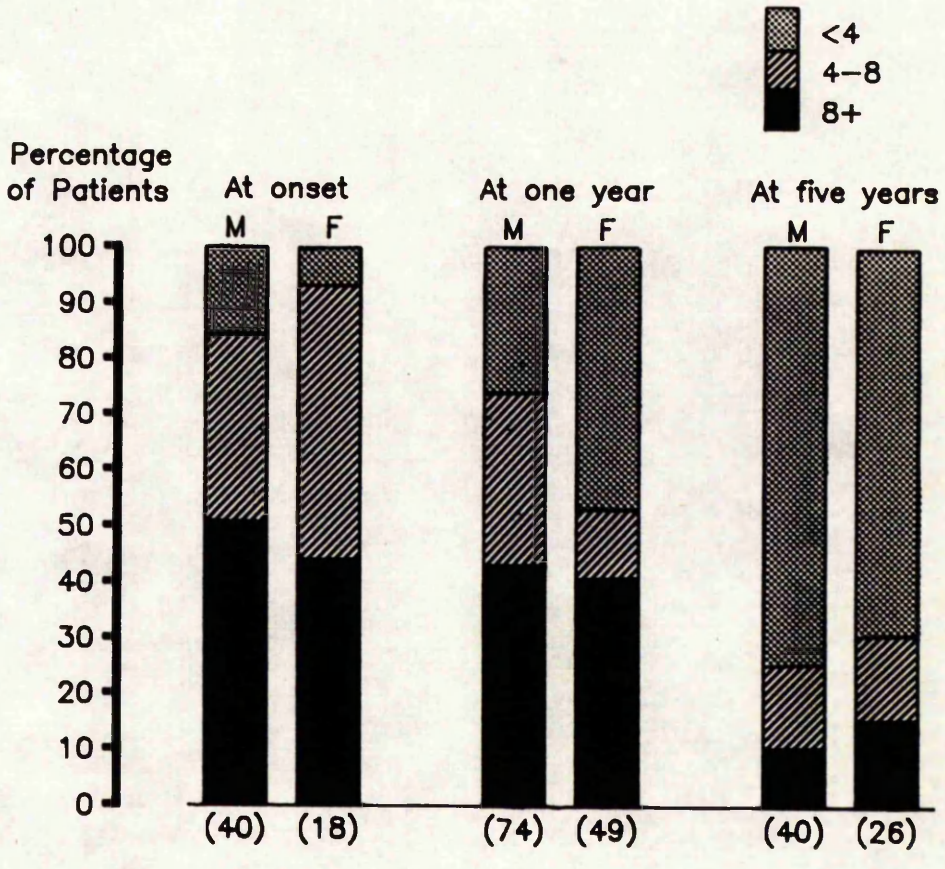


FIG. VII(b)

Nephrotic syndrome onset -

Distribution of:

(b) urinary protein/creatinine ratio for males and females at onset and first and fifth onset anniversaries.



(Fig. VIII), levels were compared between the sexes after correction for weight and for surface area. Males had significantly higher levels at each anniversary from one to five years (Mann-Whitney U-test, maximum $P<0.05$).

The relationship between prevailing heavy urinary protein loss and plasma creatinine level at onset, one and five years, is shown in Table VIII. Plasma creatinine levels were significantly higher for those patients with persisting very heavy proteinuria (urinary protein/creatinine ratio =4) at one and at five years.

3.2.1.3 Histopathology

The histopathological spectra were very similar for males and females (data not shown).

Kaplan-Meier estimates for ESRF were calculated for the seven main clinico-pathological groups. Table IX shows the results at 5 and 10 years. There were statistically significant differences between the curves (Mantel-Cox $p<0.001$). Minimal change nephropathy had the best prognosis; no patients in this group progressed to ESRF. Prognosis for mesangiocapillary disease was the worst, but it was not possible to analyse Type I and Type II disease separately, since only five of these patients had had electron microscopy. The remaining groups did not differ significantly ($p>0.1$), although two of the group sizes were small. These findings were not influenced by exclusion of the patients with underlying systemic diseases.

Patients with Minimal Change nephropathy and mesangiocapillary disease differed from the remainder at one year: then the percentages with urinary protein creatinine ratio above 4 and plasma creatinine level above 0.2 mmol/l

FIG. VIII

Nephrotic syndrome onset -

Distribution of plasma creatinine for males and females, at onset and first and fifth onset anniversaries.

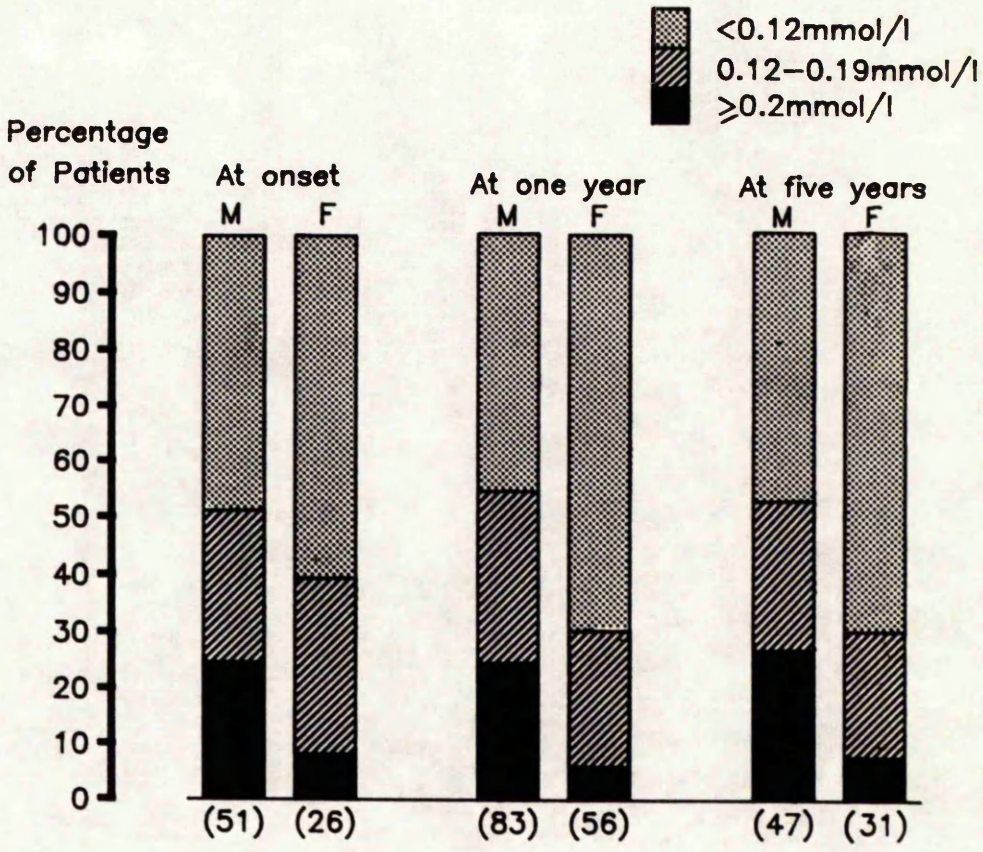


TABLE VIII
Nephrotic syndrome onset - relationship between prevailing urinary protein/ creatinine ratio and plasma creatinine level

	Plasma Creatinine Level at:		
	Onset:	**One year:**	**Five years:**
Prevailing urinary protein/creatinine ratio:			
<4	0.10* 0.08-0.24 (6)	0.09 0.04-0.34 (41)	0.10 0.04-0.69 (48)
4+	0.12 0.05-0.53 (47)	0.12 0.02-1.13 (80)	0.14 0.06-0.97 (18)
	NS+	P=0.012	P=0.007

* median, range, (n)

\+ comparison by two-tailed Mann-Whitney U-test.

NS = Not significant

TABLE IX

Nephrotic syndrome onset -
Kaplan-Meier estimates of renal survival for
the seven main histopathological groups

	Number:	At five years:	At ten years:
No light microscopic change (not MCN)	23	86%	78%
Minimal change	33	100%	100%
Membranous nephropathy	65	89%	87%
Mesangial proliferative	65	84%	75%
Focal segmental proliferative	9	(88%)	(88%)
Diffuse proliferative	7	(86%)	(86%)
Mesangiocapillary	21	65%	57%

were 43% (of 14) and 7% (of 15) for MCN, 80% (of 10) and 21% (of 14) for MCap and 66% (of 92) and 19% (of 86) for the remainder.

The survival curves for patients with systemic lupus erythymatosus (15) and amyloidosis (11) was compared with the remainder. (The other systemic disease groups were too small for analysis). There was a significant difference between the three groups (P=0.001). Renal survival was similar in the two systemic disease groups, and was worse than in the remaining patients. Most (7) of the amyloid patients were in the 'other' histopathological group, which was not included in the comparisons in Table IX. Patients with systemic lupus erythematosus were dispersed throughout the biopsy classifications shown.

This series of univariate survival analyses had confirmed the importance of the variables we had chosen, but there were obvious interrelationships between the variables. These were explored further in a series of Cox regression analyses.

3.2.1.4 Multivariate analysis

Multivariate analysis was carried out using the Cox proportional hazards regression model, with end-point ESRF, for patients with complete data for the covariates sex, age, plasma creatinine, moderately raised diastolic blood pressure (110 mm Hg or more), heavy protein loss (urinary protein/creatinine ratio of 4 or more) and the year of onset, which ranged from 1961 to 1979 in these patients. A reciprocal transformation was used for the plasma creatinine

concentration since a preliminary analysis had shown this to be a better indicator.

At onset, the data set was incomplete, since this was frequently documented at an outlying hospital. Only 52 patients had adequate data. Of these, 47 males were analysed; none of the five females in this group progressed to ESRF. Only heavy urine protein loss at onset (urinary protein/creatinine ratio $\geq$ 4) was significantly associated with progression to ESRF (P=0.025) in these patients.

At one year, 113 patients had complete data sets. Plasma creatinine level and heavy proteinuria (ratio $\geq$ 4) were the most significant, independent factors (P<0.001; P=0.052 respectively, see Table X(a). Sex, age and hypertension were not statistically significant, neither was the year of onset, implying that the underlying prognosis had not changed over the years of the study.

An analysis at one year using only plasma creatinine and heavy proteinuria (121 patients) is shown in Table X(b). Relative risk of ESRF associated with heavy proteinuria (ratio $\geq$ 4) is exp (1.3) or 3.5.

There was no significant interaction between the two prognostic variables.

In separate analyses, 'mild' hypertension (defined according to the patients age and sex), plasma albumin and haemoglobin concentrations were analysed, together with the plasma creatinine and heavy proteinuria at one year. None of these covariates were statistically significant.

'Dummy' variables (no=0; yes=1) were created to denote those patients with systemic lupus erythematosus and

TABLE X
Nephrotic syndrome onset -
Cox proportional hazards regression using
information at one year and end-point ESRF

(a) **For the 113 patients with complete data on the six variables:**

Sex:	0.127 SE 0.620	NS*
Age in years:	-0.015 SE 0.014	NS
(Plasma creatinine)$^{-1}$	-0.268 SE 0.083	P<0.001
Diastolic BP ($\geq$ 110 mm Hg):	-0.850 SE 1.046	NS
Urinary protein/creatinine ratio ($\geq$ 4):	1.286 SE 0.765	NS (P=0.052)
Year of onset:	-0.007 SE 0.063	NS

(b) **For the 121 patients with complete data on the two most important variables:**

(Plasma creatinine)$^{-1}$	-0.266 SE 0.075	P<0.001
Urinary protein/creatinine ratio ($\geq$4):	1.255 SE 0.749	P=0.049

* Coefficients with associated standard errors (SE) and significance by the likelihood ratio test

NS = Not significant

amyloidosis, and these were included in the Cox model, together with plasma creatinine and heavy proteinuria at one year. There were so few patients with these diseases (9 and 2, respectively, out of the 121) that they were combined together. Their survival did not differ significantly from the remainder ($P>0.10$) when the plasma creatinine and heavy proteinuria were taken into account.

The numbers of patients in the individual histopathologically defined groups were too small for separate multivariate analyses. Univariate analyses, however, suggested that both increased plasma creatinine and heavy proteinuria were associated with worse prognosis within the larger groups (membranous nephropathy, mesangial proliferative and mesangiocapillary), but these findings failed to reach statistical significance.

The biopsy findings were included in the Cox model, by employing a series of 'dummy' variables, for those patients with measured plasma creatinine and proteinuria at one year. No patient with minimal change nephropathy progressed to ESRF; neither did any of the very small group of patients (5) with diffuse endothelial proliferative disease. These and the 'other' group (7) were excluded, leaving 95 patients for analysis. The five remaining biopsy groups did not differ significantly overall ($P>0.10$, by the omission of four 'dummy' variables), after adjustment for plasma creatinine and heavy proteinuria, both of which were statistically significant ($P=0.004$ and $P=0.039$ respectively). This suggested that the two clinical variables may be more useful markers of outcome

than the histopathological findings, although the findings were based on relatively small group sizes.

Examination of the coefficients derived for the biopsy groups suggested that mesangiocapillary disease was associated with the greatest risk of ESRF, and this was tested separately. After allowing for plasma creatinine and urinary protein loss, a histological diagnosis of mesangiocapillary disease was associated with a worse prognosis (P=0.038).

3.2.1.5 Adequacy of the Cox model

Preliminary investigations had included plotting hazard rate estimates (piecewise-constant event rates) for patients with increasing values of plasma creatinine (0-0.11, 0.12-0.19 and 0.2+ mmol/l) and urinary protein/creatinine ratio (<4, 4+). The hazard rates confirmed a uniform increase, but this could not test the proportionality of hazards. To test this, two stratified analyses were carried out (see Methods section).

First the data were stratified by plasma creatinine, and the single covariate proteinuria was fitted; next the stratification was by proteinuria, and plasma creatinine was fitted. The two diagnostic plots are shown in Figs. IX(a) and (b). In either case, constant vertical differences should be observed between strata. It is probable that the model was satisfactory; the divergence may have reflected the small groups in the stratification and the relatively few ESRFs. The results were not conclusive.

A further stratification was carried out according to whether the patients had systemic lupus erythematosus or

FIG. IX

Nephrotic syndrome onset -

Diagnostics for the Cox proportional hazards regression model for renal survival, stratifying by:-

(a) plasma creatinine

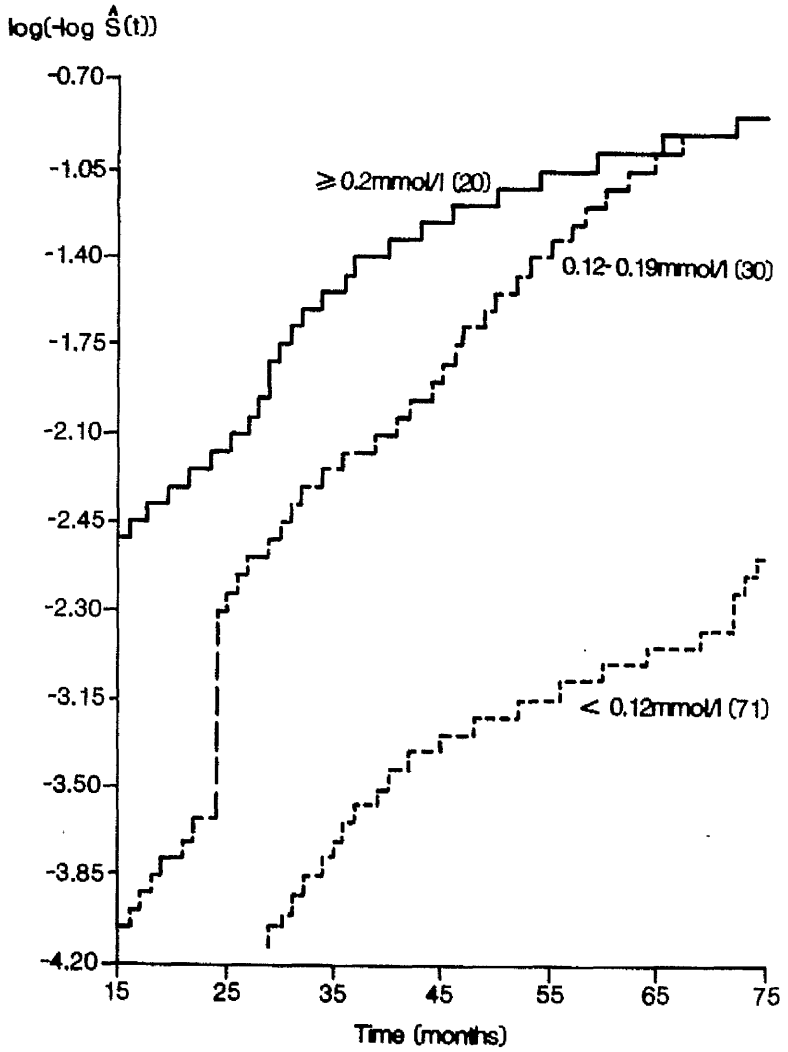
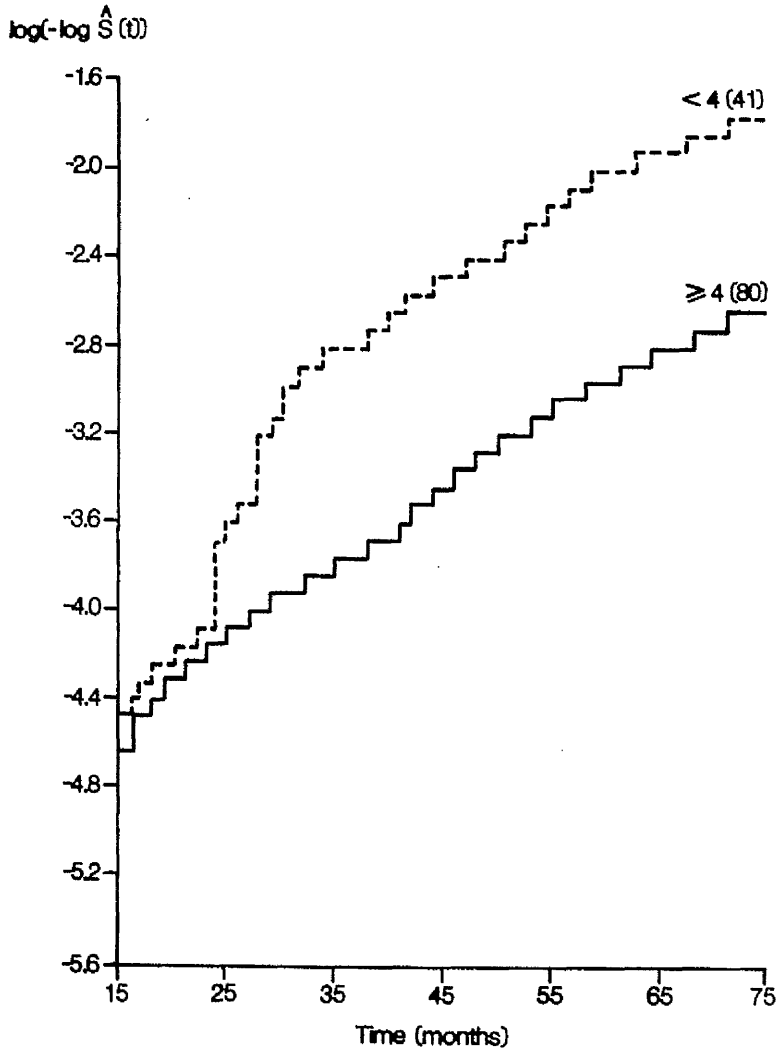


FIG. IX

Nephrotic syndrome onset -

Diagnostics for the Cox proportional hazards regression model for renal survival, stratifying by:-

(b) urinary protein/creatinine ratio



amyloidosis. This allowed for the fact that these patients may have had a different underlying hazard rate. The results confirmed the importance of the two covariates in the model, plasma creatinine and heavy proteinuria. Furthermore, the diagnostic plots were parallel, suggesting that the underlying hazards were similar and, in addition, that the previous use of a 'dummy' variable for these diseases was appropriate.

A similar analysis confirmed the appropriateness of the 'dummy' variable used for mesangiocapillary disease (see above).

A major concern was the large amount of missing data, particularly at onset; the omission of these patients may have biased the findings. Patients with known plasma creatinine and proteinuria were compared to those where one or both of these variables were missing. This was repeated for the variables at one year. The two survival curves were virtually identical in each case.

3.2.1.6 Parametric models

3.2.1.6.1 The 'piecewise exponential' model

This method enabled the shape of the hazard rate to be determined after making adjustment for the two important covariates (that is, the reciprocal of the plasma creatinine level at one year and urinary protein/creatinine ratio of 4 or more). The cut-offs used were the actual ESRF times, and therefore the results are the same as the Cox analysis given above. The 121 patients with measurements for both

covariates were analysed. The cut-points were respectively 53, 67, 107, 109, 124, 126, 135, 137, 169, 182, 203, 222, 235, 247, 263, 295, 313, 333, 368 and 373 weeks from onset.

Figure X shows the hazard rates for the individual exponential distributions, adjusting for plasma creatinine and urinary protein/creatinine ratio. The origin for the vertical axis is the rate in the first interval. A peak hazard was observed approximately 2-3 years from onset, earlier than had been suggested by the unadjusted data. After three years, the baseline hazard rate was approximately constant.

3.2.1.6.2 <u>The log-normal model</u>

A log-normal distribution was fitted using the same covariates. Their significance was obtained, as in previous analyses, by dropping each covariate in turn and performing the likelihood ratio test.

Results of this analysis are given in Table XI. From the coefficients, lower plasma creatinine levels at one year (higher reciprocal levels) were associated with a slower rate of progression to ESRF ($P<0.001$), whilst ratios above 4 were associated with a more rapid progression ($P<0.05$). The diagnostic plot is shown in Figure XI. Standardised residuals of the logarithmically transformed survival times (z) were plotted against times which corresponded to the same survival estimates assuming a 'normal' distribution ($\Phi^{-1}(1 - \hat{S}(z))$). Since this plot was an approximate straight line, the fit was good.

There is a logistical problem here, which does not arise with the Cox proportional hazards model. The covariates lengthen or shorten the time to failure. In this

FIG. X

Nephrotic syndrome onset -

Results of fitting the piecewise-exponential model

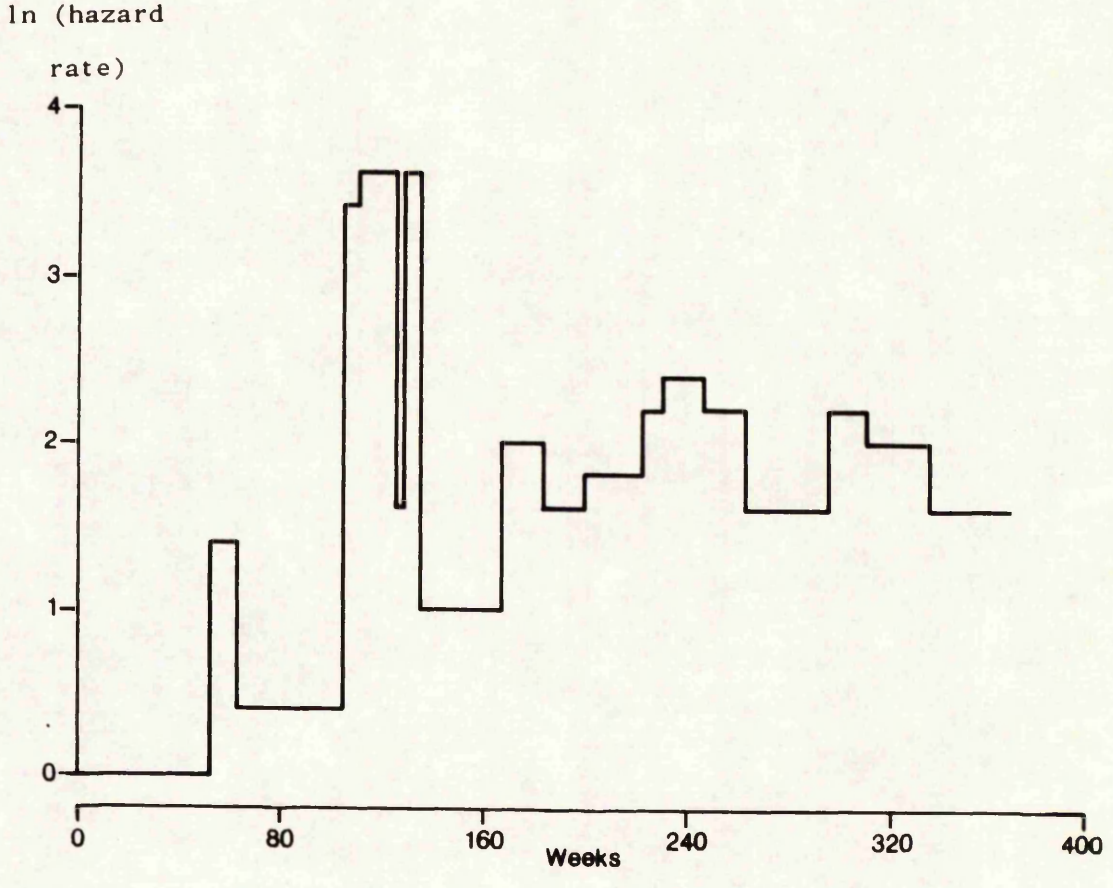


TABLE XI

Results of fitting a log-normal model

(121 patients)

Term	Coefficient	Significance
At one year:		
1/(plasma creatinine)	0.151 (SE 0.010)	P<0.001
urinary protein/creatinine ratio (4+)	-0.683 (SE 0.125)	P<0.05
constant	6.015 (SE 0.153)	

TABLE XII

Results of fitting a log-logistic model

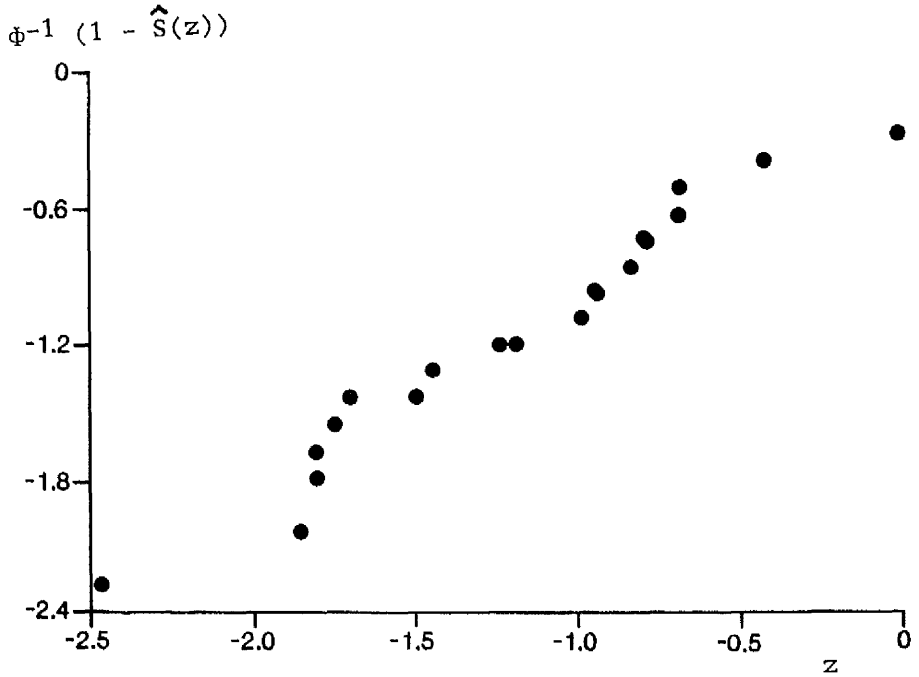
(121 patients)

Term	Coefficient	Significance
At one year:		
1/(plasma creatinine)	-0.335 (SE 0.089)	P<0.001
urinary protein/creatinine ratio (4+)	1.481 (SE 0.810)	P<0.05
ln(time)	1.629 (SE 0.295)	
constant	-9.579 (SE 1.840)	

FIG. XI

Nephrotic syndrome onset -

Diagnostic plot after fitting the log-normal model



case covariate values at one year after onset are used, therefore we need to consider whether the covariates altered the time which elapsed from onset to ESRF or that which elapsed from when they were measured. In the Cox regression analysis, shortening of all the survival times by one year makes no difference to the results; however, in accelerated failure-time models, the results would be affected.

The analysis was repeated using the time from which the covariate values were obtained. In this analysis, the urinary protein creatinine ratio (≥ 4) was significant at a higher level than before ($P<0.005$), but the diagnostic plot was curved indicating that the model fitted less well.

3.2.1.6.3 <u>The log-logistic model</u>

Estimates of log-odds were calculated from the Kaplan-Meier survival estimates. These should be linearly related to ln(time) if a log-logistic model is appropriate. Furthermore, subgroups of patients, defined by covariate values, should yield parallel lines. This was approximately true for the subgroups based on the two covariates (data not shown).

The log-logistic model was fitted to the same 121 patients. The results are shown in Table XII.

Both covariates at one year were statistically significant. The positive coefficient for the urinary protein/creatinine ratio (above 4) indicated an increased odds-on death ($P<0.05$); the negative coefficient for the reciprocal plasma creatinine indicated decreased odds-on death for lower plasma creatinine levels ($P<0.001$). These

findings were consistent with the results obtained by fitting the log-normal model.

The two diagnostic plots are shown in Figure XII(a) and (b). Each should be a straight line with a unit slope if the fit is good. Both the plots indicated that the fit was satisfactory.

From the estimated coefficients, the average time when the hazard is maximal can be estimated. For a patient with heavy proteinuria at one year (ratio ≥ 4), and with a plasma creatinine level of 0.3mmol/1, therefore, the maximum risk is at 215 weeks from onset, whereas, if the plasma creatinine level is 0.12mmol/1, the time is 601 weeks. With less proteinuria (ratio <4) and a plasma creatinine level of .12mmol/1, the time is 1491 weeks. As the time increases, the maximum hazard itself is decreased, thus for the latter patients, ESRF is most unlikely to occur.

3.2.2 Sequential collected data used as time-dependent covariates

3.2.2.1 First remission of proteinuria

Cox's proportional hazards regression was used with a 'dummy' variable to denote a first spontaneous, complete remission of proteinuria (see Methods section for details); patients were in state 0 before remission and 1 afterwards.

Remission of proteinuria was significantly related to survival (P<.001). A negative coefficient (-2.441 SE 0.743) for remission indicated that once remission had occurred the risk of ESRF was reduced. The findings were similar when the plasma creatinine concentration at one year was taken into account.

FIGURE XII
Nephrotic syndrome onset -
Diagnostic plots after fitting the log-logistic model
(a) untransformed

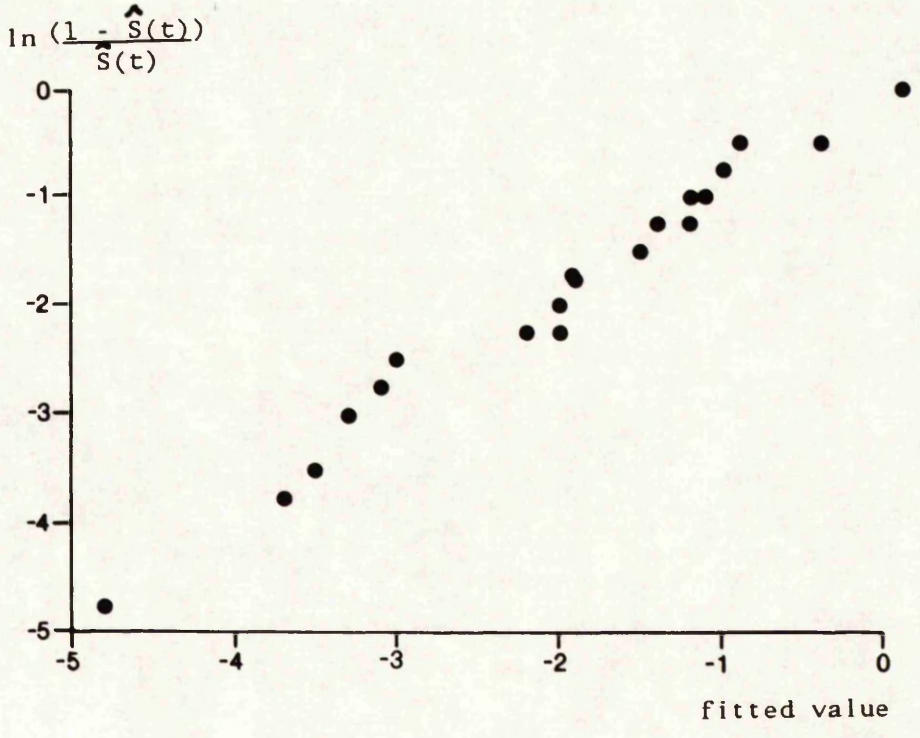
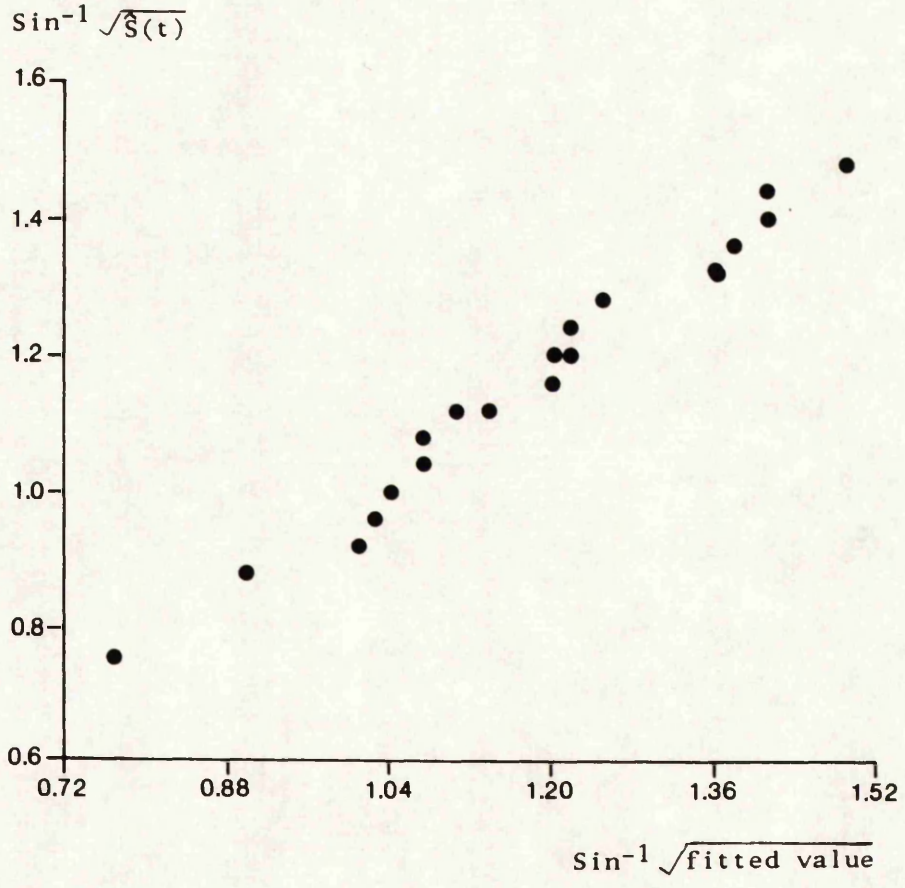


FIGURE XII

Nephrotic syndrome onset -
Diagnostic plots after fitting the log-logistic model
(b) after arc-sine transformation



One third of the patients who remitted had a subsequent relapse (proteinuria >1g/24h). Most (93%) of these patients maintained stable plasma creatinine levels; 75% of them had levels which remained within the normal range (<.12 mmol/l).

3.2.2.2 Gail's method

Gail's method was used to compare the risks of ESRF associated with different prevailing, or preceding, covariate states.

In the first analysis, the marker status was defined according to the prevailing urinary protein loss. The urinary protein/creatinine ratio was used in preference to 24 hour protein loss since this did not tend to decrease for the patients approaching ESRF (see Table XIII), as renal function became impaired, which may have invalidated the analysis.

The software written for this analysis included an optional stratification by sex (see Appendix III). In the results presented below, however, this has been overridden; preliminary analyses suggested that the stratification was unnecessary, and, furthermore, the small number of valid, informative deaths for the women led to some computational difficulties.

The results of the analysis are shown in Table XIV(a). The baseline state for comparison (state 0) was a ratio less than 4. Two other states, 'heavy' and 'very heavy' proteinuria (4-7.99 and 8+), were defined. The coefficient for state 0 is, by definition, equal to 0, thus the positive coefficients for states 1 and 2 ('heavy' and 'very heavy', respectively) indicate that these protein losses were

TABLE XIII
Urinary protein loss in patients approaching ESRF

Months prior to ESRF (+/- 2)	Urinary protein loss (g/24h)	Urinary protein/ creatinine ratio
12	11.70* 3.15-32.70 (31)	9.68 1.92-27.12 (26)
8	11.45 1.60-32.40 (36)	11.28 3.54-29.16 (32)
4	9.86 2.24-25.00 (37)	10.51 2.34-24.46 (36)
0	8.40 0.42-26.73 (35)	10.07 1.20-25.55 (32)
Comparison by Friedman's test	P=0.005	NS

* Median Range (n)

associated with increased risk of ESRF when compared with state 0. 95% confidence intervals (coefficient $\pm$ 1.96 SE) would exclude 0 in each case. The 'relative risks' are shown and emphasise that the risk was maximal when proteinuria was very heavy. The likelihood ratio test showed significant differences between the three states overall ($P<0.001$). Findings were similar when time lags 4, 8, 12 or 24 months were introduced; thus heavy proteinuria was associated with increased risk of ESRF, up to at least two years from the time it was observed.

The next stage was to take into account the prevailing plasma creatinine level in the definition of marker status.

In the first instance, six states were defined according to whether the protein/creatinine ratio was less than 4 or 4+, and whether plasma creatinine concentration was less than 0.12, between 0.12 and 0.19, and 0.2 or more mmol/1. A lag of eight months was used, since the effect of heavy proteinuria seemed greatest at this lag (see Table XIV(a)). Statistical analysis could not be performed, however, for this data set, since no ESRFs occurred in associated with plasma creatinine levels below 0.2 mmol/1 and ratios less than 4. Subsequently a lag of 12 months was considered; since no ESRFs occurred in association with the intermediate plasma creatinine group, both of the groups less than 0.2 mmol/1 were combined.

Table XIV(b) documents this last analysis. The baseline state for the analysis was a ratio less than 4 together with a plasma creatinine of less than 0.2 mmol/1; other states were defined as shown in the table. There was a statistically

TABLE XIV

Sequentially measured urinary protein/creatinine ratio as a marker for ESRF

(a) **Marker state defined only by urinary protein/creatinine ratio**

Marker States: *0 = ratio <4*
1 = ratio 4-7.99
2 = ratio 8+

Lag in Months	N+	State 1	State 2	Significance
0	30	1.360 SE 0.585 (4)*	2.500 SE 0.487 (12)	P<0.001
4	34	2.051 SE 0.618 (8)	2.994 SE 0.566 (20)	P<0.001
8	31	2.755 SE 0.789 (16)	3.407 SE 0.763 (30)	P<0.001
12	26	1.244 SE 0.787 (3)	2.964 SE 0.619 (19)	P<0.001
24	22	1.854 SE 0.717 (6)	2.690 SE 0.664 (15)	P<0.001

(b) **Marker state defined by urinary protein/creatinine ratio and plasma creatinine**

Marker States: *0 = Plasma creatinine <0.2 mmol/l, ratio <4*
1 = Plasma creatinine <0.2 mmol/l, ratio 4+
2 = Plasma creatinine ≥0.2 mmol/l, ratio <4
3 = Plasma creatinine ≥0.2 mmol/l, ratio 4+

Lag in Months	N+	State 1	State 2	State 3	
12	26	2.806 SE 1.101 (17)	3.968 SE 1.158 (53)	4.857 SE 1.078 (129)	P<0.001

\+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

highly significant difference between the four states. A high plasma creatinine (0.2 mmol/1 or more) was associated with an increased risk, whether or not the urinary protein loss was heavy. Heavy proteinuria also increased the risk, but this effect was less marked when the plasma creatinine level exceeded 0.2 mmol/1.

The latter analysis was repeated excluding patients with MCN lesions and patients with other systemic diseases and the findings were very similar.

Other covariates which were measured four-monthly were considered for analysis. An obvious consideration was the diastolic blood pressure.

Table XV(a) documents the analyses using, as definitions of marker state, diastolic blood pressure of less than 100 (state 0 - the baseline), between 100 and 109 (state 1), and 110 mm Hg or more (state 2).

The association between blood pressure and ESRF diminished as the time lag increased; differences between the three states were statistically significant up to a lag of 12 months. The coefficients for states 1 and 2 were similar.

Although diastolic blood pressure at one year had not emerged as a significant predictor of ESRF, the 'prevailing' blood pressure status was found thus to have prognostic importance, and 100, rather than 110 appeared to be a better cut-off for analysis. This was used in the second analysis, which incorporated plasma creatinine concentration, and used a lag of 12 months (Table XV(b)). A blood pressure of 100 or more was associated with worse prognosis, whether or not

TABLE XV

Hypertension as a marker for ESRF

(a) Marker state defined by diastolic blood pressure

Marker States: *0 = blood pressure <100*
1 = blood pressure 101-109
2 = blood pressure 110+

Lag in Months	N+	State 1	State 2	Significance
0	38	1.082 SE 0.394 (3)*	1.772 SE 0.385 (6)	P<0.001
4	39	1.234 SE 0.356 (3)	1.220 SE 0.440 (3)	P<0.001
8	38	0.793 SE 0.417 (2)	1.543 SE 0.376 (5)	P<0.001
12	34	1.058 SE 0.411 (3)	1.446 SE 0.430 (4)	P<0.005
24	25	0.347 SE 0.558 (1)	1.210 SE 0.479 (3)	NS

(b) Marker state defined by blood pressure and plasma creatinine

Marker States: *0 = Plasma creatinine <0.2 mmol/l, BP <100*
1 = Plasma creatinine <0.2 mmol/l, BP 100+
2 = Plasma creatinine ≥0.2 mmol/l, BP <100
3 = Plasma creatinine ≥0.2 mmol/l, BP 100+

Lag in Months	N+	State 1	State 2	State 3	
12	31	2.306 SE 0.838 (10)	4.089 SE 0.763 (60)	4.277 SE 0.784 (72)	P<0.001

\+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

BP = Blood Pressure

plasma creatinine was high. The effect of hypertension, however, was less marked when the plasma creatinine exceeded 0.2 mmol/l.

It must be stressed that the latter analysis could only go part way towards an adjustment for plasmas creatinine; for example, the two blood pressure groups may have differing plasma creatinine levels, even whilst within the range 0 to 0.19 mmol/l. In a cross-section of such patients at one year post onset, the patients with diastolic blood pressures of 100 mm Hg. or more had significantly higher plasma creatinine levels than those with blood pressures below 100 (Two-tailed Mann-Whitney U-test, $P<0.025$).

Since so many patients received antihypertensive agents and/or thiazides, the analyses in Table XV(a) were taken a stage further to try to establish whether a normal or an elevated diastolic blood pressure carried a different risk depending on whether current antihypertensive therapy was given. The aim was to determine whether hypertension per se carried the increased risk, or whether therapeutically 'controlled' levels below 100 mm Hg also carried an increased risk.

Table XVI documents the results obtained when diastolic blood pressure and current antihypertensive/thiazide therapy were used to define the prevailing marker status.

Each of the states 1,2 and 3 was associated with increased risk of ESRF. The coefficients for state 1, suggested that a need for therapy was associated with

TABLE XVI

Hypertension, with and without current antihypertensive or thiazide therapy, as a marker for ESRF

Marker States: *0 = diastolic blood pressure <100, not on any therapy*
1 = diastolic blood pressure <100, on therapy
2 = diastolic blood pressure 100+, not on any therapy
3 = diastolic blood pressure 100+, on therapy

Lag in Months	N	State 1	State 2	State 3
0	38	1.059 SE 0.461 (3)	1.360 SE 0.529 (4)	2.067 SE 0.425 (8) P<0.001
4	39	1.495 SE 0.452 (5)	1.795.SE 0.503 (6)	1.941 SE 0.458 (7) P<0.001
8	38	0.813 SE 0.444 (2)	0.556 SE 0.579 (2)	1.891 SE 0.390 (7) P<0.001
12	34	1.584 SE 0.487 (5)	1.798 SE 0.522 (6)	1.962 SE 0.508 (7) P<0.001

+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

increased risk, even when the hypertension was kept under control.

The differences between states 1 and 3, suggested that uncontrolled hypertension carried a further increase in risk. The differences between states 2 and 3 may merely be a reflection of the levels of blood pressure at which antihypertensive therapy was instigated.

The above conclusions were conjectural; the approach was naive in the sense that no information was available regarding the quantity of antihypertensive agent received, and 'control' would more properly be defined in terms of adequate therapy over a period of time. Some clinical judgement may be necessary.

The analysis was refined, as shown in Table XVII, by calculating the mean of any diastolic blood pressure measurements documented over the preceding 12 months, and noting whether any antihypertensive therapy was received during that period, and using these factors to define marker state. The period of 12 months was chosen so that there would be an adequate number of blood pressure measurements (approximately three to four) per patient. The coefficient for state 1 and the difference between 1 and 3 lead to similar conclusions to those above.

Next, the haemoglobin concentration was used to define marker status. The upper limits of normal (13 g/dl for males; 11.5 g/dl for females) were used to define 'mild' anaemia, and 8 g/dl was used to define 'severe' anaemia. The separate

TABLE XVII

Hypertension defined by mean diastolic blood pressure over the preceding twelve months, with and without current antihypertensive therapy, as a marker for ESRF

Marker States: *0 = diastolic blood pressure <100, no therapy*
1 = diastolic blood pressure <100, any therapy
2 = diastolic blood pressure 100+, no therapy
3 = diastolic blood pressure 100+, any therapy

Lag in Months	N	State 1	State 2	State 3
0	40	1.687 SE 0.422 (5)	0.793 SE 1.064 (2)	2.378 SE 0.445 (11) P<0.001

+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

results for males and females are shown in Table XVIII(a). Analyses at lags 0 and 24 were not possible for females, since there were too few observations in states 1 and 2. For both sexes, differences between the three states diminished as the lag time increased; thus anaemia was mainly a 'short-term' marker of ESRF.

Since haemoglobin correlated with plasma creatinine (see Results section 3.2.1.1), the level of anaemia may merely reflect declining renal function. Anaemia with a lag time of 12 months still had some prognostic importance, and, for males, this was combined with the prevailing plasma creatinine concentration to define marker status (see Table XVIII(b)).

Although the coefficient for state 1 at lag 8 was high; so also was its standard error. State 2 differed from state 3 at this lag, suggesting that anaemia may have some additional prognostic value. At lag 12, the dominant factor was clearly the concentration of plasma creatinine.

A further analysis was considered, but proved impossible to implement. Anaemia, in men, was combined with prevailing heavy proteinuria (urinary protein/creatinine ratio 4+); no ESRFs occurred for patients with normal haemoglobin concentrations and ratios less than 4.

For the first analysis in the section (Table XIII), no account was made of any treatment that might affect the level of proteinuria level, other than by the simple exclusion of patients with minimal change nephropathy. Approximately two thirds of the patients had received prednisolone, or other

TABLE XVIII

Sequentially measured haemoglobin concentration as a marker of ESRF

(a) Marker state defined by haemoglobin concentration

MALES:
Marker States: *0 = haemoglobin 13+ (normal)*
1 = haemoglobin 8-12.9
2 = haemoglobin <8

Lag in Months	N+	State 1		State 2	
0	32	3.691 SE 1.055	(40)*	6.797 SE 1.070	(896) P<0.001
4	27	3.087 SE 0.405	(22)	5.858 SE 0.871	(350) P<0.001
8	27	2.607 SE 0.521	(14)	4.439 SE 0.829	(85) P<0.001
12	23	1.351 SE 0.447	(4)	2.707 SE 0.851	(15) P<0.005
24	18	0.303 SE 0.534	(1)	2.238 SE 1.083	(9) NS

FEMALES:
Marker States: *0 = haemoglobin 11.5+ (normal)*
1 = haemoglobin 8-11.4
2 = haemoglobin <8

Lag in Months	N+	State 1		State 2	
4	10	3.841 SE 1.119	(47)	5.163 SE 1.162	(175) P<0.001
8	10	3.363 SE 0.849	(29)	3.798 SE 1.073	(45) P<0.001
12	9	2.328 SE 0.786	(10)	3.881 SE 1.041	(49) P<0.001

+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

TABLE XVIII

Sequentially measured haemoglobin concentration as a marker of ESRF

(b) **Marker state defined by haemoglobin concentration and plasma creatinine**

MALES:
Marker States:

0 = Plasma creatinine <0.2 mmol/l, haemoglobin 13+
1 = Plasma creatinine <0.2 mmol/l, haemoglobin <13
2 = Plasma creatinine >/0.2 mmol/l, haemoglobin 13+
3 = Plasma creatinine >/0.2 mmol/l, haemoglobin <13

Lag in Months	N	State 1	State 2	State 3
8	27	1.943 SE 1.010 (7)	2.803 SE 0.919 (17)	4.182 SE 0.778 (66) P<0.001
12	22	0.168 SE 1.103 (1)	2.040 SE 0.683 (8)	2.731 SE 0.562 (15) P<0.001

+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

immunosuppressive therapy, at some stage, usually early, in their disease, but often had not received at what might be considered a clinically 'effective' dose. In general, the level of proteinuria irrespective of any therapy was the important factor with respect to ESRF, as can be seen from inspection of the coefficients in Table XIX.

Data were scanned to find those, other than those with minimal change nephropathy or no light microscopic change, who had received at least 60 mg/day over a two month period, or, equivalently 100 mg on alternate days over the same time. A small number of these (5) showed a complete remission of proteinuria to less than 0.3 g/24h within approximately two months; three of these had mesangial proliferative lesions which might have been equivocal minimal change lesions, and the remainder had membranous nephropathy.

3.3 Patients with Asymptomatic Proteinuria Onset

3.3.1 Analyses with fixed covariates

3.3.1.1 Preliminary findings

'Onset' for the analysis of these 145 patients was taken to be the date that proteinuria was first detected. Clearly this is an imprecise definition, since the proteinuria may have started much earlier. Approximately half of the patients (73) in this group later developed oedema. The median time from onset to the first documented episode of oedema was eighteen weeks (range 1 week to 12 years), but the interval was longer than two years in approximately one quarter of the patients. This would suggest that the follow-up on these

TABLE XIX

Heavy proteinuria by mean urinary protein/creatine ratio over the preceding twelve months, with and without any prednisolone/ immunosuppressive therapy, as a marker for ESRF

(patients with other systemic diseases or with minimal change nephropathy excluded)

Marker States:
0 = mean ratio <4, no therapy
1 = mean ratio <4, any therapy
2 = mean ratio 4+, no therapy
3 = mean ratio 4+, any therapy

Lag	N	State 1	State 2	State 3
0	40	0.091 SE 0.876 (1)	2.255 SE 0.801 (10)	2.105 SE 0.758 (8) P<0.001

+ N = Number of valid, informative ESRFs

* Coefficient for each state, with associated standard error (SE)

Relative risks in parenthesis.

Overall significance by likelihood ratio test.

patients had begun at an earlier stage than for the nephrotic syndrome onset patients. A number of patients, however, had received thiazide diuretics and/or steroids and immuno-suppressive agents before the oedema developed, and therefore the oedema may not be necessarily comparable.

A larger proportion of patients progressed to ESRF (42 or 29%) than the 'nephrotic onset' patients. 81 patients were alive with their own functioning kidneys when last seen, and the rest died from other causes (ischaemic heart disease 7, hypertension complications or cerebro-vascular accident 2, malignancy 1, others 9, unknown cause 3). 28 patients had systemic diseases (systemic lupus erythematosus 11, amyloidosis 6, Henoch Schonlein purpura 7, polyarteritis nodosa 1, Alport's disease 2 and Wegener's granulomatosis 1).

Kaplan-Meier estimates for ESRF have been shown previously (Fig. III). Four patients were excluded because the month of 'onset' was not known. The asymptomatic patients had a significantly worse outcome that the patients with a nephrotic onset ($P=0.013$); the curves were close together initially but later diverged. Similar findings were obtained when the patients with MCN were omitted. All such patients presented with a nephrotic syndrome. The difference, however, was not statistically significant.

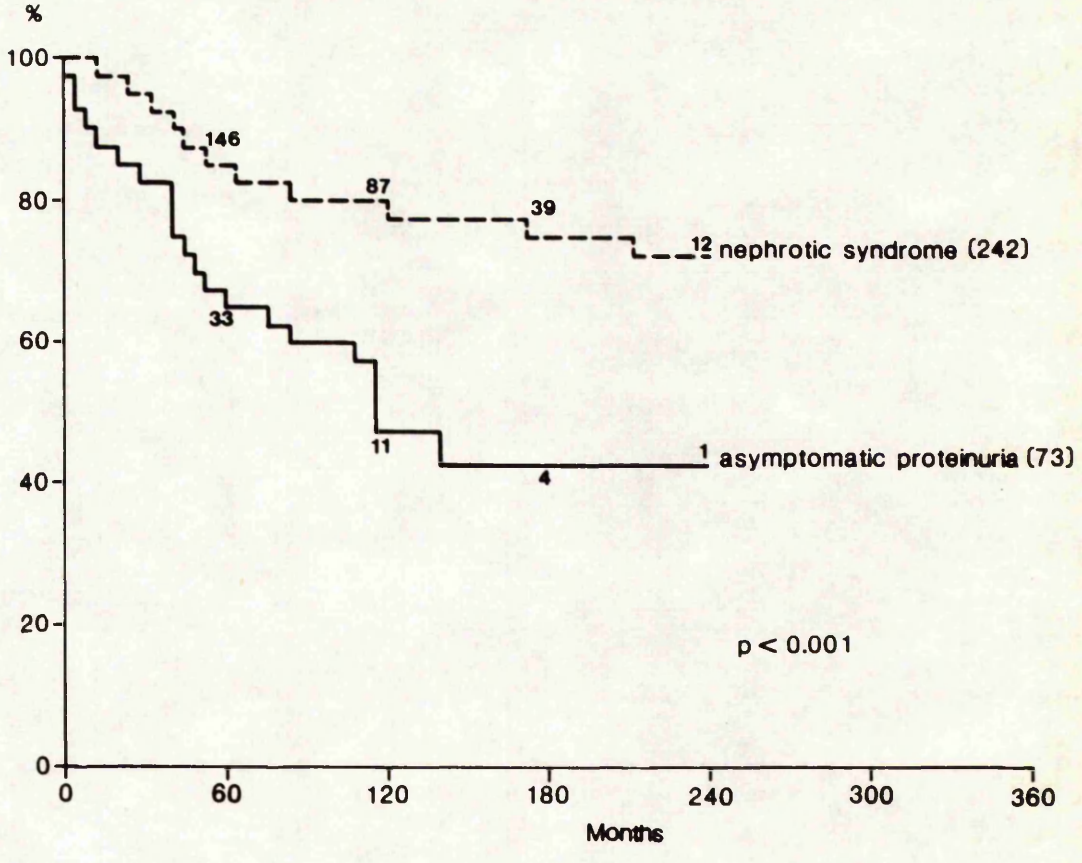
In Fig. XIII, only the patients who later had oedema have been considered, and the time interval was measured from the 'first' nephrotic syndrome. The asymptomatic proteinuria patients had significantly worse outcome than the 'nephrotic onset' patients ($P<0.001$). Even when patients in this group

FIG. XIII

Asymptomatic proteinuria onset -

Renal survival from first documented oedema compared

with patients with nephrotic onset



who had been treated in some way which might have delayed a nephrotic syndrome occurring (e.g. steroid/immunosuppressives or thiazide diuretics) were omitted, the difference was still significant (P=0.002), and also when patients with minimal change nephropathy were omitted from the 'nephrotic onset' group (P=0.021).

A possible explanation for these findings was that the disease had been 'smouldering' for some time, patients had less heavy proteinuria, and when, and if, oedema did occur, then the disease was at a more advanced stage. Support for this hypothesis comes from the fact that if oedema did occur then the patients' plasma creatinine levels (within +/- 8 weeks) were higher than those with a nephrotic onset; 31% of 56 patients had levels of 0.2 mmol/l or more compared with 18% of the 77 nephrotics (Chi-square NS but P<0.10).

No further clues could be gleaned from the hazard rate plots (see Fig. XIV); the mortality experience of this group was very different from the 'nephrotic onset' patients.

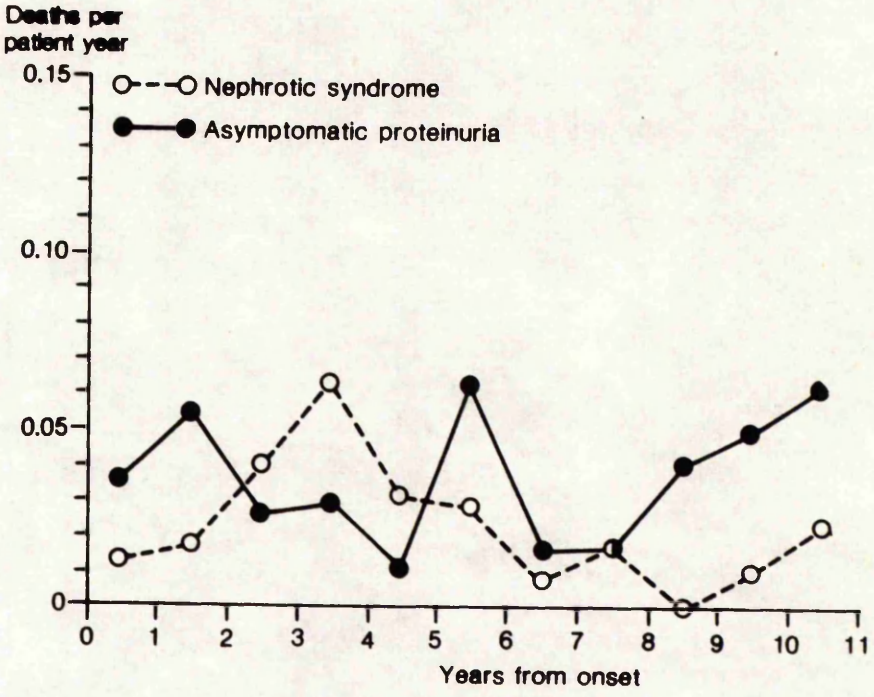
3.3.1.2 Further comparisons with patients with a nephrotic syndrome onset

The mean age when proteinuria was first detected was 36 years (SD 14) for men and 27 years (SD 11) for women; this difference which may reflect the age at the medical examination. The mean ages were slightly lower than for the 'nephrotic onset' patients, which supports the initial hypothesis that these have been detected earlier; the

FIG. XIV

Asymptomatic proteinuria onset -

Piecewise-constant event rates



difference, however, was not statistically significant.

The proportion of males increased with age, from 50% in the under 20 age group to 88% in the 50 plus age group. The overall male to female ratio, however, was approximately 2:1 (Table XX), the same as for the nephrotic onset patients.

Further comparisons with the 'nephrotic onset' patients were made with respect to the main variables: plasma creatinine, diastolic blood pressure, 24 hour urinary protein and urinary protein/creatinine ratio. The data are summarised in Table XX which may be compared with Table V of section 3.2.1.1.

The percentage of patients with high plasma creatinine levels (i.e. 0.2 mmol/1 or more) was very similar to the 'nephrotic patients'. (At onset 23% had high levels compared with 18% and at one year these figures were 20% and 18%).

The percentages with moderately raised diastolic blood pressure (110 mm Hg or more) were almost identical to the 'nephrotic onset' patients (at onset 18% compared with 17%; at one year 8% compared with 8%).

As expected, fewer patients had heavy proteinuria. For example at onset, 54% had 24 hour urinary protein losses of 5g or more compared with 79% of the 'nephrotic onset' patients (Chi-squared test $P<0.005$). At one year the percentages were 42% and 60% ($P<0.05$).

Results using the urinary protein/creatinine ratio were similar.

Levels of proteinuria tended to fall with time for both sexes (Fig. XV), and this compared with the findings in

section 3.2.1.2 (Fig. VII(a)). Plasma creatinine levels tended to stay steady (Fig. XVI). Interestingly, plasma creatinine levels for the sexes did not differ significantly (Mann-Whitney U-test P>.10 in each case) in contrast to the 'nephrotic onset' patients.

3.3.1.3 Univariate analyses

Comparisons between renal survival for subgroups of patients based on plasma creatinine levels, diastolic blood pressure and urinary protein loss are shown in Table XXI. (These may be compared with Table VI in section 3.2.1.1., which was a similar breakdown for the 'nephrotics'.) 5 patients went to ESRF in the first year.

There was no difference in renal survival between men and women, but as previously noted, their plasma creatinine levels were similar. Increased plasma creatinine level was associated with worse outcome. Heavy proteinuria was probably associated with a worse outcome, but this was not statistically significant, because of the small number of patients for analysis.

Moderately raised diastolic blood pressure was a much more important factor in this group than in the nephrotic onset patients (Fig. XVII), even though the incidence of raised blood pressure was the same. The six patients who were hypertensive at one year then had higher plasma creatinine levels than the remaining patients (Two-tailed Mann-Whitney U-test, NS but P=0.057). Four of these progressed to ESRF; the hypertension had been treated, but not controlled, in three of them.

TABLE XX
Asymtomatic proteinuria - groupings of variables showing numbers of patients for whom data were available

Sex:	Male		97	(67%)
	Female		48	(33%)
Age at onset (years):	Less than 20		26	(18%)
	20 to 34		58	(40%)
	35 to 49		37	(25%)
	50 or more		24	(17%)
	At onset:		**At one year:**	
Plasma creatinine (mmol/l):				
less than 0.20	33	(77%)	49	(80%)
0.20 or more	10	(23%)	12	(20%)
Total	43		61	
Diastolic blood pressure (mm Hg):				
less than 110	80	(82%)	70	(92%)
110 or more	18	(18%)	6	(8%)
Total	98		76	
24 hour urinary protein (g):				
less than 5	16	(46%)	33	(58%)
5 or more	19	(54%)	24	(42%)
Total	35		57	
Urinary protein/creatinine ratio (g/g):				
less than 4	13	(52%)	26	(54%)
4 or more	12	(48%)	22	(46%)
Total	25		48	

FIG. XV

Asymptomatic proteinuria onset -

Distribution of 24h urinary protein loss for males and females,

at onset and first and fifth onset anniversaries

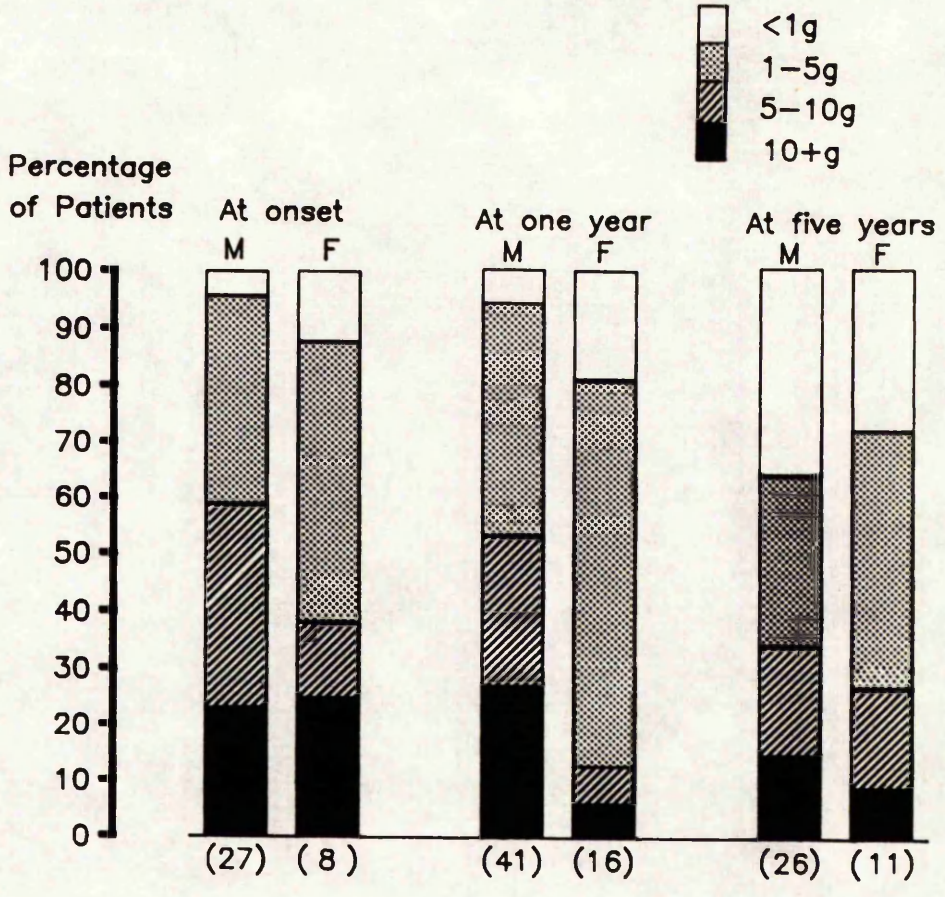


FIG. XVI

Asymptomatic proteinuria onset -

Distribution of plasma creatinine for males and females,

at onset and first and fifth onset anniversaries

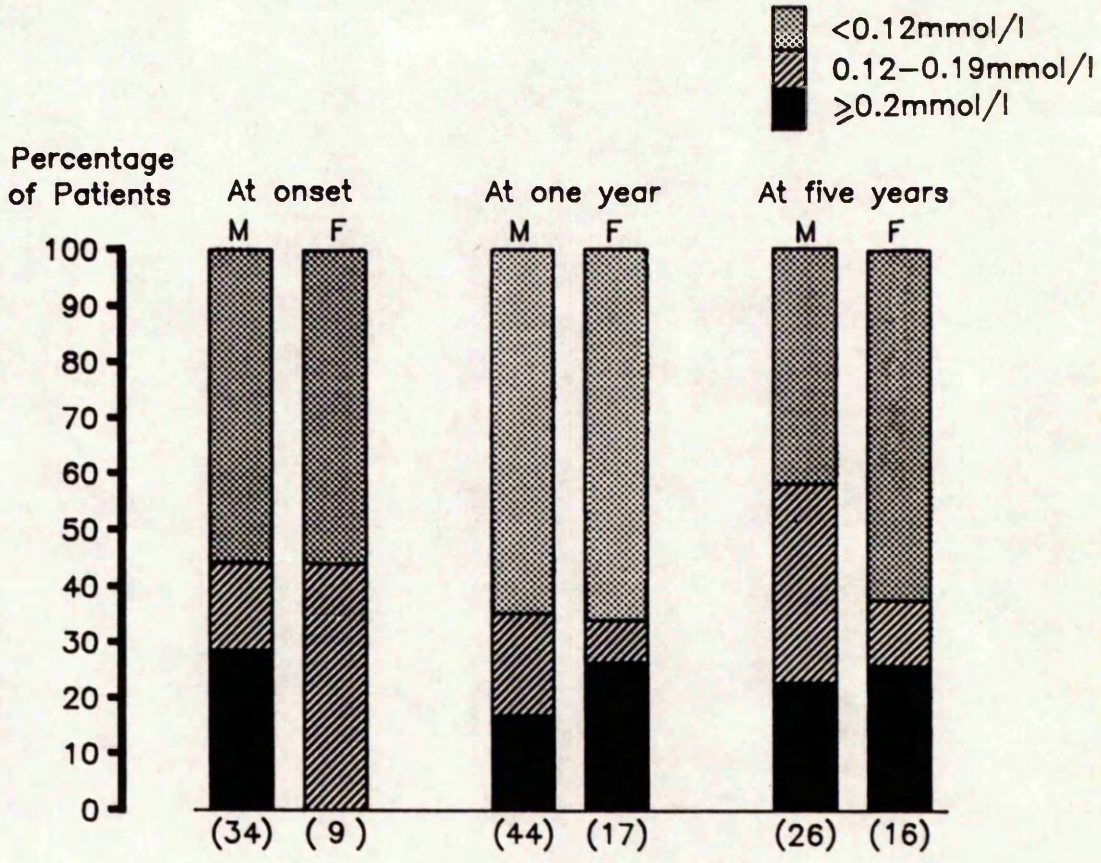


TABLE XXI
Asymptomatic proteinuria -
comparison of renal survival in subgroups of patients

Variable	Groups Compared	Significance *	Comments:
Sex	male v female	NS	worse survival for males initially
Age (years)	see table XIX	P=0.045	age group 20 - 34 worse
Plasma creatinine (mmol/l)	less than 0.2 v 0.2 or more	at onset: P<0.001 at one year: P<0.001	worse survival with increased creatinine
Diastolic blood pressure (mms Hg)	less than 110 v 110 or more	at onset: P=0.009 at one year: P<0.001	worse survival with increased blood pressure
Urinary protein (g/24hr)	less than 5 v 5 or more	at onset: NS at one year: NS (P=0.087)	worse survival with higher levels at one year only
Urinary protein/ creatinine ratio (g/g)	less than 4 v 4 or more	at onset: NS at one year: NS	worse survival with higher levels at one year only

*Significance by the Mantel-Cox test.

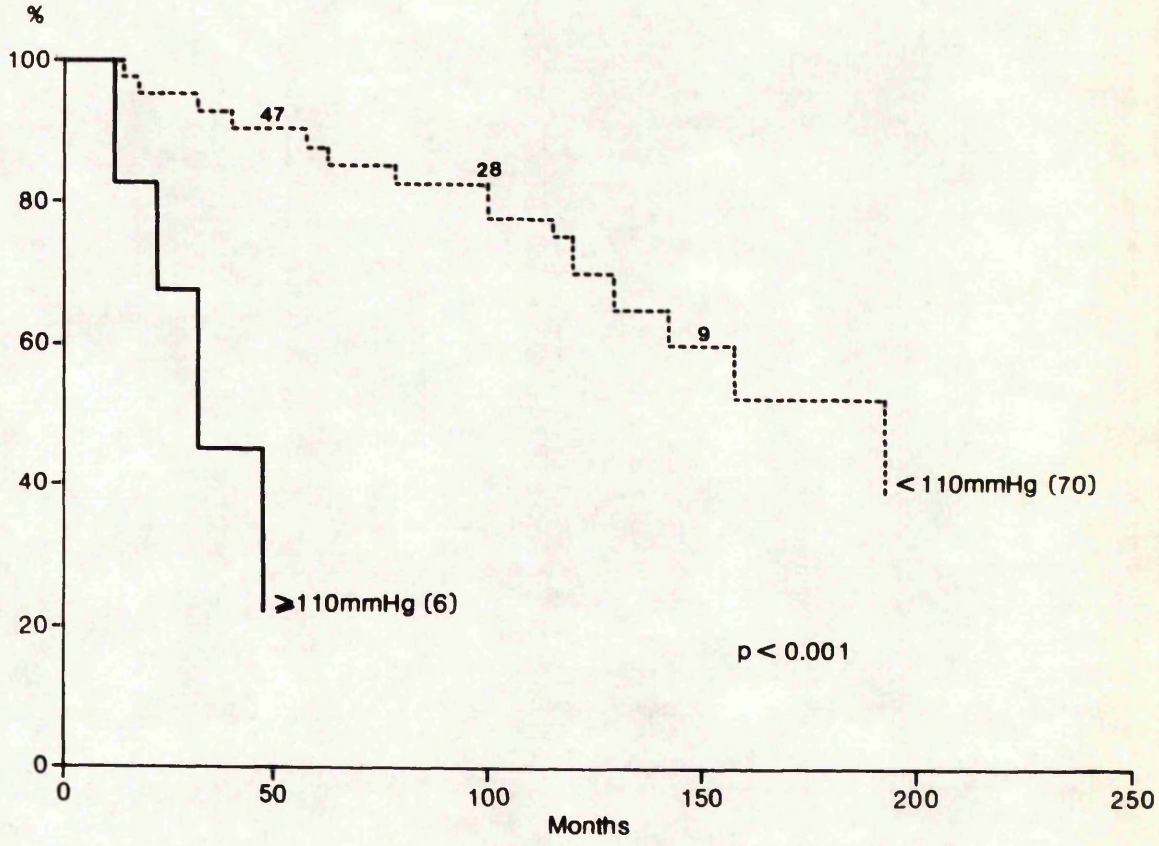
NS = Not significant

FIG. XVII

Asymptomatic proteinuria onset -

Renal survival according to diastolic

blood pressure at one year



The histopathological spectrum was similar to that for the nephrotic onset patients, except for the absence of the steroid responsive Minimal Change lesions. Renal survival estimates for the main groups at five and ten years are shown in Table XXII. The 'no light microscopic change' group had the best prognosis, but the curves did not differ significantly. (The two smallest groups were excluded from the comparison).

For completeness, the survival relating to plasma albumin and haemoglobin concentration are shown in Table XXIII, and may be compared with Table VII. Fewer patients had low plasma albumin levels than the nephrotic onset patients, reflecting the fact that the proteinuria was less heavy. Anaemia, especially for the men, was an important prognostic factor in this instance (see, for example, Fig. XVIII).

3.3.1.4 Multivariate analysis

Multivariate analysis using the Cox proportional hazards model was not appropriate for these patients, since the 'onset' was ill-defined, and the baseline hazard rate was not the same for all the patients. Whilst the log-logistic model can allow for left censoring, (occurring in this instance because the true 'onset' is unknown), here there was both left and right censoring, the timing of the measurements of the fixed covariates also presented a problem.

If it can be assumed that the current plasma creatinine concentration reflects the length of time the disease has been 'smouldering', then a stratification by plasma creatinine level can be incorporated into the Cox proportional hazards

TABLE XXII
Asymptomatic proteinuria onset -
Kaplan-Meier estimates of renal survival for
the seven main histopathological groups

	Number:	At five years:	At ten years:
No light microscopic change (not MCN)	17	100%	100%
Membranous nephropathy	28	87%	65%
Mesangial proliferative	49	88%	73%
Focal segmental proliferative	5	(80%)	(53%)
Diffuse proliferative	4	(75%)	(38%)
Mesangiocapillary	16	94%	83%

TABLE XXIII
Asymptomatic proteinuria onset - further variable groupings and comparisons of renal survival

Plasma albumin (g/l)

at onset:			
less than 30	24	(39%)	} NS but below
30 or more	38	(61%)	} 30 worse later
Total	62		
at one year:			
less than 30	18	(28%)	} P=0.035
30 or more	47	(72%)	} below 30 worse
Total	65		

Haemoglobin (g/dl)

for males:

at onset:			
less than 13	19	(34%)	} P<0.001
13 or more	37	(66%)	} below 13 worse
Total	56		
at one year:			
less than 13	8	(18%)	} P<0.001
13 or more	36	(82%)	} below 13 worse
Total	44		

for females:

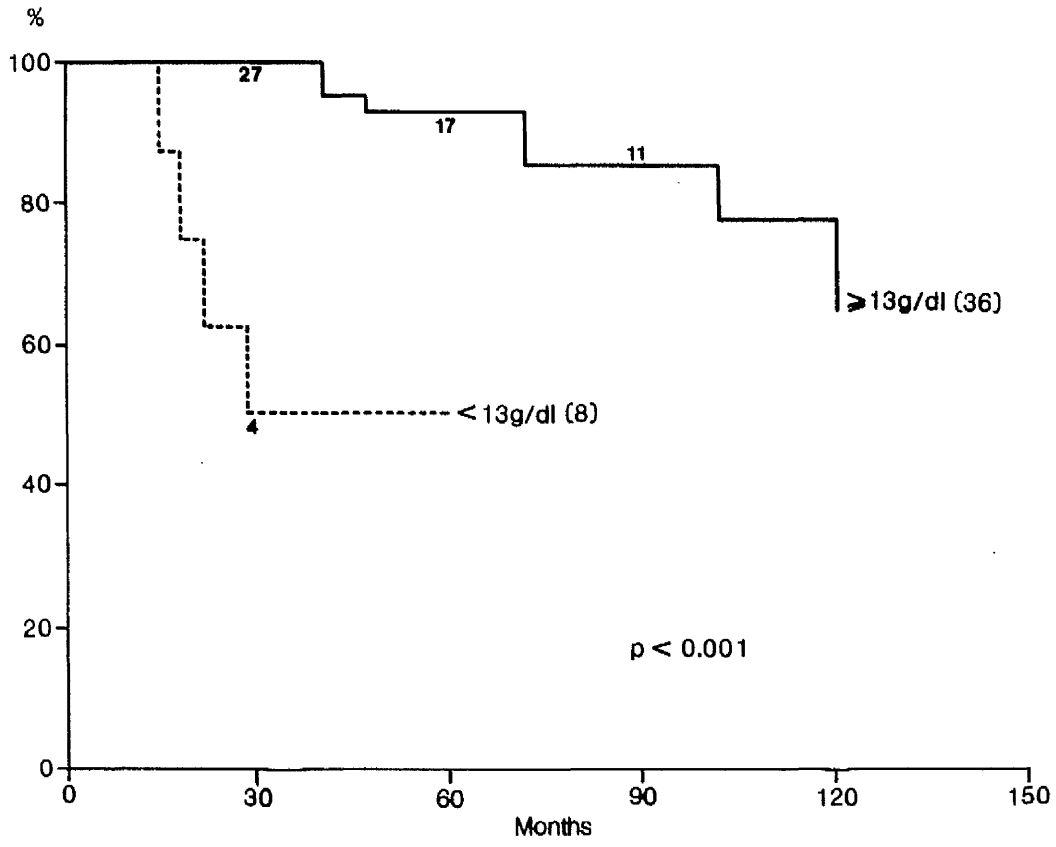
at onset:			
less than 11.5	8	(32%)	} NS
11.5 or more	17	(68%)	} below 11.5 worse
Total	25		
at one year:			
less than 11.5	4	(24%)	} NS but
11.5 or more	13	(76%)	} too few to test
Total	17		

FIG. XVIII

Asymptomatic proteinuria onset -

Renal survival according to haemoglobin

at one year for males only



model to take into account patients entering the study at different stages of disease. Such an analysis was carried out, using plasma creatinine at one year to define three strata (less than 0.12, 0.12 to .19 and 0.2+ mmol/l; 41 patients). The covariates used in the analysis were age, sex, urinary protein loss (ratio 4 or more) and hypertension (diastolic blood pressure 110 or more). No factor was statistically significant. It is probable that hypertension was statistically significant at one year in the univariate analysis above because of the association with plasma creatinine.

3.3.2 Analysis with time-dependent covariates

In the number of patients who never had documented oedema, proteinuria could still, at times, be heavy; two thirds (63%) had levels of 5 g/24h or more, and 27% had levels over 10 g/24h. Of the patients whose proteinuria remained below 5 g/24h, only one patient (4%) progressed to ESRF, compared with 9 (22%) of the remainder, and 41% of those who had documented oedema. This comparison does not take into account possibly differing follow-up periods. An attempt was made to use proteinuria first rising above 5 g/hr as a time-dependent covariate, in a similar manner to the analysis used previously to study the effect of a remission (see section 3.2.2.1). This would demonstrate that patients with losses remaining below 5g had a more benign outcome. The analysis proved to be impossible, since the times when the levels first exceeded 5 g/24hr could not be ascertained for a proportion of the patients who progressed to ESRF.

3.4 The Renal Screen

This section documents the analyses carried out on the renal screen variables. These were measured in addition to the variables described in the previous sections at irregular and much less frequent intervals.

Each subset of screen variables is considered separately and presented in two parts. First the relationships are examined with other variables of prognostic importance. For this analysis only the first measurement (or set of measurements) for each patient was used so that the results would be statistically independent. Finally, results from the application of Kay's analysis are described; this utilised all the available sequential results for each patient to assess the value of each screen variable as a market of prognosis.

Results from all the patients are reported, irrespective of first manifestation, as this gave a larger data base for analysis. Since Kay's method assumed a Markov process, the time from the onset was irrelevent. Further analyses were carried out separately for the asymptomatic proteinuria and nephrotic onset patients to test whether similar processes were acting within each group, and these are reported briefly.

3.4.1 The selectivity ratio

Selectivity ratios were available for 217 patients since 1970, and were measured on at least two occasions for 93 patients.

3.4.1.1 Relationships with other variables

The first selectivity ratio was measured at a median time of 22 months from onset (range one week to 28 years). The

results appeared to be unrelated to the time from onset, but this did not take into account the changing patient population.

The distribution of results was slightly skewed, and at this stage no transformation could be found which rendered the distribution Gaussian. Since this probably reflected the non-homogeneity of the patients, non-parametric statistical methods were used initially.

There was no significant correlation with coexistent urinary protein loss, which ranged from 0.19 to 30.54 g/24h (median 5.79) in these patients. There was, however, a significant correlation with the prevailing plasma creatinine concentration (Kendall's tau=0.28, two tailed $P<0.001$). Similar results were obtained when the nephrotic syndrome onset and the asymptomatic proteinuria patients were analysed separately.

Sequential changes in the selectivity ratio may reflect changes in GFR. Amongst the 93 patients with two measured ratios, the difference between the two could be appreciable, and exceeded 0.2 for one fifth of the patients. This difference was correlated weakly with the change in reciprocal plasma creatinine (Kendall's tau=-0.14, two-tailed NS but $P=.054$), whilst was unrelated to the time interval between (median two years, range two weeks to six years).

Fourteen patients were moderately hypertensive (diastolic blood pressure 110 mg Hg or more) when the first selectivity ratio was measured. These patients had significantly higher selectivity ratios than the remainder (median 0.35 compared with median 0.26, two-tailed Man-Whitney U-test, $P=0.003$), but

also had significantly higher plasma creatinine levels ($P<0.001$). Urinary protein loss and urinary protein/creatinine ratios did not differ significantly between the two groups.

The median (first) selectivity ratios are documented in Table XXIV for each of the main biopsy groups. There were some differences between the groups (Kruskal-Wallis one-way analysis of variance, NS but $P=0.084$). The patients with minimal change nephropathy had more selective proteinuria than patients in each of the other groups. The differences were statistically significant except for diffuse and focal segmental proliferative (two-tailed Mann-Whitney U-test, maximum $P<0.025$). The same findings were observed within the subgroup of patients with a nephrotic syndrome onset, as shown by other authors (Cameron, 1968). A correlation between the selectivity ratio and plasma creatinine concentration was observed with each of the main biopsy groups.

In summary, the selectivity ratios were associated with three variables: plasma creatinine concentration, severe hypertension (diastolic blood pressure 110 mm Hg or more) and a clinicopathological diagnosis of minimal change nephropathy. In Figures XIX, the selectivity ratios were plotted against the reciprocal of plasma creatinine, using separate symbols for (a) patients with severe hypertension and (b) minimal change nephropathy. Proteinuria from patients with minimal change nephropathy was more selected, even after adjustment for plasma creatinine, since the corresponding points on the graph were lower at any plasma creatinine level. Conversely,

TABLE XXIV

First selectivity ratio for the main biopsy groups

	Median:	Range:	N:
Minimal change	0.11	0.04-0.31	8
No light microscopic change (not MCN)	0.25	0.06-0.72	26
Membranous nephropathy	0.28	0.05-0.68	49
Mesangial proliferative	0.28	0.02-0.71	82
Focal segmental proliferative	0.26	0.04-0.30	7
Diffuse proliferative	0.22	0.11-0.48	5
Mesangiocapillary	0.27	0.07-0.63	23

FIG. XIX

Relationship between selectivity and plasma creatinine for patients with:

(a) Moderate hypertension

(diastolic blood pressure $\geq$ 100)

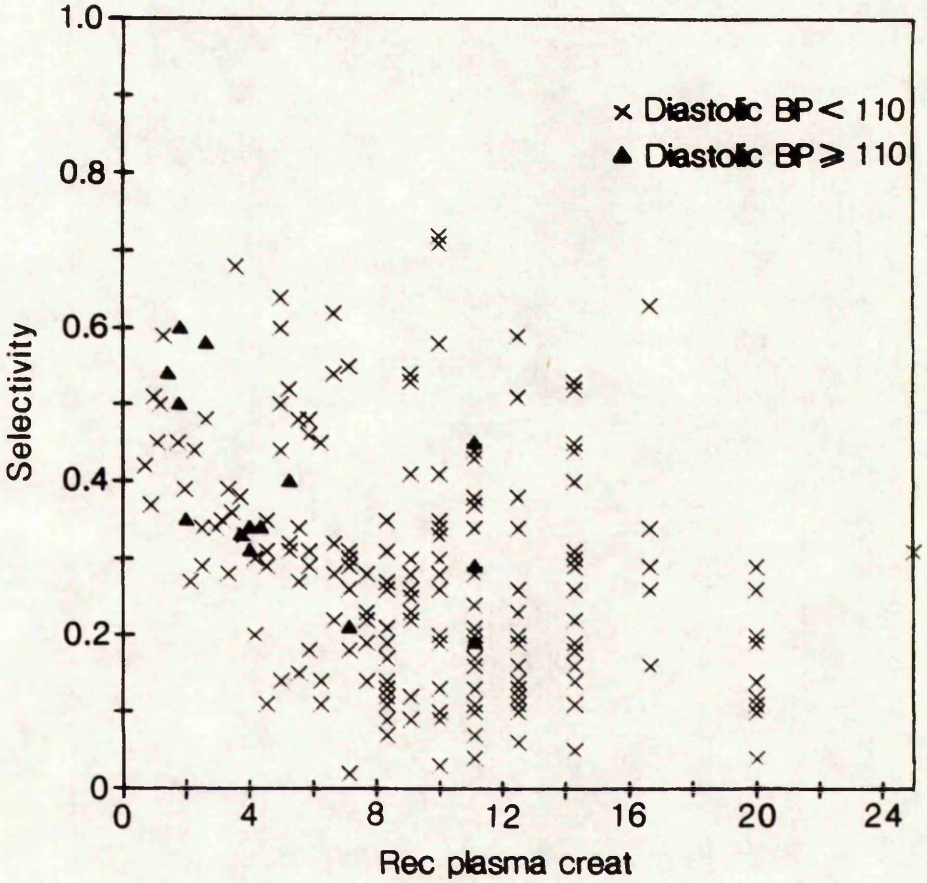
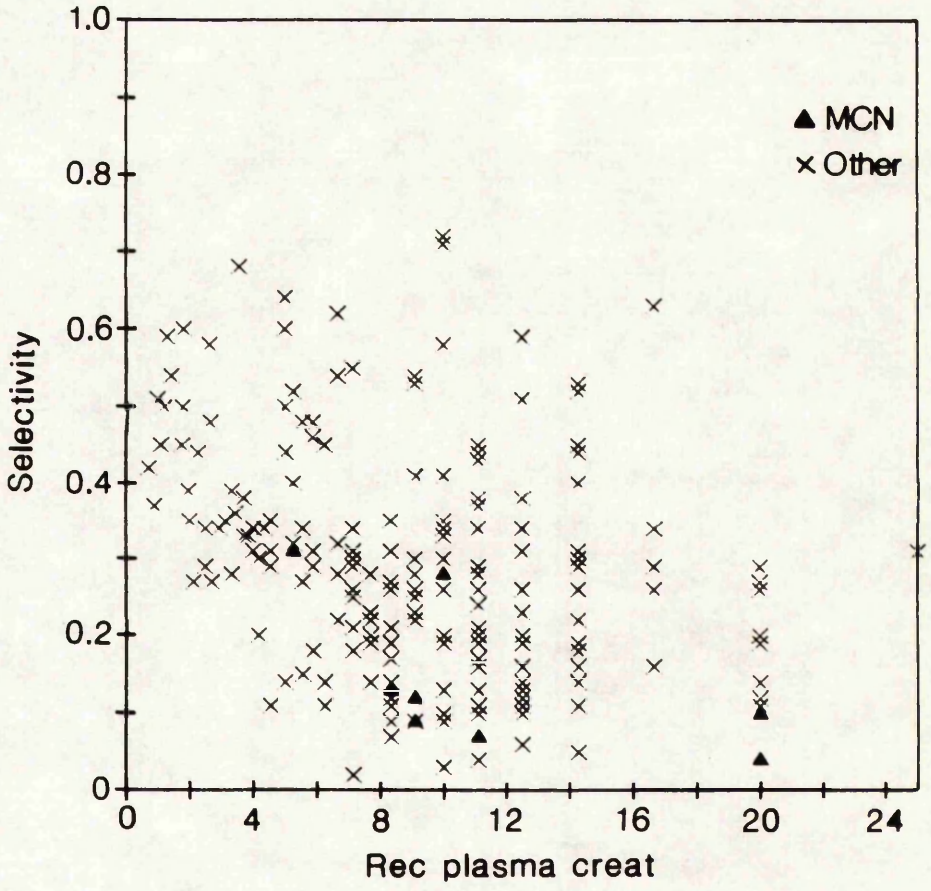


FIG. XIX

Relationship between selectivity and plasma creatinine for patients with:

(b) MCN



the poorly selected proteinuria in the hypertensive patients may merely reflect their higher plasma creatinine levels.

Multiple linear regression analyses was undertaken to examine the relationships between three independent variables (plasma creatinine, hypertension and minimal change nephropathy) and the selectivity ratio. A transformation was sought for the dependent variable, the selectivity ratio, which normalised the residuals but maintained an approximately linear relationship with the reciprocal plasma creatinine. The Box-Cox family of transformation were considered (Box and Cox, 1964). A GLIM macro was used to fit the model and determine the 'best' transformation for the selectivity (GLIM, 1986), which proved to be a power transformation of 0.45. (An analysis of the residuals confirmed this to be reasonably satisfactory). The ensuing regression analysis confirmed the relationship with plasma creatinine ($P<0.001$). After adjustment for plasma creatinine, the selectivity ratios for the hypertensive patients did not differ significantly from the remainder ($P>0.10$), whilst those for patients with minimal change nephropathy were lower ($P=0.008$).

3.4.1.2 Analysis of sequentially collected data

Kay's method (see Methods section 2.3.7 and Appendix V) utilised all the selectivity ratios measured for each patient. One patient, for example, had selectivity measured on five occasions, but, in general, the patients had only one or two measurements, which made the Markov assumption impossible to verify.

Two marker states were defined, according to whether or not the selectivity was below a certain 'cut-off' point. Four cut-off points were explored, namely 0.1, 0.2, 0.3 and 0.4, which corresponded to the 8th, 34th, 59th and 79th percentiles respectively. The rate of transition to ESRF was always greatest for the patients with more selected proteinuria; the difference was most significant for ratios above 0.3 ($P<0.001$), but was also significant for 0.2 ($P<0.005$) and 0.4 ($P<0.025$). Fig. XX shows the transition rates between the two states, and from each state to ESRF, which were obtained using the 0.3 cut-off.

Patients with a nephrotic syndrome and asymptomatic proteinuria onset were analysed separately. There were some differences between the two groups of patients in the rates of transition to ESRF; rates for more selected proteinuria were lower in the nephrotic syndrome patients than the asymptomatic proteinuria patients, whilst rates for less selective proteinuria were higher. None of the differences, however were statistically significant.

3.4.2 Serum Immunoglobulins

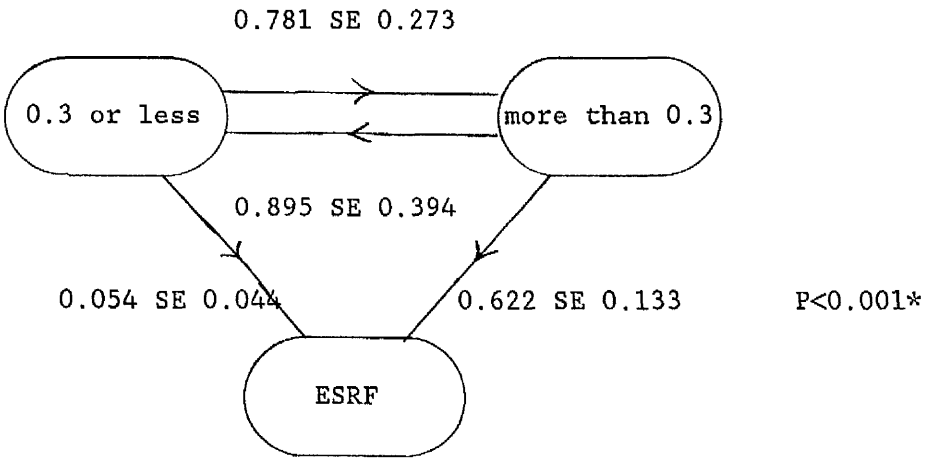
Serum immunoglobulin concentrations were measured from 1967 onwards. Only IgG was measured in the early phase of the study but subsequently all three immunoglobulins were measured simultaneously. There were 287 patients with at least one documented immunoglobulin level.

3.4.2.1 Relationships with other variables

The first result, or set of results, was used for each patient, as before.

FIG. XX

Selectivity Ratio -
transition rates (x 100) between marker states
and from each state to ESRF
(113 patients)



* Comparison between rates of transition to ESRF

The IgM concentrations were above the upper limit of the reference range in approximately one quarter (26%) of the patients, and the IgG concentrations were below the lower limit in one third (32%). The distribution of IgA concentrations was similar to that of a normal population.

The distributions of IgM, IgG and IgA were each positively skewed. A logarithmic (base 10) transform rendered the distribution closer to a Gaussian for IgM, but was found to be unsuitable for IgA, since there were zero values, and for IgG. For simplicity, non-parametric statistical tests were used throughout.

The period of time elapsing from 'onset' to the first immunoglobulin measurement was variable, ranging from zero weeks to 33 years (median 113 weeks). Serum IgM and IgA concentrations were unrelated to the time from onset, whilst serum IgG appeared to increase (Kendall's tau=.10, n=278, two-tailed p=0.014). The interpretation of this was difficult because the composition of the study group changed with time. Table XXV illustrates within-patient changes over intervals of not less than one year, and confirms that serum IgM and IgA concentrations tended to remain constant whilst serum IgG increased.

Returning to analysis of the first set of measurements for each patient, the serum concentration of IgM was not significantly correlated with prevailing urinary protein loss, but was negatively correlated with plasma creatinine (n=256, tau=-0.14, two-tailed P<0.001). The range of plasma creatinine levels represented was wide, 0.03 to 1.38 mmol/l.

TABLE XXV

Changes in Total Immunoglobulin
(results within one year excluded)

(a) Total Serum IgM (g/l)

First Level:	Second Level: 0-0.99	1-1.99	2+	Total
0-0.99	13*	5	0	18
1-1.99	8	13	3	24
2+	1	9	17	27
Total	22	27	20	69

(b) Total Serum IgG (g/l)

First Level:	Second Level: <6	6-9.99	10+	Total
<6	11	12	3	26
6-9.99	4	9	10	23
10+	1	2	19	22
Total	16	23	32	71

(c) Total Serum IgA (g/l)

First Level:	Second Level: <1.6	1.6-2.39	2.4+	Total
<1.6	13	6	1	20
1.6-2.39	5	14	2	21
2.4+	0	3	25	28
Total	18	23	28	69

* Numbers of patients in each subgroup

In contrast, the serum IgG was uncorrelated with the prevailing plasma creatinine concentration, but was negatively correlated with 24 hour urinary protein loss (n=258, tau=-0.422, P<0.001; urinary protein losses ranged from 0 to 28.98g, median 3.80g) and urinary protein/creatinine ratio (n=241, tau=-0.428, P<0.001).

Since the IgG molecule is small, the fall in serum IgG may merely have reflected the leakage to the urine, and therefore the fall with time from 'onset' (see above) would be consistent with a decline in proteinuria. If the selectivity ratio can be regarded as a measure of the 'leakiness' of the kidney, then an inverse relationship might be expected between serum IgG concentration and the prevailing selectivity ratio. No such relationship was found, however (n=124, tau=0.08 NS).

Serum IgM was uncorrelated with urinary protein loss but was weakly correlated with the selectivity ratio (n=123, tau=-0.10, NS but two-tailed P=0.094). Both IgM and selectivity ratio were correlated with plasma creatinine; when this was adjusted for by 'partialling', then the tau was reduced to -0.05.

The serum IgA level was not significantly related to either the plasma creatinine level, urinary protein loss or selectivity ratio.

The first immunoglobulin levels for each of the main biopsy groups are shown in Tables XXVI (a)-(c). Patients with messangial proliferative lesions were subdivided according to their immunofluorescence (IF): 'IgA' indicated a predominance of IgA deposits in the mesangium, 'IgM' indicated IgM deposits

Table XXVI(a)
First documented serum immunoglobulin concentrations for each of the main biopsy groups

Group	Median:	Range:	N:
(a) Serum IgM (g/l)			
Minimal change	1.40	0.30-2.50	20
No light microscopic change not (MCN)	1.45	0.17-3.00	31
Membranous nephropathy	1.25	0.28-4.60	61
Mesangial proliferative			
IF IgA	1.25	0.40-2.95	23
IF IgM	1.55	0.45-4.35	31
IF other	1.25	0.26-3.30	19
IF not done	1.60	0.15-4.20	31
All patients	1.38	0.15-4.35	104
Focal segmental proliferative	0.80	0.30-3.25	11
Diffuse proliferative	0.95	0.50-2.80	5
Mesangiocapillary	1.55	0.55-3.70	22

Table XXVI(b)
First documented serum immunoglobulin concentrations for each of the main biopsy groups

Group	Median:	Range:	N:
(b) Serum IgG (g/l)			
Minimal change	8.65	1.85-15.80	21
No light microscopic change (not MNC)	10.35	3.95-61.25	31
Membranous nephropathy	6.35	1.05-23.30	65
Mesangial proliferative			
IF IgA	10.95	4.15-21.60	23
IF IgM	9.83	1.85-54.00	32
IF other	8.80	1.96-30.18	20
IF not done	11.40	1.45-27.60	31
All patients	10.18	1.45-54.00	106
Focal segmental proliferative	10.50	3.50-17.35	11
Diffuse proliferative	9.55	5.25-21.00	5
Mesangiocapillary	10.08	3.20-15.30	24

Table XXVI(c)
First documented serum immunoglobulin concentrations for each of the main biopsy groups

Group	Median:	Range:	N:
(c) Serum IgA (g/l)			
Minimal change	2.10	0.15-5.10	20
No light microscopic change (not MCN)	2.10	0.15-8.25	31
Membranous nephropathy	2.18	0.29-7.50	62
Mesangial proliferative			
IF IgA	3.70	1.90-7.85	23
IF IgM	2.30	0.20-6.20	31
IF other	2.49	0.42-4.40	19
IF not done	2.60	0-17.00	31
All patients	2.65	0-17.00	104
Focal segmental proliferative	2.10	0.50-5.50	11
Diffuse proliferative	2.90	1.45-3.60	5
Mesangiocapillary	1.95	0.10-3.95	23

and 'other' indicates +/- other immunoglobulin deposits or mixed deposits.

For serum IgM (Table XXVI (a)), there was no significant differences between the biopsy groups (Kruskal-Wallis one-way analysis of variance) and, furthermore, the three IF subgroups of mesangial proliferative ('IgA','IgM', and 'other') did not differ significantly.

In contrast, levels of serum IgG (Table XXVI (b)) differed significantly between the main groups ($P<0.001$). Amongst the mesangial proliferative group, levels of IgG in the 'IgA' IF subgroup were higher than in both the 'IgM',and the 'other' subgroups (two-tailed Mann-Whitney U-tests; NS but $P=0.063$ and $P=0.014$ respectively). This analysis did not take into account the time from onset.

Finally, there were differences in serum IgA (Table XXVI (c)) between the main biopsy groups ($P=0.042$). Amongst the mesangial proliferative group, patients in the 'IgA' IF subgroup had significantly higher levels than those in the 'IgM' and 'other' ($P<0.001$ and $P=0.007$ respectively).

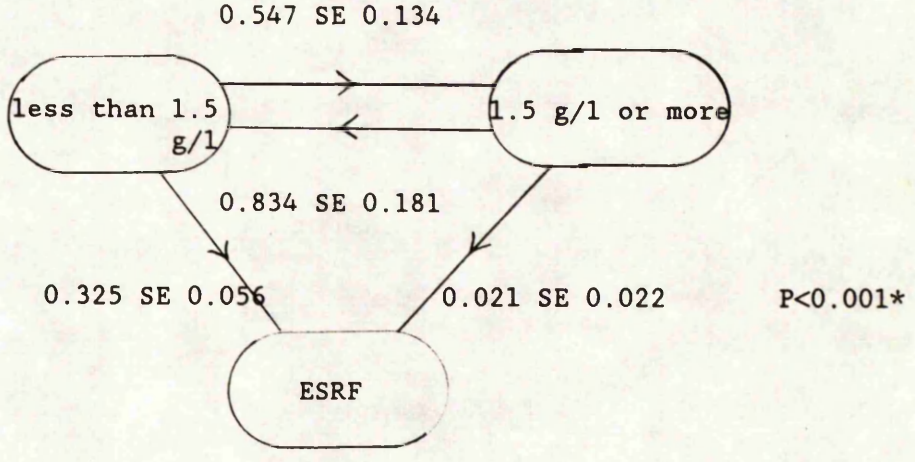
3.4.2.2 Analysis of sequentially collected data

3.4.2.2.1 Serum IgM

A series of applications of Kay's method were carried out using cut-off points of 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 and 2.0 g/l. These corresponded to the 14th, 25th, 38th, 50th, 62nd, 72nd and 79th percentiles. Higher levels of IgM were associated consistently with a decreased transition rate of ESRF. The difference was most significant for a cut-off of 1.5 g/l ($P<0.001$, see Fig. XXI). Similar results

FIG. XXI

Serum IgM -
transition rates (x 100) between marker states
and from each state to ESRF
(168 patients)



* Comparison between rates of transition to ESRF

were obtained when the asymptomatic and nephrotic patients were considered separately.

Immunoglobulins were measured more frequently than the selectivity ratio, and attempts were made to check the Markov assumption using the 1.5 g/l cut-off. The time when the patient first entered the lower IgM state (state 1) was estimated by linear interpolation. The times of the transitions from this time to the death state were calculated and these were compared between those patients who had previously moved from state 1 to state 2 (the higher IgM state) and those who had not. (Under the Markov assumption these should be the same.) There was no significant difference in the time of transition from state 1 to ESRF between those patients who had and those who had not previously moved to the state 2 (by logrank test), Likewise there was no difference in time taken to change from state 2 to ESRF in those who had previously changed to state 1. No evidence, therefore, could be found from these analyses to show that the Markov assumption was being violated. The interpolation, however, was probably inaccurate, and, in some instances previous transitions to the other state may have been missed.

Some further analyses were undertaken for specific histopathological groups where IgM has been implicated as playing a part in the disease process. These were membranous nephropathy and mesangial proliferative with 'IgM' IF. (A third group, 'no light microscopic change' was too small for analysis).

Amongst the membranous nephropathy group (n=40), six patients progressed to ESRF, and all transitions to ESRF occurred from levels below 1.25 g/1. Although this was not contradictory to hypothesis that lower levels carried a worse prognosis, further analyses using cut-off points of 1.5 or 1.25 were impossible. For the mesangial proliferative 'IF IgM' group (n=21), only 5 patients progressed to ESRF, and the most recent result for each patient was less than 1.5 g/1, so that analysis using this cut-off was not possible. The results using cut-offs of 1.25 and 1.0 g/1 showed a worse prognosis for low IgM levels (NS but $P<0.10$).

3.4.2.2.2 Serum IgG

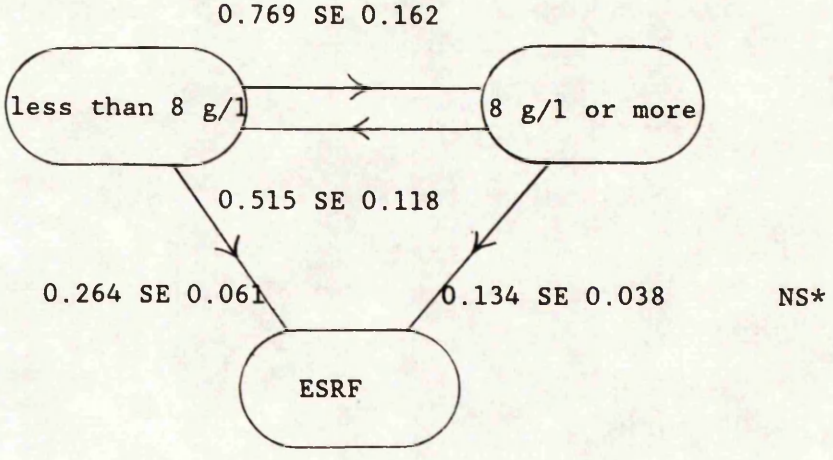
Analyses were carried out using cut-off points 4, 6, 8 and 10 g/1, which were the 14th, 27th, 41st and 58th percentiles.

Although rates of transition to ESRF were lower in patients with higher IgG levels (see, for example, Fig. XXII), none of the differences were statistically significant.

There were some differences in the transition rates to ESRF between the asymptomatic proteinuria and the nephrotic onset patients, but these were not statistically significant. Within the patients with a nephrotic onset, the contrast between the transition rates from each of the two states to ESRF was sharper; a statistically significant difference was observed using a cut-off of 8 g/1 ($P<0.025$, and for patients with levels of 10 or more there were no transitions to ESRF.

FIG. XXII

Serum IgG -
transition rates (x 100) between marker states
and from each state to ESRF
(172 patients)



* Comparison between rates of transition to ESRF

3.4.2.2.3 Serum IgA

Analyses were carried out using cut-off points 0.8, 1.2, 1.6, 2.0 and 2.4 g/l, which were the 12th, 21st, 33rd 45th and 58th percentiles.

Rates of transition to ESRF were lower in the higher IgA state, but the difference was statistically significant only for a cut-off of 2.0 g/l (see Fig. XXIII). Similar trends were observed within both the asymptomatic proteinuria and the nephrotic syndrome onset patients, but none of the sets of results were statistically significant.

3.4.3 Serum complement components

A total of 346 patients had some or all of the serum complement components measured. The results for C3, C4 and Factor B were restricted to the years where there was no change in method (that is, up to the end of 1980 for C4 and Factor B, and 1971 to 1980 for C3).

3.4.3.1 Relationships with other variables

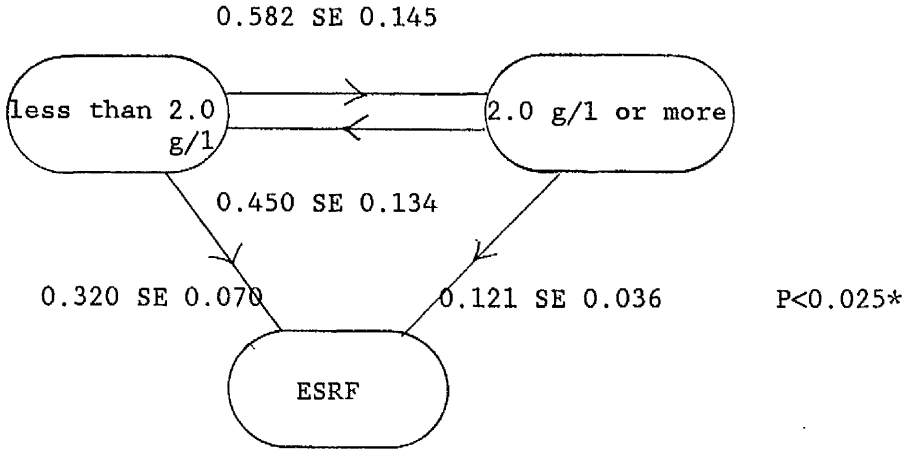
The first set of measurements were used for each patient. The median time from onset was 14 months (range 0 weeks to 28 years).

One fifth (22%) of the patients had a total haemolytic complement (CH50) which was below the lower limit of the reference range, whilst the distributions for C1q, C4, C3 and Factor B were broadly similar to a normal population.

The distribution of total haemolytic complement (CH50), C1q, C3 and Factor B were not significantly different from Gaussian (one-sample Kolmogorov-Smirnov test). The distribution of C4 was positively skewed, but could be rendered approximately Gaussian by the use of a logarithmic

FIG. XXIII

Serum IgA -
transition rates (x 100) between marker states
and from each state to ESRF
(169 patients)



* Comparison between rates of transition to ESRF

(base 10) transformation. Non-parametric correlation coefficients for the relationships with other variables, however, since these too were non-Gaussian.

CH50 was uncorrelated with 24 hour urinary protein loss but was weakly correlated with the prevailing plasma creatinine concentration (n=312, Kendall's tau=0.10, two-tailed P=0.016). The results for C1q were similar (for plasma creatinine, n=144, tau=0.10, NS but P=0.078).

Both C3 and C4 were significantly correlated with urinary protein loss (using the urinary protein/creatinine ratio: for C4, n=141, tau=0.27, P<0.001 and, for C3, n=298, tau=0.13, P<0.001), whilst only C4 was correlated with plasma creatinine (n=149, tau=0.12, P=0.030). Factor B correlated neither with urinary protein loss nor with plasma creatinine concentration.

There were statistically significant differences in levels of C3 between the main biopsy groups (One-way analysis of variance, P=0.011, see Table XXVII), but not for the other complement components. The mean C3 was lowest for the mesangiocapillary group; multiple comparison procedures by Scheffés method (using a 5% level of significance) showed that this group was significantly different from the 'no light change (not MCN)' group.

Patients with SLE had significantly lower serum complement levels of CH50, C4 and C3 (see Table XXVIII), suggesting the possible exclusion of patients with SLE in the sequential analyses below.

Table XXVII
Mean of first serum level of C3 for each of the main biopsy groups

Group	C3 (mg/dl)
Minimal change nephropathy	145 SD 43 (29)
No light microscopic change (not MCN)	161 SD 47 (39)
Membranous nephropathy	147 SD 35 (31)
Mesangial proliferative	145 SD 48 (119)
Focal segmental proliferative	135 SD 53 (12)
Diffuse proliferative	137 SD 34 (10)
Mesangiocapillary	119 SD 50 (31)

Table XXVIII
Mean of first serum level of CH50, C4 and C3
according to whether or not the patient had SLE

	SLE		
	No.	Yes	Difference
CH50 (units)	55 SD 15 (311)	47 SD 19 (20)	P=0.019*
C4 (%)	118, 32-500**(147)	67, 16-202 (11)	P=0.050
C3 (mg/dl)	146 SD 45 (319)	121 SD 53 (22)	P=0.012

* Significance by two-tailed Student's t-test;
log transformed results used for C4
Number of patients in parenthesis

** geometric mean and range

3.4.3.2 Analysis of sequentially collected data

The analysis proceeded using Kay's method to utilise all the available measurements for each individual.

3.4.3.2.1 Total haemolytic complement (CH50)

Cut-offs of 40, 50, 60 and 70 units were investigated; these corresponded to the 14th, 38th, 68th and 87th percentiles. The risk of ESRF was independent of the prevailing CH50 level (see, for example, Fig. XXIV), despite the fact that CH50 had been found to correlate weakly with the prevailing plasma creatinine level (see above). The findings were similar when patients with SLE were excluded.

3.4.3.2.2 C1q

Cut-offs 80, 90, 100 and 120 were used, corresponding to the 17th, 30th, 43rd, 68th and 80th percentile. The risk of ESRF was independent of the level of C1q in each case.

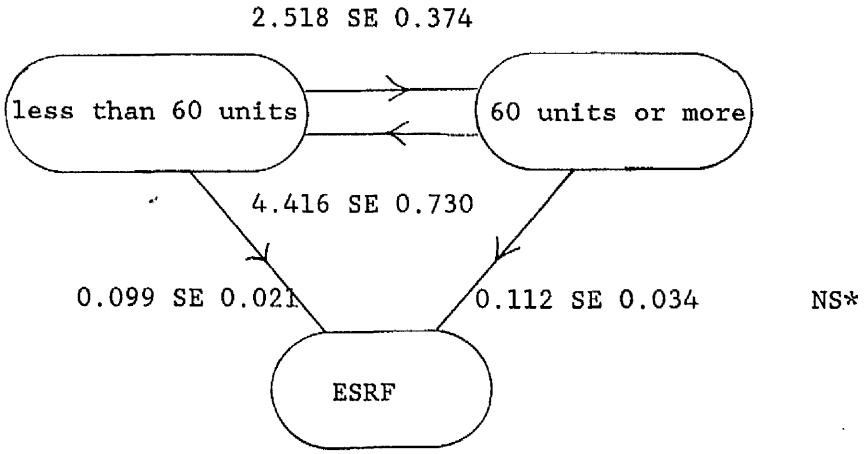
3.4.3.2.3 C4

Cut-offs of 75, 100, 125 and 150 were used; these were the 23rd, 43rd, 60th and 73rd percentiles respectively. A higher level of C4 was associated with a greater risk of ESRF in each case. The most significant difference was obtained using 100 as the cut-off ($P<.001$, see Fig. XXV). The results were similar when patients with SLE were omitted.

The cut-off of 100% was used to try to check the Markov property, using the same approach as the one adopted for serum IgM (see section 3.4.2.2.1). No evidence could be found to show the Markov assumption was being violated.

FIG. XXIV

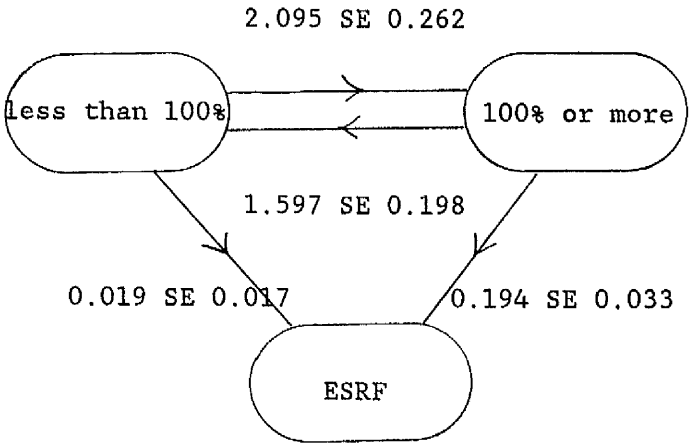
Total haemolytic complement -
transition rates (x 100) between marker states
and from each state to ESRF
(312 patients)



* Comparison between rates of transition to ESRF

FIG. XXV

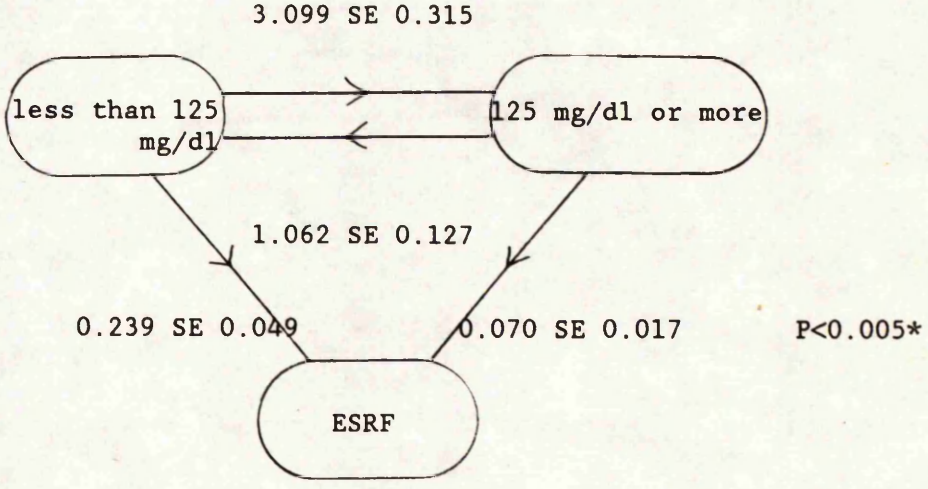
Serum C4 -
transition rates (x 100) between marker states
and from each state to ESRF
(272 patients)



* Comparison between rates of transition to ESRF

FIG. XXVI

Serum C3 -
transition rates (x 100) between marker states
and from each state to ESRF
(309 patients)



* Comparison between rates of transition to ESRF

3.4.3.2.4 C3

The cut-offs used for these analyses were 100, 125, 150 and 175 md/dl, corresponding to the 13th, 34th, 60th and 79th percentiles. For these three analyses, lower levels of C3 carried the greatest risk of ESRF. The most significant difference was attained using a cut-off of 125 (see fig. XXVI). The results were unchanged when patients with SLE were excluded.

There were differences, in this instance, between patients with an asymptomatic proteinuria 'onset' and patients first presenting with the nephrotic syndrome (see Fig. XXVII). A low C3 carried a worse prognosis in the former group, but not in the latter.

3.4.3.2.5 Factor B

The cut-offs used were 80, 100, 120 and 140%, corresponding to the 24th, 49th, 72nd and 86th percentiles. There was no significant relationship between level of Factor B and ESRF.

3.4.4 Lipids and lipoproteins

A much larger data base was available than for the other screen variables; 430 patients had serum cholesterol and/or L, M or S particle 'scores' measured on at least one occasion.

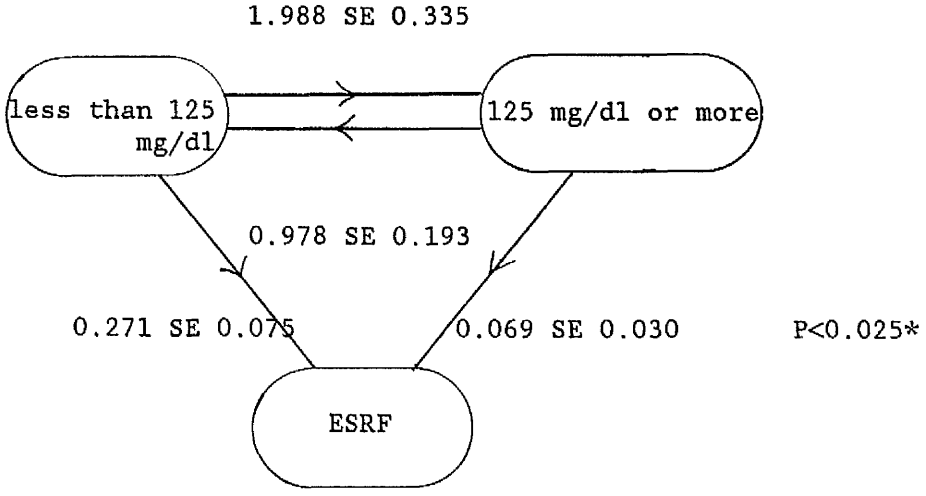
3.4.4.1 Relationships with other variables

The first measurement or set of measurements was used for each patient. This was at a median time from onset of 12 weeks (range 0 to 17 years).

FIG. XXVII

Serum C3 -
transition rates (x 100) between marker states
and from each state to ESRF
Asymptomatic proteinuria and nephrotic
syndrome onset patients shown separately

(a) Asymptomatic proteinuria (101 patients)

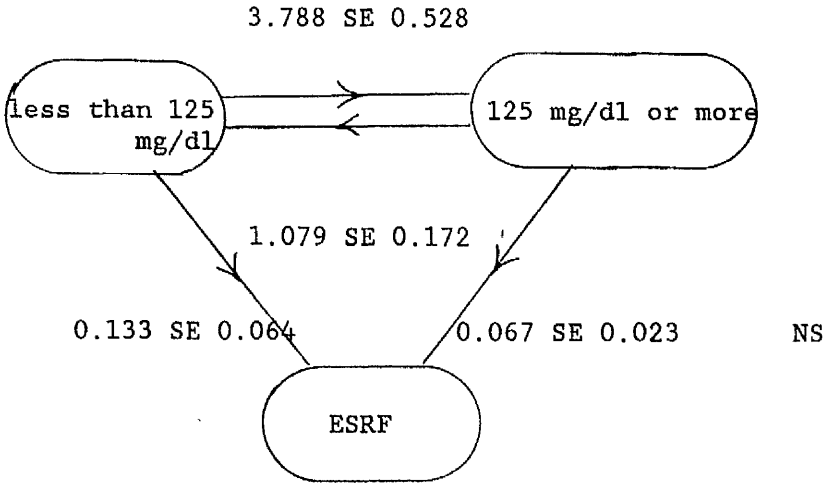


* Comparison between rates of transition to ESRF

FIG. XXVII

Serum C3 -
transition rates (x 100) between marker states
and from each state to ESRF
Asymptomatic proteinuria and nephrotic
syndrome onset patients shown separately

(b) Nephrotic syndrome (176 patients)



* Comparison between rates of transition to ESRF

A large number of patients had levels above the upper limit of the reference distributions; the percentages were 63% for serum cholesterol, 55% for the S particle 'scores', 56% and 72% for the male and female M particle 'scores' respectively, but only 22% for the L particle 'scores'.

Both serum cholesterol and S 'scores' had distributions which were positively skewed, in contrast to a normal population; in both instances a logarithmic (base 10) transformation rendered the distribution approximately Gaussian.

The relationships between the above variables (serum cholesterol and the three particle scores) and each of urinary protein loss, plasma creatinine concentration, plasma albumin, plasma fibrinogen (see next section) and selectivity were explored. Since some of the latter variables were non-Gaussian, it was more convenient to use non-parametric correlation coefficients throughout. Correlation coefficients for the M scores were calculated separately for males and females, since the reference ranges were different. All the variables were significantly correlated with urinary protein loss (for example, using urinary protein/creatinine ratio, tau ranged from 0.18 n=104 two-tailed P=0.007 for L score to 0.38 n=160 P<0.001 for serum cholesterol) and, correspondingly, were negatively correlated with plasma albumin concentration. In addition, there were weak negative correlations with prevailing plasma creatinine concentration for all variables, except M score for females; the correlation coefficient was significant only for serum cholesterol where the group size was larger (n=229, tau=-0.09, P=0.044).

With the exception of the L score, the variables were positively correlated with plasma fibrinogen (tau ranged from 0.19 n=54 P=0.042 for M score for males to 0.33 n=94 P<0.001 for serum cholesterol), which itself was negatively related to plasma albumin concentration (tau=-0.49, two-tailed P<0.001). Further analysis of plasma fibrinogen is documented in a subsequent section.

None of the variables correlated significantly with the selectivity ratio, but the group sizes were small in each case (50 or less).

There was a very strong correlation, as expected, between S score and total serum cholesterol (n=120, tau=0.89, P<0.001). For the remainder of this section, the results of the analyses using the S score were found to be so very similar to those using serum cholesterol that only the latter have been documented.

Table XXIX shows the mean serum cholesterol and particle scores for each of the main biopsy groups. The serum cholesterol levels differed significantly between the groups (one-way analysis of variance using the logarithmically transformed values, P<0.001); multiple comparison procedures using Scheffés test (5% significance) showed pairwise differences between the mesangial proliferative group and both the membranous and minimal change groups, and also between the latter group and the mesangiocapillary group. Although some differences were indicated between the larger groups for the L scores (P=0.040) and for the M scores for males (P=0.023), none of the pairwise differences were significant using Scheffés test.

TABLE XXIX

Mean serum cholesterol and lipoprotein scores for each of the main biopsy groups

(a) Group	Serum Cholesterol (mmol/l)	L Score
Minimal change	11.5, 5.1-20.2**(32)	3.8 SD 1.5* (2)
No light change (not MCN)	8.6, 3.8-22.0 (47)	3.1 SD 1.6 (15)
Membranous	9.9, 4.1-32.3 (99)	2.9 SD 2.0 (25)
Mesangial proliferative	7.9, 3.0-25.4 (140)	2.1 SD 1.2 (47)
Focal segmental proliferative	7.2, 3.4-36.2 (17)	1.6 SD 0.8 (4)
Diffuse proliferative	9.5, 4.5-12.8 (13)	1.5 (1)
Mesangiocapillary	7.5, 3.7-16.7 (38)	1.8 SD 1.2 (10)

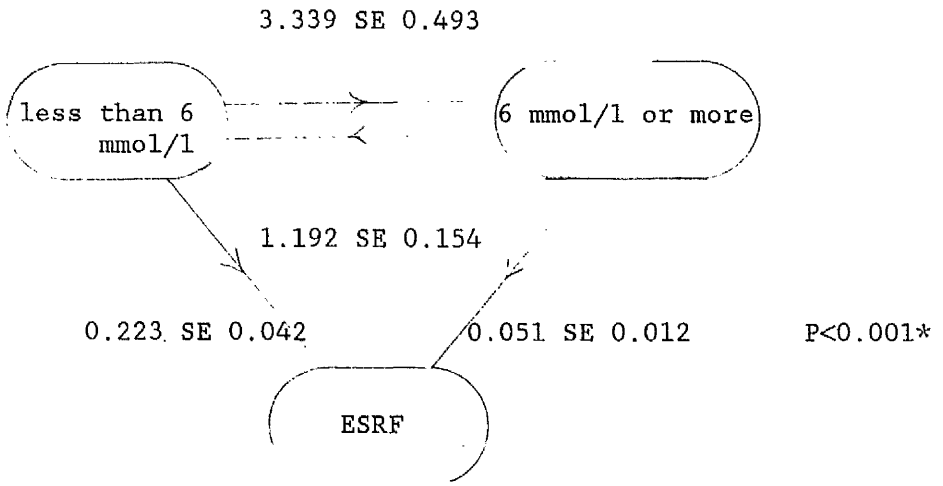
(b) Group	M Score	
	Males	Females
Minimal change	7.2 SD 0.4 (2)	-
No light change (not MCN)	4.7 SD 1.9 (10)	4.1 SD 2.8 (5)
Membranous	5.0 SD 2.3 (21)	5.1 SD 2.0 (4)
Mesangial proliferative	4.2 SD 1.8 (27)	3.1 SD 1.2 (20)
Focal segmental proliferative	2.6 SD 1.6 (3)	5.2 (1)
Diffuse proliferative	-	5.4 (1)
Mesangiocapillary	1.9 SD 0.2 (3)	3.0 SD 1.4 (7)

* Mean and standard deviation
Number of patients in parenthesis

** Geometric mean and range

FIG. XXVIII

Serum Cholesterol -
transition rates (x 100) between marker states
and from each state to ESRF
(381 patients)



* Comparison between rates of transition to ESRF

3.4.4.2 Analysis of sequentially collected data

Next Kay's method was applied, using all the available observations for each patient.

3.4.4.2.1 Serum Cholesterol

Cut-offs of 6, 7, 8, 9 and 10 mmol/1 were investigated; these were the 21st, 34th, 46th, 58th and 67th percentiles. In each instance, a significantly greater risk of ESRF was associated with the lower serum cholesterol level. The difference was most significant using a cut-off of 6 mmol/1, and details are shown in Fig. XXVIII. The result was unchanged after exclusion of the minimal change nephropathy patients. The Markov assumption was tested using this cut-off (see section 3.4.2.2.1); there was no evidence from this analysis to show that the Markov assumption was being violated.

3.4.4.2.2 M Score

Separate analyses were carried out for males and females using cut-offs of 3, 4, 5 and 6. (The 37th, 54th, 67th and 79th percentiles respectively). For male patients, lower levels were associated with a worse prognosis, but the differences were not statistically significant. For females the converse was true, with a statistically significant difference occurring with a cut-off of 4 (see Figs. XXIX(a) and (b)).

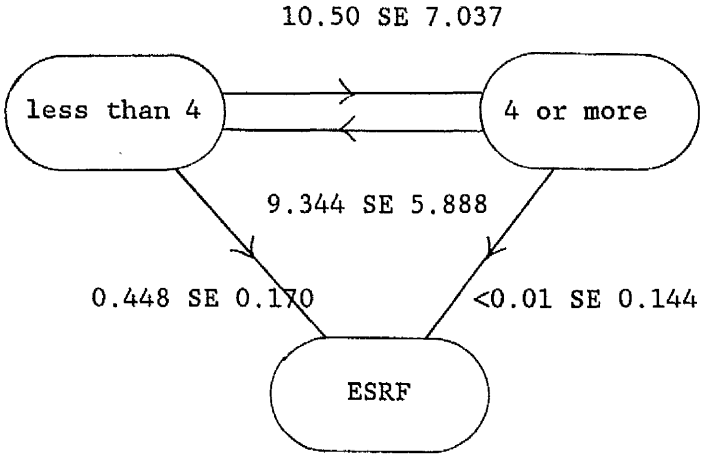
3.4.4.2.3 L Score

Separate analyses were carried out using cut-offs of 1, 2, 3 and 4 (the 22nd, 47th, 69th and 84th percentiles).

FIG. XXIX

M Particle scores -
transition rates (x 100) between marker states
and from each state to ESRF

(a) Males (146 patients)

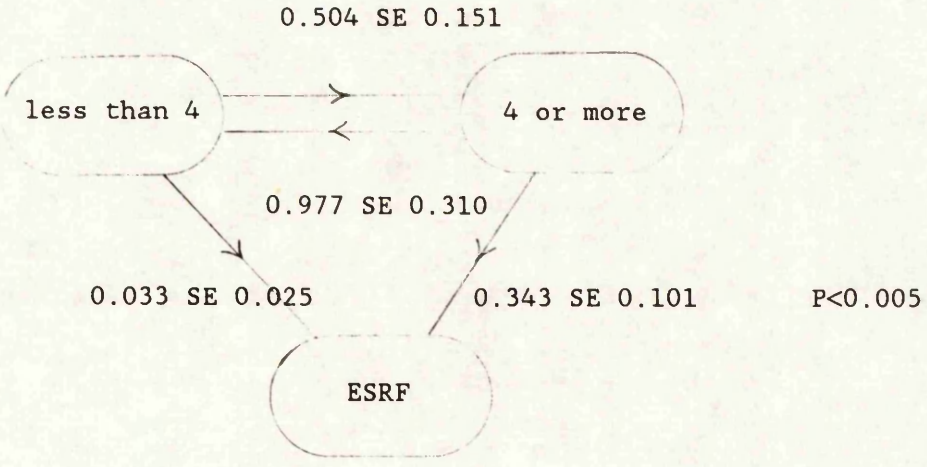


* Comparison between rates of transition to ESRF

FIG. XXIX

M Particle scores -
transition rates (x 100) between marker states
and from each state to ESRF

(b) **Females** (82 patients)



* Comparison between rates of transition to ESRF

There was a trend towards better prognosis with higher scores, but in each case the difference was not statistically significant (see, for example, Fig. XXX).

3.4.5 Plasma fibrinogen

A total of 340 patients had plasma fibrinogen measured at least once.

3.4.5.1 Relationship with other variables

The first measurement was at a median time from onset of 13 months (range 0 weeks to 26 years).

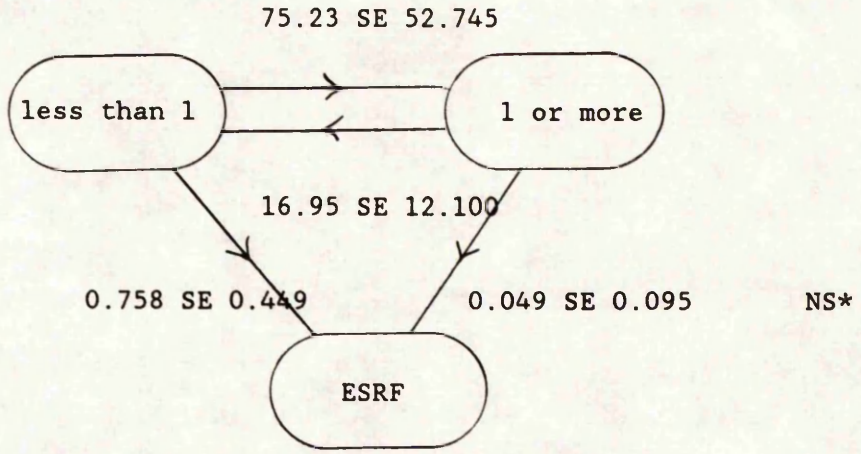
Two thirds (63%) of patients had concentrations above the upper limit of the reference range.

The distribution was positively skewed. Although a logarithmic (base 10) transformation could render the distribution approximately Gaussian, non-parametric methods were used for the correlations with the other variables. Plasma fibrinogen was positively correlated with urinary protein loss (for example, with the urinary protein/creatinine ratio, $n=300$ $tau=0.45$, two-tailed $P<0.001$) and with the prevailing plasma creatinine concentration ($n=323$ $tau=0.16$ $P<0.001$).

Table XXX shows the mean plasma fibrinogen concentrations for each of the main biopsy groups. There were differences between the groups (one-way analysis of variance using logarithmically transformed values $P=0.023$). None of the pairwise differences, however, were statistically significant (Scheffés test).

FIG. XXX

L Particle scores -
transition rates (x 100) between marker states
and from each state to ESRF
(228 patients)



* Comparison between rates of transition to ESRF

Table XXX
Mean plasma fibrinogen
for each of the main biopsy groups

(a) Group	Plasma fibrinogen (mg/100ml)	
Minimal change	380, 144-826	(24)*
No light change (not MCN)	448, 220-1100	(40)
Membranous	512, 252-1166	(74)
Mesangial proliferative	451, 184-1288	(120)
Focal segmental proliferative	390, 211-890	(12)
Diffuse proliferative	477, 390-609	(10)
Mesangiocapillary	449, 186-1024	(30)

* Geometric mean and range

Number of patients in parenthesis

3.4.5.2 Analysis of sequentially collected data

For the applications of Kay's method, cut-offs of 300, 400, 500, 600 and 700 were explored. These were the 11th, 34th, 57th, 71st and 81st percentiles. In all cases except the first, the risk of ESRF was higher for higher plasma fibrinogen levels. For the first, there were no transitions to ESRF from the levels below 300. This did not contradict the other results, but meant that Kay's method could not be applied. Where the method could be applied, the most significant difference was obtained using a cut-off of 400 (see Fig. XXXI).

3.5 Interrelationships between prognostic variables

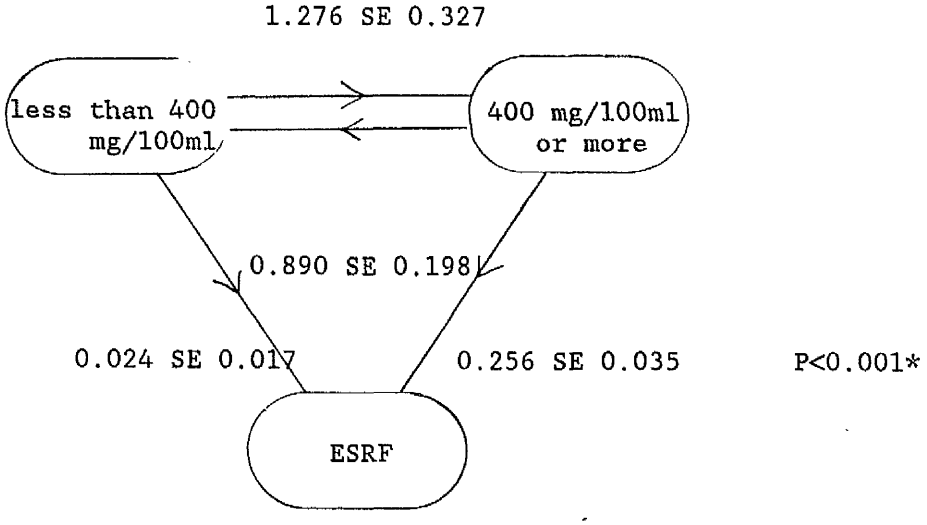
Table XXXI gives a summary of the variables emerging from all the analyses as the most important markers of eventual ESRF. The variables omitted were either not significantly associated with prognosis (sex, age, serum IgG, CH50, C1q, Factor B and L score) or there were conflicting results for men and women (M scores).

The subgroups of values shown in parentheses in Table XXXI were found to be associated with a greater risk of ESRF than the remainder. In the case of the renal screen variables the subgroups were defined somewhat arbitrarily and it was not possible to test whether the risk of ESRF increased or decreased linearly over the continuous range of variable values.

There were obvious interrelationships between the marker variables; for example, the selectivity ratio, serum IgM, serum C4 and plasma fibrinogen were found each to be correlated with the prevailing plasma creatinine

FIG. XXXI

Plasma fibrinogen -
transition rates (x 100) between marker states
and from each state to ESRF
(264 patients)



* Comparison between rates of transition to ESRF

concentration, and therefore their perceived prognostic value merely may reflect this correlation. The final exercise was to explore in more detail the interrelationships between the variables in Table XXXI, and, in addition, the plasma albumin.

A total of 148 patients had at least one complete set of these variables, and the first such set was used for each patient. Approximately one third (30%) of these were within one year; 51% were more than three years from onset. Transformations were used to render the variables approximately 'normal'; the logarithmic transformation was used in this instance for serum IgA and urinary protein/creatinine ratio, since there were no zero values.

The correlation matrix is shown separately for men and women in Table XXXII. These represent variable associations across the patients, and are not concerned with the relationships with prognosis.

Table XXXII suggested that there were clusters of variables which were highly correlated. The first cluster contained urinary protein loss, plasma albumin, serum cholesterol, plasma fibrinogen, and also possibly C4 and serum IgA. The second contained plasma fibrinogen, serum C3 and C4 and possibly plasma creatinine. The third contained the selectivity ratio, haemoglobin concentration, diastolic blood pressure, plasma creatinine and possibly the serum IgM. A factor analysis with a varimax rotation was used to confirm these clusters.

Table XXXIII shows the separate factor analyses carried out for men and women. The sample size for women was small

TABLE XXXI

A summary of the variables found to be the most significant markers of ESRF

From the follow-up data:-

Plasma creatinine	
Urinary protein/creatinine ratio	($\geq$ 4)
Diastolic blood pressure	($\geq$ 110 mm Hg)*
Haemoglobin	(<13 g/dl for men;* <11.5 for women)

From the 'renal screens':-

Selectivity ratio	(>0.3)
Serum IgM	(<1.5 g/l)
Serum IgA	(<2 g/l)
Serum C4	($\geq$ 100%)
Serum C3	(<125 mg/dl) *
Serum cholesterol	(<6 mmol/l)
Plasma fibrinogen	($\geq$ 400 mg/100 ml)

* A more important marker in patients whose disease first manifest as asymptomatic proteinuria

TABLE XXXII

Matrix of correlation coefficients between the screen variables (men and women shown separately)

* Males n = 94
** Females n = 54
highlighted if two-tailed P<0.05

	1/PC	BP	HB	SEL	UP	PA	CHO	FIB	C3	C4	IgM
BP	**-0.29***										
	-0.28**										
HB	**0.52***	-0.19									
	0.37**	-0.00									
SEL	**-0.47***	**0.38***	**-0.32***								
	-0.41**	**0.44****	-0.20								
UP	-0.09*	0.15*	-0.06*	0.10*							
	-0.21**	0.02**	-0.09**	0.08**							
PA	-0.11*	0.09*	0.05*	0.19*	**-0.71***						
	0.06**	0.11**	**0.39****	0.01**	**-0.71****						
CHO	0.10*	-0.05*	**0.28***	-0.14*	**0.59***	**-0.63***					
	-0.20**	0.13**	-0.21**	**0.37****	**0.51****	**-0.53****					
FIB	**-0.23***	0.02*	-0.18*	-0.00*	**0.64***	**-0.60***	**0.47***				
	-0.27**	-0.03**	-0.24**	0.20**	**0.31****	**-0.30****	**0.59****				
C3	-0.06*	-0.11*	-0.02*	-0.14*	0.08*	-0.00*	0.14*	**0.36***			
	0.22**	-0.14**	**0.30****	-0.09**	-0.02**	**0.27****	0.12**	**0.47****			
C4	**-0.26***	0.10*	-0.13*	0.13*	**0.31***	-0.20*	0.19*	**0.45***	**0.31***		
	-0.32**	0.20**	0.03**	0.22**	0.25**	-0.07**	**0.45****	**0.57****	**0.34****		
IgM	0.20*	-0.19*	**0.28***	-0.19*	0.01*	0.17*	0.12*	-0.03*	-0.13*	-0.14*	
	0.18**	-0.09**	0.19**	-0.18**	0.13**	-0.14**	-0.11**	-0.16**	-0.08**	-0.13**	
IgA	-0.06*	**0.24***	-0.12*	0.12*	-0.11*	**0.28***	**-0.24***	**-0.25***	0.08*	0.07*	-0.14*
	0.07**	-0.06**	0.11**	-0.18**	-0.19**	0.13**	-0.14**	0.02**	0.18**	0.07**	0.10**

KEY

1/PC	Reciprocal Plasma Creatinine	PA	Plasma albumin
BP	Diastolic Blood Pressure	CHO	Log_{10} (serum cholesterol)
HB	Haemoglobin	FIB	Log_{10} (plasma fibrinogen)
SEL	Selectivity ratio to power 0.45	C3	Serum C3
		C4	Log_{10} (serum C4)
UP	Log_{10} (urinary protein/ creatinine ratio)	IgM	Log_{10} (IgM)
		IgA	Log_{10} (IgA)

TABLE XXXIII

Factor analysis of the screen variables*

(i) Rotated factor matrix for 94 men (54% of variance explained)

	I	II	III	IV
(Plasma Creatinine)$^{-1}$	-**	-0.55	0.37	-
Diastolic blood pressure	-	0.55	-	-
Haemoglobin	-	-0.27	0.96	-
Selectivity ratio (to power 0.45)	-	0.73	-	-
Log_{10} (urinary protein/ creatinine ratio	0.81	-	-	-
Plasma albumin	-0.94	-	-	-
Log_{10} (serum cholesterol)	0.69	-	0.27	-
Log_{10} (plasma fibrinogen)	0.67	-	-	0.52
Serum C3	-	-	-	0.71
Log_{10} (serum C4)	0.27	-	-	0.46
Log_{10} (serum IgM)	-	-	-	-
Log_{10} (serum IgA)	-0.27	-	-	-

(ii) Rotated factor matrix for 54 women (60% of variance explained)

	I	III	IV	II
(Plasma Creatinine)$^{-1}$	-	-0.57	0.36	-
Diastolic blood pressure	-	0.61	-	-
Haemoglobin	-	-	0.82	-
Selectivity ratio (to power 0.45)	-	0.66	-	-
Log_{10} (urinary protein/ creatinine ratio	0.77	-	-	-
Plasma albumin	-0.96	-	-	-
Log_{10} (serum cholesterol)	0.58	0.27	-	0.45
Log_{10} (plasma fibrinogen)	0.28	-	-0.31	0.83
Serum C3	-	-0.27	-	0.74
Log_{10} (serum C4)	-	0.31	-	0.62
Log_{10} (serum IgM)	-	-	0.26	-
Log_{10} (serum IgA)	-	-	-	-

* Maximum likelihood method, followed by varimax rotation

** Only coefficients >0.25 are shown, and are highlighted if >0.50

and therefore the results were likely to be less reliable, but they nevertheless confirmed the results found for the men. Four significant factors were indicated; the first factor was strongly correlated with urinary protein loss, plasma albumin, serum cholesterol and plasma fibrinogen, the second was correlated with plasma creatinine, diastolic blood pressure and selectivity ratio, the third factor was correlated with haemoglobin and the fourth with serum C3, serum C4 and plasma fibrinogen.

The factors were independent of one another since a varimax rotation had been used. This rotation, however, tried to force each variable to be highly correlated with only one factor, and it may be that the variable was related to more than one cluster of variables. Mixed interaction modelling was used therefore to study the variable interactions in a more meaningful way.

The matrix of partial correlations is shown separately for men and women in Table XXXIV.

These partial correlations removed the effect of all other variables; a better approach would have been to eliminate variables by the backward selection procedure offered by MIM (Edwards, 1987), but there were computational dfficulties due to the large number of variables involved. This method was used for some subsets of the variables, but there were conflicting results depending on which variables were 'partialled out'.

If the interrelationships between the variables were the same for men and women, then the results in Table XXXIII should be confirmatory. Some simplification was possible by

TABLE XXXIV

Matrix of partial correlations between screen variables (men and women shown separately)

* Males n = 94
** Females n = 54
Highlighted if two-tailed P<0.05

	1/PC	BP	HB	SEL	UP	PA	CHO	FIB	C3	C4	IgM
BP	-0.10*										
	-0.06**										
HB	**0.42**	-0.01									
	0.32	0.04									
SEL	**-0.26**	0.19	-0.14								
	-0.19	**0.32**	-0.10								
UP	0.00	0.15	0.09	**0.25**							
	-0.38	0.06	**0.32**	-0.01							
PA	**-0.25**	0.10	**0.36**	**0.26**	**-0.46**						
	-0.38	0.12	**0.40**	0.18	**0.69**						
CHO	-0.11	0.02	**0.42**	0.02	0.20	**-0.37**					
	0.09	0.06	0.08	**0.35**	0.06	-0.33					
FIB	**-0.22**	0.02	-0.08	-0.06	**0.30**	**-0.26**	0.03				
	-0.29	-0.10	-0.19	0.03	-0.17	-0.28	0.27				
C3	0.05	-0.14	-0.04	-0.18	-0.05	**0.27**	0.15	**0.39**			
	0.43	-0.13	0.09	-0.06	**0.31**	**0.49**	0.03	**0.59**			
C4	**-0.13**	0.00	0.08	0.08	0.01	-0.05	-0.01	**0.24**	0.16		
	-0.21	0.17	0.16	-0.05	0.08	-0.08	0.20	0.29	0.08		
IgM	-0.06	-0.10	**0.24**	-0.02	-0.07	-0.19	-0.05	-0.01	-0.09	-0.09	
	0.05	-0.01	0.19	-0.01	0.07	-0.14	-0.11	-0.04	-0.03	-0.04	
IgA	0.06	0.20	-0.12	-0.04	0.17	0.12	-0.07	**-0.26**	0.17	0.16	-0.00
	-0.09	0.03	0.04	-0.12	-0.21	-0.12	-0.07	-0.03	0.16	0.11	0.11

KEY

1/PC	Reciprocal Plasma Creatinine	PA	Plasma albumin
BP	Diastolic Blood Pressure	CHO	Log_{10} (serum cholesterol)
HB	Haemoglobin	FIB	Log_{10} (plasma fibrinogen)
SEL	Selectivity ratio to power 0.45	C3	Serum C3
		C4	Log_{10} (serum C4)
UP	Log_{10} (urinary protein/ creatinine ratio)	IgM	Log_{10} (IgM)
		IgA	Log_{10} (IgA)

choosing to ignore any significant partial correlations where the results for the two sexes were dissimilar. Figure XXXII shows the simplified structure; variables were joined by a line if they were unconditionally related.

A number of features were suggested by Fig. XXXII:-

First, urinary protein loss was 'conditionally independent' from both serum cholesterol and plasma fibrinogen given plasma albumin; this means that plasma albumin was needed to 'explain' the relationship between urinary protein loss and both serum cholesterol and plasma fibrinogen. Perhaps the liver was stimulated to produce more cholesterol and fibrinogen as it synthesised albumin to compensate for that which was lost. Serum C4 was related to these four variables only via its correlation with fibrinogen (see below).

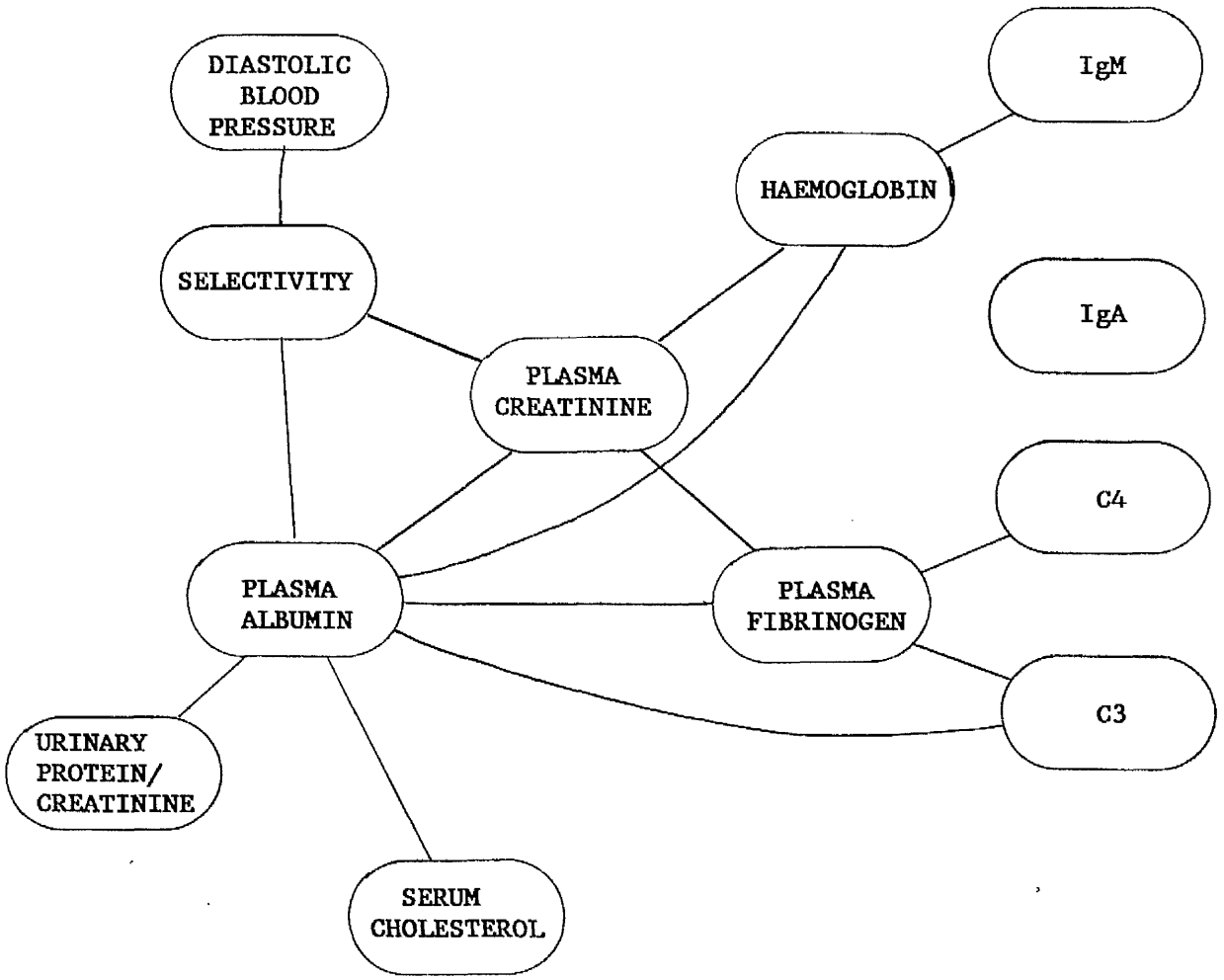
Secondly, the diastolic blood pressure and plasma creatinine concentration were conditionally independent given the selectivity ratio; the latter was needed to 'explain' the correlation between blood pressure and plasma creatinine, and by inference GFR.

Thirdly, the haemoglobin concentration was unconditionally correlated with plasma creatinine. This was perhaps to be expected since both reflected damage done to the kidney. Serum IgM was related to plasma creatinine via haemoglobin concentration.

Fourthly,plasma fibrinogen (and plasma albumin) was unconditionally correlated with plasma creatinine. Both serum C3 and C4 were related to each other and to plasma creatinine only via plasma fibrinogen. Again,

FIG. XXXII

Conditional independence graph derived from the partial correlation matrix for the 'screen' variables



this may suggest liver involvement as both C3 and C4 are made by the liver.

Finally, serum IgA was unrelated to any of the other variables.

CHAPTER FOUR

DISCUSSION

4.1 General

The main aim of this study was to apply statistical failure-time methods to look for markers of prognosis in patients with glomerular disease with proteinuria. A number of clinical variables were identified which may help identify patients with a high risk of developing ESRF. Some of the interrelationships between these variables were studied. These variables need re-examining on a prospective series for confirmation (see below).

A number of different approaches were adopted in the analyses; these approaches represented a pragmatic response to the complex nature of the disease under study and the follow-up procedures which had been adopted. The multivariate Cox proportional hazards method was used extensively. This is in line with its increasing usage in other studies published since 1984 (Magil, Ballon, Chan, et al., 1984; D'Amico, Minetti, Ponticelli, et al., 1986; Beukhof, Kardaun, Schaafsma, et al., 1986; Gerstoft, Balsolv, Brahm, et al., 1986; Heilman, Offord, Holley and Velosa, 1987).

As emphasised initially, precise definitions of start and end-points were necessary for the type of statistical analyses undertaken. It was felt more appropriate in this study to take as the 'start' date the earliest date the disease manifested. Whilst the 'start' was easier to define for patients first presenting with a nephrotic syndrome, this, of

course, may not represent the true onset. Half of the patients with asymptomatic proteinuria later had a nephrotic syndrome, so the disease may have 'smouldered' for a while without any symptoms. Interestingly, the survival of the latter group of nephrotics was much worse than that of the former even when patients with MCN were excluded. The 'onset' for asymptomatic proteinuria patients is unknown and was taken, arbitrarily, as the date that the proteinuria was first apparent.

Blainey and co-workers (Blainey, Brewer and Hardwicke, 1986) also chose to analyse non-nephrotics separately but, in general, comparisons with other studies are difficult if such a separation has not been made. Some authors used the date of biopsy as the 'start'; we chose not to use this date as it was often some considerable time after onset. In general, the main histopathological classification does not change, whilst the clinical variables, such as urinary protein loss, do. A case for using the date of first biopsy may be when fine biopsy detail is included (see below), since such detail may vary with time and need to be considered together with clinical variables measured at the same time. It may be possible to take into account disease progression by stratifying by the time which has elapsed from the first manifestation of disease.

The end-point used here was ESRF, in line with most of the more recent studies reviewed by this author; 'renal survival' was examined rather than actual survival. In this study, the risk of death from other causes increased with

age, as might be expected. The total number of such deaths, however, was significantly greater than the number expected for such a cohort in this region. The deaths included deaths which may have been related to the consequences of prolonged renal disease, such as ischaemic heart disease or cerebrovascular disease. There was a suggestion that the excess of such deaths decreased over the study period. These findings need further study, perhaps with a comparison of the numbers of deaths from each cause with the cause-specific expected rates.

In the study from four Copenhagen Hospitals (Braham, Balslov, Brun, et al., 1985; Gerstoft, Balslov, Brahm, et al., 1986), plasma creatinine was used to define end-point. Times of transitions between three plasma creatinine states and uraemia were modelled, sometimes with fairly small cohorts. The covariates used were variables measured at the time of the biopsy, rather than the start of each transition period, and therefore the authors ignored any changes in these variables. The present study shows how alteration in risk can be modelled by time-dependent covariates and, furthermore, that such approaches are very flexible. Plasma creatinine was used here as a covariate marker of eventual ESRF. Although plasma creatinine rises as ESRF is approached, a rise in plasma creatinine did not necessarily mean that ESRF was inevitable. Some patients had concentrations which remained above 0.2 mmol/l for several months and later fell.

The emphasis of the present study was on the clinical variables, and so patients were analysed from across the

histopathological spectra, without the deliberate exclusion of systematic diseases. The policy, rightly or wrongly, was to avoid subdividing the data set. Even idiopathic biopsy subgroups may eventually turn out to be non-homogeneous subsets; for example, Beukhof and his co-workers, suggested that idiopathic IgA nephropathy with and without macroscopic haematuria may represent two different diseases (Beukhof, Kardaun, Scaafsma, et al., 1986).

Amongst other natural history studies, the prognosis was found to be best for a histopathological diagnosis of minimal change nephropathy, followed by, in order, mesangial proliferative disease, mesangiocapillary disease, focal segmental glomerular sclerosis and membranous nephropathy (see Cameron, 1979). Other authors have shown the prognosis of mesangiocapillary to be poor, and worse than that of membranous nephropathy (see Brahm, Balslov, Brun, et al., 1985 for a review). For the nephrotic syndrome patients analysed in the present study, there were differences in renal survival between the main biopsy groups. The prognosis was best for MCN and worst for mesangiocapillary disease. Differences between the other groups, as far as one could tell from the small groups analysed, could be explained by differences in the other clinical variables associated with prognosis, namely plasma creatinine and urinary protein loss. Furthermore, spontaneous remission of proteinuria occurred more slowly in the mesangiocapillary group than in the other groups (the cumulative percentage of patients who had not remitted by five years was 80% for mesangiocapillary, compared to between 26% and 70% in the other groups).

The authors of the Copenhagen study initially suggested that light microscopy was important in determining prognosis, but later qualified this by stating that biopsy does give new prognostic information. This was the case, however, only for patients with plasma creatinine concentrations of less than 0.2 mg/100 ml. Mesangiocapillary disease had a greater risk of eventual increase in plasma creatinine than their baseline, mesangial proliferative disease group, and by implication the risk of ESRF or recovery had changed in these patients. Tomura and co-workers (Tomura, Tsutani, Sakuma and Takeuchi, 1985) found that patients with mesangiocapillary disease had both the highest mean plasma creatinine concentrations and systolic blood pressures relative to the other groups they analysed.

Tomura's figures also showed that the mean serum cholesterol concentration was highest for MCN and focal glomerular sclerosis, in parallel with heavier urinary protein losses and lower mean plasma albumin levels in these groups. Amongst the nephrotic syndrome onset patients analysed here, the mean plasma albumin was lowest in the MCN patients only at onset (data not shown); by two years, however, this difference was reversed, and the mean for this group was highest. In addition at two years the median 24 hour urinary protein loss was significantly lower in the MCN group than in the other groups. Although time related studies were not carried out with serum cholesterol, the findings are likely to be similar, since serum cholesterol was shown to be highly correlated with plasma albumin.

If accurate prediction of biopsy is possible from clinical and laboratory variables, as claimed by Tomura, then this may argue against the case for a renal biopsy. Despite its apparently poor value in prognosis, the biopsy is important in the patient management, since it can indicate whether a good therapeutic response is likely (minimal change nephropathy and vasculitic syndromes). Furthermore, fine biopsy detail, which was not available for the present series (other than for a small subset, see O'Donoghue, Lawler, Hunt, Acheson, et al.), is highly likely to refine the prognosis. A number of authors have looked at such morphological changes as glomerular sclerosis and obsolescence, tubulo-interstitial inflammation, and interstitial fibrosis, for individual biopsy groups, sometimes using their own system of grading, and have correlated the findings with outcome and with other clinical variables (see, for example, Nicholls, Fairley, Dowling and Kincaird-Smith, 1984; D'Amico, Minetti, Ponticelli, et al., 1986; Beukhof, Kardaun, Schaafsma, Poortema, et al., 1986; Heilman, Offord, Holley and Velosa, 1987; Magil and Ballon, 1987; Schmitt and Bohle, 1987; Schumm, Wehrmann, Bogenschutz, et al., 1987; Wehrmann, Schumm, Bogenschutz, et al., 1987). The results seem encouraging.

Amongst the nephrotic patients here, renal survival was worse for patients with SLE and amyloidosis. The difference, however, was not significant when other clinical variables were taken into account. The Copenhagen study excluded amyloidosis, but included connective tissue disorders (SLE, polyarteritis nodosa and Wegener's granulomatosis), reporting

that the poor prognosis in these groups was due to more deaths from other causes than uraemia. In the present study outcome with respect to ESRF was of major importance; patients with SLE and also MCN were omitted from some analyses where it was thought relevant. The omission seemed, in general, to make little difference to the findings, but this may be because of the small numbers of patients involved; the overall findings obviously reflected the largest subgroup.

Although this is a single-centre study, there may have been changes in the patient management care when the patients were referred to the MRI, and these were not taken into account. A small proportion of results which were measured at outlying centres were included; there may have been variation between results obtained from different hospital laboratories, but it was not possible to make adjustment for this in the retrospective assessment. The proportion of results referred to is likely, in any case, to be small, and would have included early determinations of urinary protein (which were usually by dipstick and were disregarded in the present analysis in favour of the more accurate quantitative assessment), concentrations of both plasma creatinine and plasma albumin and blood pressure.

4.2 Findings from the study

4.2.1 Choice of Model

The shape of the hazard function for the nephrotic syndrome patients was 'peaked' and the Weibull model, therefore, was disregarded. This is in contrast to Austin

and co-workers who used it, albeit without justification, in a subset of patients with SLE (Austin, Muenz, Joyce, et al., 1983). Peak risk of ESRF was three to four years from onset. This is in general agreement with the Copenhagen study where most of the changes in renal state took place within five years from entry.

The shape of the hazard rate was very different in the patients who first presented with asymptomatic proteinuria, reflecting the non-homogeniety of this group and the uncertain disease onset. This group should be analysed separately from the nephrotic onset patients. For the former group the Cox model is inappropriate.

Returning to the nephrotic onset patients, the log-normal and log-logistic models both fitted well, and confirmed the results which were obtained using Cox proportional hazards, despite depending on different assumptions.

Since the log-logistic model can cope with left censoring, it was thought that this may be applicable for the asymptomatic proteinuria patients where the 'true' onset is not known. Questions arose as to when the covariates should be measured, and whether one could take into account time-varying covariates; these remain problems for the statistical theoretician.

Since the Cox model could accommodate time-varying covariates, and had a wider scope of application, it was found to be the most useful approach here. The proportional hazards assumptions should be reassessed with a larger series.

4.2.2 Analysis with fixed and time-varying covariates

Initial univariate analysis of the patients with a nephrotic syndrome onset suggested that a number of variables were related to an increased risk of ESRF; these were male sex, and an increased age, plasma creatinine concentration, diastolic blood pressure and urinary protein loss (equivalent to 5 g/24 h or more). An analysis of the data at one year proved to be the most satisfactory. Only plasma creatinine and urinary protein loss were significant in the multivariate analysis, which took all these variables into account.

There was a preponderance of men, (in line with other UK studies; see Mallick, Short and Hunt, 1987), and their renal survival was worse than that of women across most of the histopathological spectrum. Men were found to have heavier protein losses and higher plasma creatinine concentrations than women, however, even after correcting for differences in body size. Thus, if these variables were taken into account, the risk of ESRF was no longer significantly worse in men. It is not known why glomerular diseases are more likely to manifest in men, nor why the patient's condition is likely to be worse when it does occur.

The initial suggestion also that younger patients had slightly better prognosis was not substantiated in the multivariate analysis. In a study of IgA nephropathy (D'Amico, Minetti, Ponticelli, et al., 1986) age was suggested to be an important prognostic indicator in the univariate analysis, but its effect disappeared in a multivariate analysis. The authors concluded that only urinary protein loss and fine biopsy detail were important. In a further study of IgA

nephropathy (Beukhof, Kaudaun, Schaafsma, et al., 1986), the effect of age was removed when the GFR was corrected for age.

The plasma creatinine level was expected to be associated with survival, since it rises as GFR falls. Urinary protein loss may denote present or past disease activity. Both of these variables have been reported as markers, either of eventual ESRF or of a further increase in GFR, by a number of authors across the histopathological groups (more recent examples include Beukhof, Kardaun, Schaafsma, et al., 1986; O'Donoghue, Lawler, Hunt, et al., Heilman, Offord, Holley and Velosa, 1987; Donadio, Torres, Velosa, et al., 1988). The definitions and the cut-offs used tended to vary, however.

Both of these variables were statistically significant markers of eventual ESRF in this study. Amongst the surviving patients, protein levels fell with time. This suggests that the lesion healed gradually. Initial analysis showed that patients with heavy urinary protein losses had higher plasma creatinine concentrations; a subsequent detailed study of the intercorrelation structure, however, suggested that, in fact, coincidental measurements of these two variables were independent of one another. Both, as far as one could tell, appeared to be related independently with renal survival. Perhaps the one preceded the other, so that a heavy protein loss gave rise to a subsequent increase in plasma creatinine. The answers to such questions may be found by using Time Series Analysis (see below). In this study, analyses with time-dependent covariates were able to confirm that heavy protein losses were associated with an increased risk of ESRF, up to at least two years subsequently.

If increased urinary protein loss was associated with increased risk of ESRF, then MCN was the exception, since some of the heaviest losses were found in this group yet the prognosis was very good. These patients, however, were defined clinically by their prompt remission of proteinuria. In other patients with no light microscopic change disease, the proteinuria was not controllable and the prognosis was not as good. A spontaneous remission of proteinuria significantly improved the prognosis in non-MCN patients.

A detailed study of prednisolone or other steroid therapy was not undertaken since the policy at the MRI was not to treat patients unless they had potentially steroid responsive lesions. It became evident, however, that a large proportion of patients had received prednisolone or other steroid therapy at some time in the course of their disease, often by their referring General Practitioner, and usually at a less than effective dose. Cameron described a study of the 'natural history' of glomerulonephritis as really being an 'unnatural history' (Cameron, 1979), and referred to its 'obscuring overlay of treatment'. Analysis with time-dependent covariates in the present study suggested that the risk of ESRF was increased with heavy protein losses, irrespective of whether there had been any therapy over the preceding 12 months but no account was taken of dose or duration in this analysis.

It may be that if proteinuria were reduced, either spontaneously or by therapy, then the risk of ESRF was coincidently diminished. This could be the result of healing of the lesion or by a reduction of the damage caused. It has

been suggested that the proteinuria itself may cause irreversible glomerular damage (Cameron, 1982). Prolonged, severe proteinuria is associated with a poor prognosis irrespective of histopathology.

For patients other than MCN, there may have been small, subtle reductions in the urinary protein loss due to therapy, which at most gave rise to a partial remission. The effects, if any, of such changes on prognosis would be difficult to model statistically.

The presence of hypertension may indicate a poorer prognosis if it occurs as a result of damage to the kidney, but its prognostic value was difficult to assess here as treatment usually intervened. Analysis with time-dependent covariates showed that a raised diastolic blood pressure was associated with an increased risk of ESRF up to at least one year subsequently, but it was difficult to separate this effect from that of a high plasma creatinine concentration which tended to occur concurrently.

The authors of two independent studies of IgA nephropathy tried to look at the effect of 'uncontrolled' hypertension (Beukhof, Kardaun, Schaafsma, et al., 1986; Payton, McLay and Boulton Jones, 1988). There were some differences between the two studies in the definition of hypertension, as well as in statistical approach. In the first study, hypertension was defined as a diastolic blood pressure exceeding 97 mm Hg, and such hypertension was regarded as being treated adequately if it fell and remained below this level; these two occurrences were included as 'dummy' variables in a Cox model. In the second study, the mean blood pressure over the period of the

study was calculated and a level in excess of 95 mm Hg was used to define hypertension, and comparisons were made using the logrank test. The findings from both studies were essentially the same, namely that prognosis for patients with 'insufficiently treated' hypertension was poor, but if there was adequate treatment then renal survival did not differ significantly from that of non-hypertensive patients. In the latter study, patients with uncontrolled hypertension had higher plasma creatinine concentrations than those with controllable hypertension, although this difference was not statistically significant; this may suggest that a poorer control actually reflected a more advanced disease. The finding in the present study that the second and subsequent periods of hypertension took longer to control than the first may support this hypothesis. However, in the first study described above, the effect of insufficient hypertension control was statistically significant even after adjustment for the initial GFR.

In the present study, an analysis with time-dependent covariates was used to try and take into account antihypertensive therapy. The very tentative findings suggested that the need for antihypertensive therapy was associated with increased risk of ESRF, even when hypertension was controlled (diastolic blood pressure less than 100 mm Hg), but that the risk was greatest when hypertension was uncontrolled. The results, however, did not taken into account the dose and type of antihypertensive therapy received, and whether the therapy was adequate.

There were further trends towards increased risk with low plasma albumin creatinine concentrations and with anaemia (haemoglobin concentration less than 13 for men or 11.5 for women). These were not statistically significant, and may have resulted from relationships of these variables with others, since plasma albumin concentration tends to fall with increased proteinuria, and haemoglobin falls through loss of erythropoietin as the kidney is progressively damaged. The analysis with time-dependent covariates, however, showed that anaemia was a significant short-term marker of ESRF; a reduced haemoglobin level was associated with an increased risk of ESRF up to one year subsequently.

Some interesting features emerged from the study of the patients presenting initially with asymptomatic proteinuria, despite the more restricted analysis. Renal survival for men and women did not differ significantly, but here their plasma creatinine levels were similar. Urinary protein losses, as expected, were less for the patients in this group than the nephrotics, and prognosis was best for those whose proteinuria remained below 5 g/24h and was worse for the patients whose levels were heaviest enough to cause oedema. Renal survival amongst the latter group of patients was worse than the nephrotic onset patients even when MCN patients were excluded. This suggested a more advanced disease which had been 'smouldering', rather than an early detection of patients who would later become nephrotic; possibly dietary albumin or other factors had prevented earlier heavy proteinuria from becoming symptomatic. At one year, moderate and uncontrolled

hypertension and anaemia defined patients with significantly increased risk of ESRF.

4.2.3 Problems encountered with the 'screen' variables

The ad hoc timing of the 'renal screens', together with sparse data collection, precluded the use of Cox's model for these variables, and only two statistical approaches were possible. Either one could use the first variable measurement for each patient and calculate Kaplan-Meier survival estimates from the time the variable was measured and compare these ignoring all subsequent measurements, or one could use Kay's method based on a continuous-time Markov model which used most of the measurements, but which was not in general use and whose merits were relatively unknown. The second of the two approaches was felt likely to be the more satisfactory and was adopted for this study. The first method was used a confirmatory check for the first two of variables found to be statistically significant markers of ESRF, namely the selectivity ratio and the serum IgM (see below).

Kay's method depends upon a Markov assumption which is impossible to verify satisfactorily; a patient moves from covariate state to covariate state and the risk of ESRF is dependent only on the prevailing state. It may be possible subsequently to allow for the transition rates to further vary with time from onset, and this may be more comparible with the Cox approach which allows for a changing underlying baseline hazard.

Initially a discrete-time Markov approach (a Markov 'chain') was considered for the four-monthly data, using the method described by Myers and co-workers (Myers, Paulson,

Berry, et al., 1980). This was discarded, mainly because of its restriction to stationary (non-changing) transition probabilities. The authors were continuing their investigations into parametrising the time-dependence and the dependence on the covariates, and their approach may lend itself to future studies of glomerular diseases.

The main disadvantage with Kay's method was that it was a univariate procedure. There were interrelationships between the variables and it was impossible to examine their independent effects. These need to be studied prospectively, with a better structured data collection, particularly with regard to the timing of the measurements (see below).

The second disadvantage was that the variable range had to be dichotomised; if the effect of a variable was not linear, for example, if the risk of ESRF is increased for very high AND very low values,but not for intervening values, then this effect may be missed. Where this is suspected, a three state model could be used (see Kay, 1986).

An obvious application of Kay's method would be to see whether values either above or below the reference range were associated with adverse prognosis. There were computational difficulties if relatively few of measurements were abnormal. A series of cut-offs were used, therefore, which spanned the whole range of values. Indeed, changes which occur within the reference range may be more important clinically (see, for example, Mallick, Eyres, Acheson, et al., 1981).

The first renal screen was usually carried out when the patient was biopsied but the subsequent screens were carried out apparently 'if proteinuria persisted'. This was a cause

for statistical concern since the second and subsequent results were likely to be biased towards the patients with more active disease. There may be a problem particularly with the variables which are correlated with urinary protein loss. In practice however, urinary protein losses at these times appeared to span a wide range which included levels below 1 g/24h, and so it was likely that such a bias was minimal. At worst the conclusions should be modified: thus a variable is a significant marker for ESRF 'conditionally, given that proteinuria persists'.

4.2.4 Findings from analyses of the 'screen' variables

Selectivity ratios above 0.3 were associated with increased risk of ESRF. Such levels previously have been associated with non steroid-responsive lesions (Hardwicke, Cameron, Harrison, et al., 1970). In the present study, the selectivity ratio was found to correlate with the prevailing plasma creatinine, which itself was a marker of prognosis. Patients with MCN, however, had more selective proteinuria than the other groups even after adjustment for the prevailing plasma creatinine. Moderately hypertensive patients (diastolic blood pressure $\geq$110 mm Hg) had less selective proteinuria than the other patients, but this difference disappeared on making a statistical adjustment for their higher plasma creatinine levels. Later analysis, however, which used the absolute blood pressure measurements (see Results section 3.5), suggested that selectivity ratio may be needed to explain the relationship between blood pressure and plasma creatinine.

Concentrations of individual immunoglobulins in the blood might be expected to fluctuate in response to antigens, but

overall neither total serum IgM nor total IgA changed markedly over long periods in a given patient and both were found to be of value in prognosis.

Approximately one quarter of patients had serum IgM concentrations which were above the reference range. In a study of IgA nephropathy (Nicholls, Fairley, Dowling and Kincaid-Smith 1984), serum IgM levels were raised in 43% of patients. The authors claimed that the serum level was not associated with outcome, but did not specify the cut-offs they used. In the present series, lower levels, particularly those under 1.5 g/l, were associated with increased risk of ESRF; results within the reference range (0.5-2 g/l) may still be associated with an adverse outcome. The serum IgM was also inversely related to plasma creatinine, possibly via haemoglobin.

One third of patients had concentrations of IgG below the reference range, because of an inverse relationship with urinary protein loss. Serum IgG was not related to prognosis, but tended to increase as proteinuria diminished. Apparent relationships with biopsy (low levels with membranous nephropathy and MCN) therefore need to be interpreted with caution since the measurements used were not necessarily taken at the time of the biopsy. The relationship of serum IgG with urinary protein loss did not appear to be the result of the 'leakiness' of the kidney since neither IgG nor urinary protein/creatinine ration correlated significantly with the selectivity ratio.

The serum IgA concentration did not seem to be related to any of the other variables, particularly plasma creatinine; low levels (in particular, <2g/l), however, were associated with increased risk of ESRF. Patients with mesangiocapillary

disease, however which had the worst prognosis of the main biopsy groups, also had the lowest serum IgA levels.

Amongst the mesangial proliferative groups, serum levels of IgA were higher in IgA nephropathy patients than in IgM nephropathy. These may need further study since the serum levels were not necessarily measured at the same time as the biopsy. Two studies of IgA nephropathy (Nicholls, Fairley, Dowling and Kincaid-Smith 1984; D'Amico, Minetti, Ponticelli et al 1986) both failed to show any relationship between serum IgA levels and outcome. The first reported further that 21% of patients had increased serum IgA levels, and that this occurred more frequently in men, but the serum level was not related to the amount deposited in the biopsy. In a study of IgM nephropathy (O'Donoghue, Lawler, Hunt et al), IgA deposits in the biopsy were associated with a more favourable prognosis.

One fifth of the patients had CH50 (total haemolytic complement) levels below the reference range. Although CH50 and serum C1q were weakly correlated with the prevailing plasma creatinine, neither were significant markers of ESRF. Factor B was neither related to the prevailing plasma creatinine, not was it found to be associated with ESRF in this work. Earlier work carried out by Dr. Mallick and colleagues at the MRI (Mallick, Eyres, Acheson et al 1981), using a discriminant analysis, had suggested that Factor B (and serum C3, see below) could predict a subsequent rise in plasma creatinine.

High levels of C4 ($\geq$ 100%) were associated with increased risk of ESRF. This was consistent with C4 being positively correlated with the prevailing plasma creatinine concentration and urinary protein loss (probably via the plasma fibrinogen

concentration); increased levels of each of these are significant markers of ESRF.

Conversely, for C3, low levels (<125 mg/dl) were associated with increased risk of ESRF. This finding was difficult to explain because it was inconsistent with other findings in the study; although C3 was not related to the prevailing plasma creatinine, it was positively correlated with C4, plasma fibrinogen, and (probably via these), to urinary protein loss. The mean C3 concentration was lowest for mesangiocapillary disease, which had the worst prognosis. Dr. Mallick had previously reported persistently low C3 levels in this group; serum C3 was found to correlate with glomerular deposition and, in both this group and the diffuse proliferative group, the deposition correlated significantly with the rate of rise of the plasma creatinine concentration.

It is possible that the inconsistent result for C3 was a statistical artifact. The omission of patients with SLE, who had significantly lower C3 levels, left the findings unchanged. In a study of diffuse lupus nephritis (Magil, Ballon, Chan et al 1984), there was a trend towards increased risk of ESRF with a serum C3 below 45 mg/dl at the time of biopsy, as well as a greater likelihood of declining renal function with serum C4 9 mg/dl or more; neither of these findings, however, were statistically significant.

Two thirds of the patients had serum cholesterol concentrations above the reference range, over half (55%) had raised S particle scores and over half (males 56%; females 72%) had raised M particle scores (VLDL). Concentrations of cholesterol, LDL and VLDL have previously been reported to be high in patients with the nephrotic syndrome, on account of the increased production of the liver in response to fall in

albumin. Here this was supported by significant correlations between these variables and plasma albumin concentration (inversely), urinary protein loss, and plasma fibrinogen, which is also made by the liver; further work with serum cholesterol suggested that this variable was related to urinary protein loss and plasma fibrinogen via plasma albumin. The L particle scores (chylomicrons), which are made in the gut, were only raised in one quarter of patients and were not correlated with plasma fibrinogen.

Serum cholesterol and the three particles scores were only weakly negatively correlated with plasma creatinine. The serum cholesterol was measured more frequently and low levels (<6 mmol/l) were associated with increased risk of ESRF. This must reflect the correlation with the prevailing plasma creatinine, as one might have predicted a reverse finding because of the relationship with proteinuria. Visual examination of the data suggested that the levels above 6 mmol/l tended to fall shortly before ESRF; this therefore needs further, time related study. Lipid levels, particularly serum cholesterol, were low for patients with mesangiocapillary disease, who had a poor prognosis.

The findings for the M score were difficult to explain; levels above 4 were associated with increased risk of ESRF only in women. The cut-offs for men and women were not equivalent, but similar trends were not observed using higher and equivalent cut-offs for men.

Finally, two thirds of patients had abnormally high plasma fibrinogen concentrations. Fibrinogen was positively correlated with urinary protein loss, probably via plasma albumin, as well as with plasma creatinine. Increased levels

($\geq$ 400 mg/100ml) were associated with increased risk of ESRF.

In summary, there were a number of variables which had prognostic importance. In addition there was a complex network of interrelationships between these variables. There were probably interactive effects on survival which were impossible to determine. The subsets of intercorrelated variables described in section 3.5 may give a key to future studies in this area. This is discussed further in the concluding section.

4.3 Summary of this study

The aims of this study were to explore the applications of failure-time analysis in glomerular disease and search for markers of prognosis. All currently available failure-time methods, therefore, have been reviewed and, where relevant, have been applied in a search for markers of ESRF.

Previous workers have used only the Cox proportional hazards regression analysis with 'fixed' covariates. This method was used in the present study and confirmed the importance of the covariates plasma creatinine and urinary protein loss when measured in the early phase of the disease.

'Accelerated failure-time' models were also investigated, and it seems likely that these models may be more appropriate models for glomerular disease, but this will need confirmation in a larger series. Here, such models served to confirm the importance of the covariates indicated above.

Analyses using time-dependent covariates have been explored for the first time in the study of glomerular disease. These were possible only for the variables measured at frequent, regular intervals. Gail's method was used

extensively since the data set was large and incomplete. Software was developed to restructure the data and carry out the analyses. The results showed that prevailing heavy proteinuria, hypertension and anaemia were significant markers of subsequent ESRF. Their independent, prognostic value, however, could not be determined. Whilst there were obvious correlations with plasma creatinine and with the other variables, a multivariate analysis was not possible since the data were too often incomplete.

The renal screen variables presented a problem for analysis since these were measured on an ad hoc basis. Kay's method was found to be the most suitable method for analysis. This is a univariate procedure and has not been used previously in glomerular studies. A list of the most significant variables was obtained (Table XXXI), although it was difficult to verify the Markov assumption satisfactorily.

The main problems encountered in the study have arisen from the timing of the data collection. Some recommendations for a further prospective study, therefore, are outlined below.

4.4 Some implications for future work

This study was concerned with markers of prognosis. The list of variables used was not exhaustive; there were others which the author would have liked to have included but there were restraints which prevented this. For example, changes in the laboratory methods affected the reference range. Further fine biopsy detail was not available for most patients. Of the variables which were studied, those which did not appear to have value in prognosis may in fact have done so indirectly

by yielding information about the mechanisms of glomerular damage. A further dimension may be time; the length of time a variable remained persistently high or low (for example proteinuria) may be important.

It was hoped that a **predictive index** could be derived for the patients in the study. The problem was that the complex framework of interrelated variables could not be analysed simultaneously because of the timing of the measurements. This suggest **a further prospective study**, where changes, especially of the screen variables, can be looked at together at corresponding and equally-spaced time intervals, say every four or six months.

A single centre would be preferable or else a few geographically and clinically related centres, so that the consistency of laboratory methods and results could be assured. It would also be important to measure variables even when patients are in remission. Although this may not be helpful in patient management, it does ensure that the data set does not represent a biased group of patients (the worst), and therefore permits a valid study of the relationships between proteinuria and other variables. This would enable sensible comparisons to be made between patients in relapse and remission.

The statistical analysis of such a study could focus firstly on verifying the clusters found amongst the variables (Results section 3.5, and then, perhaps, the first principal components could be used as composite variable score from each cluster. Time series analysis may help unravel the effects of changes in the plasma albumin (and associated cholesterol/ fibrinogen/proteinuria) on subsequent renal function (plasma creatinine and associated blood pressure and selectivity).

Questions that might be posed are whether these effects are modified by therapy, and, if so, whether the progression is thereby halted.

In time series analysis, it is important that the data are collected at precise, equally-spaced intervals. Algorithms are available which use continuous-time autoregressive models to study relationships between variables measured at unequally spaced intervals (Jones, 1983). These were explored in the present study but were not found to be computationally robust. Other workers are attempting to revise the algorithms (Tunnicliffe-Wilson, 1989, personal communication).

The Cox proportional hazards regression could be repeated with an expanded list of covariates. It would be interesting to include, as covariates in the model, specific attributes reported by the pathologist, such as deposits of IgA, C3 etc., glomerular obsolescence, and interstitial fibrosis etc. If the laboratory results are measured sufficiently frequently to enable interpolation, Gail's method could be used; with a little imagination this approach can be very fruitful. One may choose either to stratify by the initial plasma creatinine level, or include plasma creatinine into the definition of the prevailing marker state, or to make a statistical adjustment for the prevailing reciprocal plasma creatinine level. Only the second of these approaches has been explored here.

Further statistical work may lead in time to the Cox approach being replaced by either accelerated time models or discrete Markov models; there is scope for theoretical research in both these areas. These together with judicious, regular data collection, may help unravel the extraordinary complex nature of glomerular diseases.

APPENDIX I

Some Notes on the File Structures

Some changes were made to the raw data files described previously (Hunt, 1982).

The basic data file (referred to as RENBAS DATA) contained cards 1.1 to 1.6, 4.1 to 4.3 and 6.1, as before, for each patient. Two additional cards (3.1 and 3.2), however, which contained the biopsy details were inserted.

The follow-up files were restructured to facilitate execution of the programs described in Appendices III and IV.

The first two follow-up cards (2.1 and 2.2), which contained the four-monthly data, were stored on a separate file (REN2 DATA). An additional variable was computed and stored for each follow-up for each patient; this was the time interval in weeks from the date of onset to the date of the follow-up. If the month of onset was unknown, then this was coded as 9999.

Where any of the renal screen variables were measured (that is information on cards 2.3, 2.4 or 2.5), then this information was stored on another file (REN5 DATA). A rectangular structure was used; if, for example, only cards 2.3 and 2.5 had been coded, then a 'dummy' card 2.4 was inserted which contained missing value codes for each variable field (that is, containing 9's from column 13 onwards).

To relate the renal screen variables with other follow-up data on cards 2.1 and 2.2 (for example plasma creatinine), a short FORTRAN program was executed; each sequence of cards 2.3-2.5 were preceded by cards 2.1 and 2.2 for the same follow-up date and then written to a new, temporary file (REN5X DATA).

Variables for Analysis:

Four-monthly follow-up (Cards 2.1/2.2 on file REN2 DATA):

Clinical:	blood pressure oedema and macroscopic haematuria (since last visit)	
Urine:	24 hour urine protein fibrinogen degradation products	
Blood:	haemoglobin	total protein
	white cell count	plasma urea
	packed cell volume	plasma creatinine
	plasma albumin	creatinine clearance
Therapy:	diuretics/thiazides	antihypertensives
	prednisolone	other immunosuppressives
	anticoagulants	

Renal Screen (Cards 2.1-2.5 on file REN5X DATA):

All the above measurements plus:

Urine:	selectivity ratio red and white cell counts granular casts
Blood:	immunoglobulins - IgG, IgA and IgM complements - total haemolytic complement (CH50), C3, C1q, C4 and Factor B lipids - cholesterol, L, M,S particle 'scores' plasma fibrinogen platelets erythrocyte sedimentation rate fibrinogen degradation products serum urate anti-nuclear factor anti-DNA antibody LE cells SCAT and LATEX
Miscellany:	serum and urine electropheresis serum and urine immunoelectropheresis ASOT titre throat and nose swab

The basic patient data (cards 1.1-1.6, 3.1-3.2, 4.1-4.3 and 6.1 on file RENBAS DATA):

This includes the following:

Sex:	
'Onset' details:	age date first manifestation
Histopathological diagnosis:	Light microscopy immunofluorescence electron microscopy
Status at last follow-up date:	alive with own functioning kidneys renal death (dialysis or transplantation without prior dialysis) death directly due to renal failure death due to ischaemic heart disease death due to hypertension or CVA death due to malignancy death due to other causes

APPENDIX II

Some Basic Mathematical Definitions and Formulae

Survivor function S(t)

The survivor function is the probability of surviving to at least time t. Thus, if T is the time from onset to failure, then:

$$S(t) = \text{Prob } (T>t)$$

Kaplan-Meier estimate

The Kaplan-Meier estimate $\hat{S}(t)$ is an estimate of S(t).

If t_1, t_2, t_3 etc. are the ordered times from onset, up to t, at which either a failure or a censoring occurs,

d_1, d_2, d_3 etc. are the corresponding number of failures which occurred, and

R_1, R_2, R_3 etc. are the numbers of patients alive just prior to these times (and therefore at risk at the beginning of the preceding time interval).

then $\hat{S}(t)$ is the cumulative probability of survival to time t and is calculated from:

$$\hat{S}(t) = \frac{(R_1 - d_1)}{R_1} \frac{(R_2 - d_2)}{R_2} \frac{(R_3 - d_3)}{R_3} \quad \ldots\ \text{etc}$$

Kaplan-Meier estimates are plotted against time from onset. Between failure times the graph is constant.

Hazard rate

The hazard rate is the rate of failure at time t, conditional on survival up to time t.

$$\lambda(t) = \lim_{dt \to 0} \frac{P\ (\ t < T \leqslant t+dt\ /\ T > t\ )}{dt}$$

This is plotted against the time from onset.

There is a mathematical relationship between the survivor function and the hazard rate:

$$\lambda(t) = \frac{-\frac{d(S(t))}{dt}}{S(t)} = \frac{d}{dt}\ (\ \ln S(t)\ )$$

$$S(t) = \exp\ (\ -\int_0^t \lambda(u)\ du\ )$$

The Cox 'proportional hazards' regression analysis

In this method, the hazard rate is modelled by the covariates in the following manner:

$$\lambda(t) = \lambda_0(t)\ \exp\ \bar{\beta}'\bar{X}$$

$\lambda_0(t)$ is the unknown 'baseline' hazard which is not estimated.

$\bar{\beta}'\bar{X}$ is a weighted composite score derived from the covariates x_1, x_2, x_3 etc.:

$$\bar{\beta}'\bar{X} = b_1\ x_1 + b_2\ x_2 + b_3\ x_3 + \text{etc.}$$

The coefficients b are derived in the analysis to give the 'best fit' to the data (see below). The exponential function is used so that the hazard function will always be positive.

The name 'proportional hazards' arises because if any covariate is increased by a unit amount, then the hazard rate is multiplied by a factor. The factor is peculiar to the covariate and is constant in time. Consider, as an example, the case where there is only one covariate x, so that $\bar{\beta}'\bar{X} = b\ x$, and imagine two patients with covariate values x=1 and x=2, respectively. The ratio of the hazard rates for these two individuals at any time t will be:

$$\frac{\lambda(t) \text{ for } x=2}{\lambda(t) \text{ for } x=1} = \frac{\lambda_0(t) \exp 2b}{\lambda_0(t) \exp 1b} = \exp b$$

The sign of the coefficient b, therefore, determines whether the hazard rate for the second individual would be increased (b>0) or decreased (b<0) proportionally, or unchanged (b=0), when compared with the first. Figure XXXIII gives an example in which b is positive.

To derive the coefficients, b, the following partial likelihood function is maximised (Cox, 1975):

$$L(\beta) = \prod_{i=1}^{k} \frac{\exp \bar{\beta}'\bar{X}_i}{\sum_{j \subset R_i} \exp \bar{\beta}'\bar{X}_j}$$

The product is across each of the k failure times. The summation is across all individuals in the risk set R_i (see above).

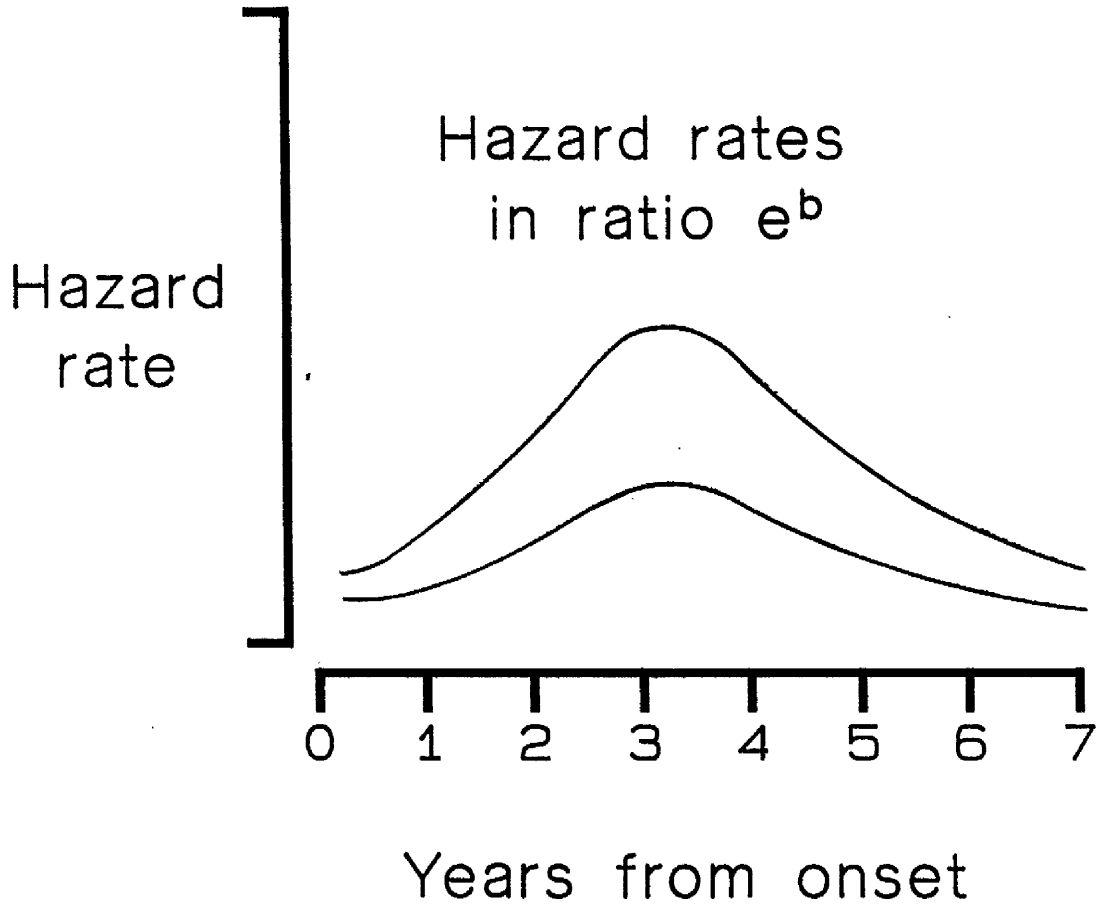
This assumes that no two patients share the same failure times. If this occurs, then the BMDP program uses Breslow's approach (Breslow, 1974) and maximises the following function:

$$L(\beta) = \prod_{i=1}^{k} \frac{\exp \bar{\beta}'\bar{S}_i}{\left[\sum_{j \subset R_i} \exp \bar{\beta}'\bar{X}_j\right]^{m_i}}$$

where $\bar{\beta}'\bar{S}_i$ is $b_1\ s_1 + b_2\ s_2 + b_3\ s_3$ etc. is a composite of the sums s of the covariates for the m individuals with tied failure times.

FIG. XXXIII

An illustration of the 'proportional hazards' assumption



Accelerated failure-time models

In these models, covariates alter the rate at which an individual proceeds along his/her time course. The general form of the hazard rate is:

$$\lambda(t) = \lambda_0(t \exp (- \bar{\beta}'\bar{X})) \exp (- \bar{\beta}'\bar{X})$$

where $\bar{\beta}'\bar{X} = b_0 x_0 + b_1 x_1 + b_2 x_2 + \text{etc.}$

and $x_0 = 1$

Examples

(a) The log-normal

If T is the time to failure, then log T has a normal distribution with mean $\bar{\beta}'\bar{X}$.

(b) The log-logistic

In this case there is proportionality of the odds on failure having occurred by time t, since:

$$\ln \frac{(1 - S(t))}{S(t)} = \varphi (\ln t + \bar{\beta}'\bar{X})$$

where φ is a constant to be estimated.

The hazard rate is as follows:

$$\lambda(t) = \frac{\varphi}{t (1 + (t \exp \bar{\beta}'\bar{X})^{\varphi})}$$

This will have a maximum if $\varphi > 1$, and it can be shown that the time at which this occurs is:

$$t = \frac{(\varphi - 1)^{1/\varphi}}{\exp \bar{\beta}'\bar{X}}$$

and then the hazard is $\exp \bar{\beta}'\bar{X} \ (\varphi - 1)^{(\varphi-1)/\varphi}$

Furthermore the ratio of two hazard rates evaluated for different values of the covariates will converge to 1 as t tends to infinity. Note that Roger and Peacock have reparametrised the problem, and have incorporated φ into the coefficients:

$$\ln \frac{(1 - S(t))}{S(t)} = \varphi \ln t + \bar{\beta}'\bar{X}$$

Their coefficients must be divided by φ to obtain the parametrisation shown above.

APPENDIX III

Programs to calculate the expected numbers of deaths for each year of the study*

Introduction and purpose:

The number of deaths observed a given year of the study is obviously determined by the age and sex composition of the study group during that year. It is possible to estimate the expected number of deaths during the period by summing the expected contributions to mortality made by each of the patients (see, for example, Hill, Laplanche and Rezvani, 1985). This program was written to calculate the overall expected mortality for each year.

Mortality rates (per million) must be supplied by the user. The rates used in this study were the death rates from all causes (the current ICD 'F' codes) obtained from the OPCS 'Registrar Generals Statistical Review' for each year up to 1973, and the OPCS 'Mortality Statistics by Area, England and Wales' thereafter (see references). In more recent years it was possible to derive, for each sex, death rates for each of the following 10 age groups: 1-4, 5-14, 15-24, 25-34, 35-44, 45-54, 55-64, 65-74, 75-84, 85+ years. In the program described below, therefore, rates were input for each of these intervals. In earlier years, some of the intervals were combined and, for these, the rates were duplicated.

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* The program documentation in appendices III, IV and V follow as closely as possible, the format used in the Journal of the Royal Statistical Society.

Language:

FORTRAN 77.

Description:

In any given year of the study, the expected contribution to mortality made by each patient was obtained by calculating his or her period of risk, and then multiplying this by the age and sex-specific death rate appropriate for that year. The age of the patient changed during the year, and, therefore, if there was a change in the relvant death rate, the contributions were calculated separately for the two ages and then added together. The contributions made by each the individuals were accumulated.

Structure and formal parameters:

PROGRAM SEXAGE

The following input/output files were defined:

Input	unit 3	RENBAS DATA	(Basic data file, see Appendix I)
Input	unit 9	SEXAGE DATA	(regional mortality rates - see below)
Output	unit 7	SEXAGE RESULTS	(results file)

Each row of the input file SEXAGE DATA contained mortality rates (per million) for the 10 age groups defined above. The rows were arranged in order of the 40 years spanned by the study (that is 1945 to 1984), and within each year by sex. The rates were stored in two two-dimensional arrays:-

Name:	Type:	Description:
RATEM (40,10)	Double Precision Array	Mortality rates for men ...

RATEF (40,10)	Double precision array	... and for women

For each patient in turn the following variables were read from the basic data file RENBAS DATA:

Name:	Type:	Description:
SNO	Integer	Study number (used to check for patient exclusions)
DB,MB,YB	Integer	Date of birth (day, month and last two digits of year)
SEX	Integer	Sex (male = 1; female = 2)
MO,YO	Integer	Month and year of onset
DE,ME,YE	Integer	Date of death or final follow-up

Two approximations were made which were not thought to adversely affect the results. The day of onset (DO) was assumed to be 15 for each patient. For the small number of patients (11) where the month of onset (MO) was not known this was assumed to be 6 (June).

The period of follow-up DO/MO/YO to YE/ME/YE was subdivided according to year. For each year, the contribution to the expected mortality made by this patient was calculated using subroutine EXS. These contributions were stored as elements 45 to 84 of a double precision array M, corresponding respectively to years 1945 to 1984 of the study. This was repeated for each patient, accumulating the contributions in a similar double precision array SUM:

Name:	Type:	Description:
SUM(84)	Double precision array	The total expected mortality for this year

For each year, from 1945 to 1985, the year and the total expected mortality were written to the output file SEXAGE RESULT.

Auxiliary algorithms:

(a) SUBROUTINE EXS (DX, MX, YX, ADD, DY, MY, EXP)

This subroutine calculated the expected contribution to the total mortality made by a patient between two dates, DX/MX/YX and DY/MY/YX, in the same year. (The year YX, must be 1945 or later).

The patients sex (male = 1; female = 2) and date of birth (DB/MB/YB), and the mortality rates (array RATEM and RATEF) were available to this subroutine through a COMMON block.

The formal parameters were:

Name:	Type:	Description:
DX,MX,YX	Integer	First date
DY,MY	Integer	Day and month of second date
ADD	Integer	Flag to denote whether the first day DX/MX/YX is to be included in the calculation (no = 0; yes = 1) (Note: that the final day DY/MY/YX is included automatically)
EXP	Double precision array	The expected contribution to mortality returned by the subroutine

Three subroutines were called by EXS. These were as follows:

(b) SUBROUTINE GRP (IA, IND)

This subroutine determined to which, of the 10 ordered aged categories defined above, a patient aged IA years belonged.

The formal parameters were:

Name:	Type:	Description:
IA	Integer	Age in years
IND	Integer	Age group (numbered 1 to 10 sequentially) returned by the subroutine

(c) SUBROUTINE DAYS (DD,MM,YY,IDAYS)

This subroutine calculated the number of days (IDAYS) from 1/1/40 to the date DD/MM/YY, and has been documented previously (Hunt, 1982).

The formal parameters were:

Name:	Type:	Description:
DD,MM,YY	Integer	The date in question
IDAYS	Integer	Days from 1/1/40, returned by the subroutine

(d) SUBROUTINE AGES (DB,MB,YB,DF,MF,YF,IAGE)

This subroutine returned the age in completed years at the date DF/MF/YF given the patient's date of birth was DB/MB/YB, and has been documented previously (see Hunt, 1982).

The formal parameters were:

Name:	Type:	Description:
DB,MB,YB	Integer	The date of birth
DF,MF,YF	Integer	The date in question
IAGE	Integer	The age in years returned by the subroutine

Listings:

The listings for this program and the associated subroutines are given in Fig. XXXIV.

FIG. XXXIV

Listing of program SEXAGE with associated subroutines

```
      PROGRAM SEXAGE
      DOUBLE PRECISION RATEM(40,10),RATEF(40,10),SUM(84),M(84),EXP
      INTEGER SNO,EXCL(22),SEX,DB,MB,YB,DO,MO,YO,DE,ME,YE,
     1        D1,M1,D31,M12,YD,ADDO,ADD1
      COMMON DB,MB,YB,SEX,RATEM,RATEF
C     PROGRAM TO CALCULATE ANNUAL CONTRIBUTIONS TO THE EXPECTED
C     TOTAL MORTALITY IN A FOLLOW-UP STUDY SPANNING 1945-1984
C     (AGES OF PATIENTS FROM 1 UPWARDS)
C     L HUNT AUGUST 1989
C     PATIENT EXCLUSIONS
      DATA EXCL/238,277,285,304,392,407,474,55,68,87,97,193,
     1 511,471,489,490,512,137,325,532,281,438/
C     READ MORTALITY RATES FOR MEN AND WOMEN FOR EACH YEAR FROM 45-84
C     NOTE THAT THE NUMBERS ACROSS THE ROWS ARE FOR AGE-GROUPS
C          1-4, 5-, 15-, 25-, 35-, 45-, 55-, 65-, 75-, AND 85-
      DO 8 I=1,40
      READ(9,1500) (RATEM(I,K),K=1,10)
      READ(9,1500) (RATEF(I,K),K=1,10)
    8 CONTINUE
C     INITIALISATION
      N=0
      DO 5  I=45,84
      SUM(I)=0.0D0
    5 CONTINUE
      DO=15
      D1=1
      M1=1
      D31=31
      M12=12
      ADDO=0
      ADD1=1
   10 READ(3,1000,END=90) SNO,DB,MB,YB,SEX,MO,YO,DE,ME,YE
      DO 15 K=1,22
      IF (SNO .EQ. EXCL(K)) GOTO 10
   15 CONTINUE
      N=N+1
      IF (MO .EQ. 99) MO=6
      IF (YO .EQ. 45) GOTO 25
      DO 20 J=45,YO-1
      M(J)=0.0D0
   20 CONTINUE
   25 IF (YE .EQ. 84) GOTO 35
      DO 30 J=YE+1,84
      M(J)=0.0D0
   30 CONTINUE
   35 IF (YO .NE. YE) GOTO 50
      CALL EXS(DO,MO,YO,ADDO,DE,ME,EXP)
      M(YO)=EXP
      GOTO 70
   50 CALL EXS(DO,MO,YO,ADDO,D31,M12,EXP)
      M(YO)=EXP
      IF ((YE-YO) .EQ. 1) GOTO 60
      DO 55 J=YO+1,YE-1
      YD=J
      CALL EXS(D1,M1,YD,ADD1,D31,M12,EXP)
      M(J)=EXP
   55 CONTINUE
   60 CALL EXS(D1,M1,YE,ADD1,DE,ME,EXP)
      M(YE)=EXP
   70 DO 75 J=45,84
      SUM(J)=SUM(J)+M(J)
   75 CONTINUE
      GOTO 10
   90 PRINT *, N, 'PATIENTS PROCESSED'
```

```
      WRITE(7,2000)
      DO 510 J=45,84
      WRITE(7,2500) J,SUM(J)
  510 CONTINUE
C     FORMAT STATEMENTS
 1000 FORMAT(I4,2X,3I2,I1,2X,2I2,11(/),6X,3I2)
 1500 FORMAT(4X,F5.0,9F7.0)
 2000 FORMAT(' YR','  TOT-EXP')
 2500 FORMAT(I3,F10.6)
      END
      SUBROUTINE EXS(DX,MX,YX,ADD,DY,MY,EXP)
      INTEGER DX,MX,YX,DY,MY,ADD,DB,MB,YB,SEX,
     1 A1,A2,DUM1,DUM2,DUM3,LEAP,IND,YXX
      DOUBLE PRECISION EXP,R1,R2,P1,P2,RATEM(40,10),RATEF(40,10)
      COMMON DB,MB,YB,SEX,RATEM,RATEF
C     SUBROUTINE CALCULATES EXPECTED CONTRIBUTION TO MORTALITY
C     FOR A PATIENT FOLLOWED UP BETWEEN TWO DATES IN THE SAME
C     YEAR (DX,MX,YX TO DY,MY,YX)
C     NOTE THE YEAR SHOULD BE BETWEEN 1945 AND 1984
      LEAP=YX-40
   75 LEAP=LEAP-4
      IF (LEAP .GE. 4) GOTO 75
      CALL AGES(DB,MB,YB,DX,MX,YX,A1)
      CALL AGES(DB,MB,YB,DY,MY,YX,A2)
      CALL DAYS(DX,MX,YX,DUM1)
      CALL DAYS(DY,MY,YX,DUM2)
      IF (LEAP .GT. 0) P1=DFLOAT(DUM2-DUM1+ADD)/3.65D2
      IF (LEAP .EQ. 0) P1=DFLOAT(DUM2-DUM1+ADD)/3.66D2
      YXX=YX-44
      CALL GRP(A1,IND)
      IF (SEX .EQ. 1) R1=RATEM(YXX,IND)
      IF (SEX .EQ. 2) R1=RATEF(YXX,IND)
      IF (A1 .NE. A2) GOTO 90
      EXP=P1*R1/1.0D6
      GOTO 100
   90 CALL GRP(A2,IND)
      IF (SEX .EQ. 1) R2=RATEM(YXX,IND)
      IF (SEX .EQ. 2) R2=RATEF(YXX,IND)
      CALL DAYS(DB,MB,YX,DUM3)
      IF (LEAP .GT. 0) P2=DFLOAT(DUM2-DUM3)/3.65D2
      IF (LEAP .EQ. 0) P2=DFLOAT(DUM2-DUM3)/3.66D2
      P1=P1-P2
      EXP=(P1*R1+P2*R2)/1.0D6
  100 CONTINUE
      RETURN
      END
      SUBROUTINE GRP(IA,IND)
      INTEGER IA,IND
      IF (IA .LE. 5) IND=1
      IF (IA .GE.  5 .AND. IA .LE. 15) IND=2
      IF (IA .GE. 15 .AND. IA .LE. 25) IND=3
      IF (IA .GE. 25 .AND. IA .LE. 35) IND=4
      IF (IA .GE. 35 .AND. IA .LE. 45) IND=5
      IF (IA .GE. 45 .AND. IA .LE. 55) IND=6
      IF (IA .GE. 55 .AND. IA .LE. 65) IND=7
      IF (IA .GE. 65 .AND. IA .LE. 75) IND=8
      IF (IA .GE. 75 .AND. IA .LE. 85) IND=9
      IF (IA .GE. 85 ) IND=10
      RETURN
      END
      SUBROUTINE DAYS(DD,MM,YY,IDAYS)
      INTEGER DD,MM,YY,IDAYS
C     SUBROUTINE TO CALCULATE DAYS FROM 1-1-40 TO DD-MM-YY
C     NB OK FOR ONSET AND FOLLOW UP DATES SINCE
C        EARLIEST ONSET YEAR IS 1945
      INTEGER ISUM(10)
```

```
      DATA ISUM/59,90,120,151,181,212,243,273,304,334/
      M=0
      IX=YY-40
   10 IX=IX-4
      M=M+1
      IF (IX .GE. 4) GOTO 10
      IDAYS=M*1461
      IF (IX .NE. 0) IDAYS=IDAYS+365*IX+1
      IF (MM .GT. 2) GOTO 50
      IDAYS=IDAYS+(MM-1)*31+DD-1
      GOTO 100
   50 IF (IX) 60,55,60
   55 IDAYS=IDAYS+ISUM(MM-2)+DD
      GOTO 100
   60 IDAYS=IDAYS+ISUM(MM-2)+DD-1
  100 RETURN
      END
      SUBROUTINE AGES(DB,MB,YB,DF,MF,YF,IAGE)
      INTEGER DB,MB,YB,DF,MF,YF,IAGE
C     SUBROUTINE TO CALCULATE AGE IN COMPLETED YEARS
C     NB IF THE YEAR OF BIRTH IS BETWEEN 95 AND 99
C        THEN THIS IS ASSUMED TO BE IN THE 1800'S
C     IT IS ASSUMED THAT ALL DATES OF BIRTH ARE KNOWN
      IF (YB .GE. 95) YB=100-YB
      IF (MF-MB) 20,10,30
   10 IF (DF .GE. DB) GOTO 30
   20 IAGE=YF-YB-1
      GOTO 50
   30 IAGE=YF-YB
   50 RETURN
      END
```

APPENDIX IV

Programs to carry out Gail's analysis

Introduction

Two programs were written to carry out Gail's analyses as described in the Methods section 2.3.6. GAIL2 was a general purpose program for use when the numbers of patients at risk were already available (see, for example, Table 1 in Gail's paper). GAILIC was written to extract the numbers at risk from the specific data files used in this study (see Appendix I) and in an appropriate form for input to GAIL2. Urinary protein/urinary creatinine ratio was used to define the marker states; variations of this program which were written, for example, to incorporate plasma creatinine into the analysis, have not been documented.

Language:

Both programs were written in FORTRAN 77.

(a) GAIL2

Description and purpose:

Gail (1981) described an application of Cox proportional hazards regression analysis with time-dependent covariates to assess the value of serial cancer markers. This program was written to carry out his suggested analysis for sparse continuous data.

His strategy was to define a vector functional $\bar{Z}(t)$ which depended on the marker history up to time t. One possible

functional was the value of the marker itself, and it was convenient to divide this into discrete categories. For K categories, the (K-1) dimensional functional was:-

$$\bar{Z}'(t) = (z_2(t), z_3(t), \ldots z_K(t))$$

where each of the $z_k(t)$ were 'dummy' variables. In the present study, for example, urinary protein/creatinine ratio was used as a marker for ESRF. Three states were defined, <4, 4-7.99 and 8 or more, corresponding to three values of the functional: (0,0), (1,0) and (0,1) respectively.

Other, initial prognostic variables could be allowed for by stratification, in which a different 'baseline' hazard was assumed for each stratum.

Since the marker state was only measured on discrete occasions, an interpolation convention was needed so that a patient's result could be estimated at any time point. Patients only contributed to the 'risk set' at a given time-point if such a result could be obtained by interpolation, that is the result was 'valid'. The key time-points in the analysis were the 'failure-times'; a failure-time was 'informative' (that is, of use in the analysis) only if the marker state could be defined for the patient who 'failed', and if marker states could be determined for at least two patients with different valid functionals.

The hazard at time t, for an individual in the ith stratum was:

$$\lambda(t) = \lambda_{0i}(t) \exp \bar{\alpha}' \bar{Z}(t)$$

where $\lambda_{0i}(t)$ was the 'baseline' hazard for the ith stratum, and $\bar{\alpha}'$ was the set of coefficients, (α_2, α_3, ... α_k)

The product $\bar{\alpha}' \bar{Z}(t)$ was 0 for state 1 (the baseline), and for other states was α_k . The α_k were the covariate effects, and were assumed to be time-invariant. They could be estimated by maximising the following log partial-likelihood function (equation 2 in Gail's paper, after correction):-

$$\sum_{i=1}^{I} \sum_{j=1}^{J_i} \left[\sum_{k=1}^{K} \alpha_k D_{ijk} - D_{ij+} \ln \sum_{l=1}^{K} N_{ijl} \exp \alpha_l \right]$$

where N_{ijk} were the numbers at risk (that is, alive, under observation with a valid functional result) in state k at the time of the jth informative death in stratum i.

D_{ijk} were the corresponding number of 'failures' occurring, and α_1 was defined to be equal to 0.

The hypothesis of no marker effect (that is $\alpha_k = 0$ for all k) was tested with the likelihood-ratio statistic; this was distributed approximately as a chi-squared with (K-1) degrees of freedom. The Mantel-Haenszel test was also calculated (see Gail 1981, equations 3 and 4); this was a 'score' statistic, derived from the vector of D_{ijk} across each of the states k=2...K, and also was approximately chi-squared.

Numerical Method:

The log partial-likelihood function (see above) was calculated, and its sign was reversed. A subroutine from the NAG library (NAG 1977), E04LAF, was used to carry out an unconstrained minimisation of the resulting scalar-valued function F:

$$F = \sum_{i=1}^{I} \sum_{j=1}^{J_i} \left[D_{ij+} \ln \sum_{l=1}^{K} N_{ijl} \exp \alpha_l - \sum_{k=1}^{K} \alpha_k D_{ijk} \right]$$

Substituting x_{k-1} for α_k, and noting that $x_0 = 0$, this was re-written as:

$$F = \sum_{i=1}^{I} \sum_{j=1}^{J_i} \left[D_{ij+} \ln \left(N_{ij1} + \sum_{l=2}^{K} N_{ijl} \exp x_{l-1} \right) - \sum_{k=2}^{K} x_{k-1} D_{ijk} \right]$$

The subroutine E04LAF returned the maximum likelihood estimates for the coefficients x_k ,k=1...K-1, that is the coefficients of the non-baseline states.

In order to use E04LAF, auxilliary subroutines were written to calculate the (K-1) dimensional gradient vector and the elements of the Hessian matrix.

The kth term of gradient vector was the first partial derivative of F with respect to x_k and, in the subroutine, was calculated from:

$$\frac{\partial F}{\partial x_k} = \sum_{i=1}^{I} \sum_{j=1}^{J_i} \left[\frac{D_{ij+} N_{ij(k+1)} \exp x_k}{\left(N_{ij1} + \sum_{l=2}^{K} N_{ijl} \exp x_{l-1} \right)} - D_{ij(k+1)} \right]$$

The Hessian matrix was the matrix of second-order partial derivatives and its terms were calculated as follows:

For the kth diagonal,

$$\frac{\partial F^2}{\partial x_k{}^2} = \sum_{i=1}^{I} \sum_{j=1}^{J_i} \left[\frac{D_{ij+} N_{ij(k+1)} \exp x_k}{\sum_K (N_{ij1} + \sum_{l=2} N_{ijl} \exp x_{l-1})} - \frac{D_{ij+} (N_{ij(k+1)} \exp x_k)^2}{\sum_K (N_{ij1} + \sum_{l=2} N_{ijl} \exp x_{l-1})^2} \right]$$

and, for the off-diagonal terms, $n \neq m$

$$\frac{\partial F^2}{\partial x_n dx_m} = - \sum_{i=1}^{I} \sum_{j=1}^{J_i} \frac{D_{ij+} N_{ij(n+1)} N_{ij(m+1)} \exp x_n \exp x_m}{\sum_K (N_{ij1} + \sum_{l=2} N_{ijl} \exp x_{l-1})^2}$$

Finally, the elements of Hessian matrix and F were recalculated using the maximum likelihood estimates of the coefficients x_k. The Hessian matrix was inverted using the NAG subroutine F01ABF to form the 'observed information matrix'; its inverse was an estimate of the variance-covariance matrix for the coefficients which could be assumed to be asymptotically multivariate normal. The value of F was recalculated setting all the coefficients equal to 0; the likelihood-ratio statistic was twice the difference between this value and F.

The Mantel-Haenszel test statistic was calculated using Gail's formulae. A vector $\bar{X}_{ij}$, of dimension K-1, was defined for each stratum (i=1...I) and each valid, informative 'failure' within each stratum (j=1...J_i); the vector contained the number of failures across each of the K-1 non-baseline states. The expected values $E\bar{X}_{ij}$, and the variance-covariance matrix V_{ij}, were calculated for each state. Elements of the variance-covariance matrix and $\bar{X}_{ij} - E\bar{X}_{ij}$ were summed across all strata and

failure-times, to define V and $\bar{Y}$ respectively. The Mantel-Haenszel statistic was computed by inverting V (using F01ABF) and computing $\bar{Y}'V^{-1}\bar{Y}$

Structure and formal parameters:

The following input/output files were defined:

Input unit 3 GAIL2 DATA (output from GAIL1C, see below)

Input unit 7 GAIL2 RESULTS (results file)

The following variables were requested from the keyboard at run-time:-

Name:	Type:	Description:
II	Integer	The number of strata
J(II)	Integer array	The number of valid, informative deaths in each stratum (total JJ, maximum 150)
KK	Integer	The number of states (note that the number of coefficients to be estimated is N=KK-1)
X(N)	Double precision array	Initial values of the coefficients (later contained the maximum likelihood estimates)

The input file GAIL2 DATA contained the numbers of failures across each of the states and the corresponding numbers of patients at risk, for each valid, informative failure-time occurring within each stratum . There were two rows of input for each failure time; the first contained the values of D_{ijk} across the states, and the second N_{ijk}.

The order of data entry was as follows :

stratum 1, data for first failure
data for second failure
data for third failure
etc.

stratum 2, data for first failure
data for second failure
etc.

stratum 3, etc.

An example is given in Fig. XXXV. This was an unstratified data set, with three states, which was generated by GAIL1C using a lag of four months. There were 34 valid and informative failures.

The data were stored as two-dimensional arrays:

Name:	Type:	Description:
D(JJ,KK)	Double precision array	The number of failures occurring
NR(JJ,KK)	Double precision array	and the number of patients at risk

The following variables were written to the output file GAIL2 RESULT, together with the final values of X(N) returned by E03LAF:

Name:	Type:	Description:
F	Double precision	The minimum value of F
IFAIL	Integer	Indicator returned by E04LAF (if the minimisation has bee successful then IFAIL=0)
G(N)	Double precision	The gradient vector (should be 0 at the minimum)
A(N,N)	Double precision array	The observed information matrix and (later) the variance-covariance matrix
LRT	Double precision	The likelihood-ratio statistic
MH	Double precision	The Mantel-Haenszel statistic

FIG. XXXV

Sample input data for program GAIL2

1	0	0	0	0	0	0	0	0
41	30	59	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0
44	28	61	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
47	22	62	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
44	30	52	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
55	31	43	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
54	29	39	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
56	21	41	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
52	20	40	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
55	18	36	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0
55	20	34	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
49	12	32	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
56	11	29	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
54	12	29	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
49	14	27	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
47	18	25	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
44	18	23	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
46	16	22	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
47	15	21	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
43	19	20	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
41	17	18	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
52	11	15	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
48	15	11	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
48	18	10	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
53	13	9	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
52	14	12	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
58	7	9	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
48	8	11	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
51	8	7	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
48	8	5	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
47	6	4	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0
35	4	3	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
30	6	2	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0
12	3	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0
10	2	2	0	0	0	0	0	0

To monitor convergence, after each iteration was carried out by E04LAF, the 'current' values of the coefficients, and the diagonal and lower triangular parts of the corresponding Hessian matrix, were written to the results file.

An example of the output generated from the sample data set (see Fig. XXXV) is given in Fig. XXXVI).

Auxiliary Algorithms

Two subroutines were called by the NAG subroutine E04LAF:

(a) SUBROUTINE FUNCT2(N,XC,FC,GC)

This subroutine calculated the value of F, FC, and the gradients for the 'current' values of the coefficients. The formal parameters were:

Name:	Type:	Description:
N	Integer	The number of coefficients to be estimated
XC(N)	Double precision	The 'current' values of the coefficients
GC(N)	Double precision	The gradient vector evaluated at the 'current' values
FC	Double precision	The value of F returned by the subroutine

(b) SUBROUTINE HESS2(N,XC,HESLC,LH,HESDC)

This subroutine calculated the elements of the Hessian matrix using the 'current' values of the coefficients. The formal parameters were:

Name:	Type:	Description:
N	Integer	The number of coefficients
XC(N)	Double precision array	The 'current' values of the coefficients

FIG. XXXVI

Sample output data for program GAIL2

```
NUMBER OF STRATA IS  1
NUMBER OF INFORMATIVE DEATHS IN EACH STRATUM  34
NUMBER OF STATES IS  3

INITIAL ESTIMATES OF COEFFS WERE     1.00000    2.00000


   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
   5.06789    7.56016
  -3.39308
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
 137.94096
  -1.71192   -2.90272
   1.00000    2.00000
   5.06789    7.56016
  -3.39308
   1.85040    2.76562
 135.89977
  -0.10792   -0.67527
   1.85040    2.76562
   5.86177    7.17182
  -4.59558
   2.03573    2.97853
 135.81446
  -0.01659   -0.03344
   2.03573    2.97853
   5.86302    6.99619
  -4.75068
   2.05063    2.99343
 135.81409
  -0.00007   -0.00018
   2.05063    2.99343
   5.86825    6.98706
  -4.76569
   2.05071    2.99351
 135.81409
   0.00000    0.00000
   2.05071    2.99351
   5.86827    6.98701
  -4.76576
ERROR TYPE  0


FUNCTION VALUE ON EXIT= 135.81409

AT THE POINT
  2.05071    2.99351

WITH CORRESPONDING GRADIENT
```

```
  0.00000    0.00000


   2.05071    2.99351
   5.86827    6.98701
  -4.76576


OBSERVED INFORMATION MATRIX
   5.86827  -4.76576
  -4.76576   6.98701


INVERSE OF INFORMATION MATRIX
   0.38203   0.26058
   0.26058   0.32086


MAXIMUM LIKELIHOOD  =-135.81409
LIKELIHOOD UNDER HO =-157.50738
LIKELIHOOD RATIO TEST STATISTIC=  43.38659
DEGREES OF FREEDOM              = 2


MANTEL-HAENSZEL TEST STATISTIC=  44.15477
```

HESLC(LH)	Double precision array	The lower triangular part of the Hessian matrix (row by row) returned by the subroutine
LH	Integer	The number of elements in the lower triangular part
HESDC(N)	Integer	The diagonal elements of the Hessian matrix, returned by the subroutine

Listings:

The listings for this program and the associated subroutine are given in Fig. XXXVII.

(b) GAIL1C

Description and purpose

This program generated a data file suitable for input to GAIL2. An appropriate 'lag', in weeks, was requested at run-time. Patients were included in the analysis if the 'first manifestation', as indicated on the basic data file (RENBAS DATA), was coded to 2 ('nephrotic syndrome'); a stratification by 'sex' (also from the basic data file) was included but, optionally, could be overridden.

Numerical method

First, for each patient, the marker status was determined at each time point which corresponded to an ESRF event. The variable which was to be used to define marker status was read from the follow-up file (REN2 DATA), together with the time in weeks from the individual patient's onset. The ESRF times, also in weeks, were read from a specially prepared input file (see GAIL1 DATA below). Assuming X was the 'lag' time, the values of the variable which were closest in time (but within eight weeks) to a date X weeks prior to each of the ESRF times were used to

FIG. XXXVII

Listing of program GAIL2 with associated subroutines

```
      PROGRAM GAIL2
      INTEGER II,JJ,KK,N,
     1 LH,LW,LIW,IFAIL,IW(12),J(10),
     2 IA,IB,IBOUND,BL(9),BU(9)
      DOUBLE PRECISION D(150,10),NR(150,10),
     1 F,G(9),X(9),W(145),
     2 HESL(36),HESD(9),A(10,9),B(9,9),Z(10),F0,F1,F2,LRT,
     3 XD(9),EXD(9),Y(9),DSUM,NRSUM,YVI(9),MH
      COMMON JJ,KK,D,NR
C
C     PROGRAM TO CARRY OUT GAILS ANALYSIS
C     BASED ON COX PROPORTIONAL HAZARDS MODEL
C     WITH TIME DEPENDENT COVARIATES
C     SEE BIOMETRICS 1982
C     PROGRAM BY LINDA HUNT FEBRUARY 1986
C     MODIFIED TO RUN ON CMS SEPTEMBER 1988
C     USE OF E04EBF REPLACED BY E04LAF APRIL 1989
C
C     READ NUMBER OF STRATA
      PRINT *, 'TYPE NUMBER OF STRATA (MAX 10)'
      READ *, II
      WRITE(7,100) II
C
C     READ NUMBER INFORMATIVE DEATHS IN EACH STRATA
      PRINT *, ' '
      PRINT *, 'TYPE NUMBER OF INFORMATIVE DEATHS FOR EACH STRATUM'
      PRINT *, 'ONE STRATUM AT A TIME'
      PRINT *, '(MAX 150 DEATHS OVER ALL STRATA)'
      JJ=0
      DO 1 I=1,II
      PRINT *,'STRATUM',I
      READ *, J(I)
      JJ=JJ+J(I)
    1 CONTINUE
      WRITE(7,110) (J(I),I=1,II)
C
C     READ NUMBER OF STATES
      PRINT *, ' '
      PRINT *, 'TYPE NUMBER OF STATES (MIN 2 MAX 10)'
      PRINT *, 'REMEMBER STATE 1 IS THE BASELINE'
      PRINT *, 'AND ITS COEFFICIENT IS FIXED AS 0'
      READ *, KK
      WRITE(7,120) KK
C
      N=KK-1
C
C     READ FROM DATA FILE THE NUMBERS OF INFORMATIVE DEATHS, ROW BY ROW,
C     AND NUMBERS AT RISK, ROW BY ROW
      PRINT *,' '
      PRINT *,'NUMBERS OF INFORMATIVE DEATHS AND '
      PRINT *,'NUMBERS AT RISK READ FROM FILE'
      DO 10 I=1,JJ
      READ(3,200) (D(I,K),K=1,KK)
      READ(3,200) (NR(I,K),K=1,KK)
   10 CONTINUE
C
C     READ INITIAL VALUES OF COEFFICIENTS
      PRINT *,' '
      PRINT *,'TYPE STARTING VALUES FOR THE COEFFICIENTS'
      PRINT *,'FOR STATES 2,3 ETC'
      PRINT *,'SELECT NON-ZERO DISTINCT VALUES TO BEGIN WITH'
      DO 11 K=1,N
      PRINT *,'STATE ',K+1
      READ *,X(K)
   11 CONTINUE
      WRITE(7,400) (X(K),K=1,N)
      WRITE(7,450)
C
```

```
C      MAXIMISE THE LIKELIHOOD FUNCTION
C      (MAKE IT NEGATIVE AND USE E04EBF
C      TO DO UNCONSTRAINED MINIMISATION)
       IFAIL=1
       IBOUND=1
       DO 12 K=1,N
       BL(K)=-1.0D7
       BU(K)=1.0D7
   12  CONTINUE
       LIW=12
       LW=145
       LH=(N*N-N)/2
       CALL E04LAF(N,IBOUND,BL,BU,X,F,G,IW,LIW,W,LW,IFAIL)
C      WRITE OUT THE RESULTS
       WRITE(7,500) IFAIL
       WRITE(7,600) F
       WRITE(7,700) (X(K),K=1,N)
       WRITE(7,800) (G(K),K=1,N)
       WRITE(7,450)
C
C      CALCULATE AND INVERT THE OBSERVED INFORMATION MATRIX
C      AT THE MAXIMUM LIKELIHOOD ESTIMATE
       CALL HESS2(N,X,HESL,LH,HESD)
       DO 13 K=1,N
       A(K,K)=HESD(K)
   13  CONTINUE
       M=0
       DO 16 K1=2,N
       DO 14 K2=1,K1-1
       M=M+1
       A(K1,K2)=HESL(M)
       A(K2,K1)=HESL(M)
   14  CONTINUE
   16  CONTINUE
       WRITE(7,900)
       DO 18 K1=1,N
       WRITE(7,1000) (A(K1,K2),K2=1,N)
   18  CONTINUE
       IA=10
       IB=9
       IFAIL=0
       CALL F01ABF(A,IA,N,B,IB,Z,IFAIL)
       IF (IFAIL .EQ. 0) GOTO 20
       WRITE(7,1100) IFAIL
       GOTO 32
   20  DO 24 K2=2,N+1
       DO 22 K3=1,K2-1
       A(K2-1,K3)=A(K2,K3)
   22  CONTINUE
   24  CONTINUE
       DO 28 K2=1,N-1
       DO 26 K3=K2+1,N
       A(K2,K3)=A(K3,K2)
   26  CONTINUE
   28  CONTINUE
       WRITE(7,1200)
       DO 30 K1=1,N
       WRITE(7,1000) (A(K1,K2),K2=1,N)
   30  CONTINUE
C
C      LIKELIHOOD RATIO TEST OF ALL COEFFICENTS=0
   32  F0=0.0D0
       DO 36 I=1,JJ
       P1=0.0D0
       P2=0.0D0
       DO 34 K=1,KK
       P1=P1+D(I,K)
       P2=P2+NR(I,K)
   34  CONTINUE
       F0=F0+P1*DLOG(P2)
```

```
   36 CONTINUE
      F=-F
      F0=-F0
      LRT=2.0D0*(F-F0)
      WRITE(7,1250) F
      WRITE(7,1300) F0
      WRITE(7,1350) LRT
      WRITE(7,1400) N
C
C     CALCULATE THE MANTEL-HAENSZEL TEST OF NO MARKER EFFECT
C     (NB THE MATRIX A CORRESPONDS TO V, AND LATER TO V INVERSE)
      DO 40 K=1,N
      Y(K)=0.0D0
      DO 38 K1=K,N
      A(K,K1)=0.0D0
   38 CONTINUE
   40 CONTINUE
      DO 65 I=1,JJ
      DSUM=D(I,1)
      NRSUM=NR(I,1)
      DO 45 K=2,KK
      DSUM=DSUM+D(I,K)
      NRSUM=NRSUM+NR(I,K)
   45 CONTINUE
      DO 50 K1=1,N
      XD(K1)=D(I,K1+1)
      EXD(K1)=DSUM*NR(I,K1+1)/NRSUM
      Y(K1)=Y(K1)+(XD(K1)-EXD(K1))
      A(K1,K1)=A(K1,K1)+DSUM*(NR(I,K1+1)/(NRSUM))
     1 *(1.0D0-NR(I,K1+1)/NRSUM)*(NRSUM-DSUM)/(NRSUM-1.0D0)
   50 CONTINUE
      DO 60 K2=1,N-1
      DO 55 K3=K2+1,N
      A(K2,K3)=A(K2,K3)-(DSUM*NR(I,K2+1)*NR(I,K3+1)*(NRSUM-DSUM)
     1 /(NRSUM*NRSUM*(NRSUM-1.0D0)))
      A(K3,K2)=A(K2,K3)
   55 CONTINUE
   60 CONTINUE
   65 CONTINUE
C     COMPUTE INVERSE OF V
      IFAIL=0
      CALL F01ABF(A,IA,N,B,IB,Z,IFAIL)
      IF (IFAIL .EQ. 0) GOTO 70
      WRITE(7,1500)
      WRITE(7,1100) IFAIL
      GOTO 6000
   70 DO 80 K2=2,N+1
      DO 75 K3=1,K2-1
      A(K2-1,K3)=A(K2,K3)
   75 CONTINUE
   80 CONTINUE
      DO 84 K2=1,N-1
      DO 83 K3=K2+1,N
      A(K2,K3)=A(K3,K2)
   83 CONTINUE
   84 CONTINUE
C     MULTIPLY Y BY THE INVERSE OF V
      DO 90 K1=1,N
      YVI(K1)=0.0D0
      DO 85 K4=1,N
      YVI(K1)=YVI(K1)+Y(K4)*A(K4,K1)
   85 CONTINUE
   90 CONTINUE
C     POST MULTIPLY PRODUCT BY THE TRANSPOSE OF Y
      MH=0.0D0
      DO 95 K1=1,N
      MH=MH+YVI(K1)*Y(K1)
   95 CONTINUE
      WRITE(7,1600) MH
C
```

```
C     FORMAT STATEMENTS
  100 FORMAT(/' NUMBER OF STRATA IS ',I2)
  110 FORMAT(' NUMBER OF INFORMATIVE DEATHS IN EACH STRATUM',10I4)
  120 FORMAT(' NUMBER OF STATES IS ',I2)
  200 FORMAT(10F5.0)
  400 FORMAT(/' INITIAL ESTIMATES OF COEFFS WERE ',9F10.5)
  450 FORMAT(/)
  500 FORMAT(' ERROR TYPE ',I2,2(/))
  600 FORMAT(' FUNCTION VALUE ON EXIT=',F10.5,/)
  700 FORMAT(' AT THE POINT '/9F10.5)
  800 FORMAT(/' WITH CORRESPONDING GRADIENT'/9F10.5)
  900 FORMAT(//' OBSERVED INFORMATION MATRIX')
 1000 FORMAT(1H ,9F10.5)
 1100 FORMAT(' MATRIX INVERSION IMPOSSIBLE-ERROR TYPE=',I2)
 1200 FORMAT(//' INVERSE OF INFORMATION MATRIX')
 1250 FORMAT(//' MAXIMUM LIKELIHOOD  =',F10.5)
 1300 FORMAT(' LIKELIHOOD UNDER HO =',F10.5)
 1350 FORMAT(' LIKELIHOOD RATIO TEST STATISTIC=',F10.5)
 1400 FORMAT(' DEGREES OF FREEDOM              =',I2//)
 1500 FORMAT(' MANTEL-HAENSZEL TEST NOT POSSIBLE')
 1600 FORMAT(' MANTEL-HAENSZEL TEST STATISTIC=',F10.5)
 6000 CONTINUE
      END
      SUBROUTINE FUNCT2(N,XC,FC,GC)
      INTEGER N,JJ,KK
      DOUBLE PRECISION XC(N),FC,GC(N),D(150,10),NR(150,10),PS1,PS2,PS3
      COMMON JJ,KK,D,NR
C     TO CALCULATE FC EQUAL TO THE VALUE OF THE FUNCTION
C     AT THE CURRENT POINT XC
C     AND TO PUT THE FIRST DERIVATIVES AT THIS POINT INTO GC
      DO 5 K=1,N
      GC(K)=0.0D0
    5 CONTINUE
      FC=0.0D0
      DO 20 J=1,JJ
      PS1=D(J,1)
      PS2=NR(J,1)
      PS3=0.0D0
      DO 10 K2=2,KK
      PS1=PS1+D(J,K2)
      PS2=PS2+NR(J,K2)*DEXP(XC(K2-1))
      PS3=PS3+XC(K2-1)*D(J,K2)
   10 CONTINUE
      FC=FC+(PS1*DLOG(PS2))-PS3
      DO 15 K3=1,N
      GC(K3)=GC(K3)+(PS1*NR(J,K3+1)*DEXP(XC(K3))/PS2)-D(J,K3+1)
   15 CONTINUE
   20 CONTINUE
C     MONITOR CONVERGENCE
      WRITE(7,2000) (XC(K),K=1,N)
      WRITE(7,2000) FC
      WRITE(7,2000) (GC(K),K=1,N)
 2000 FORMAT(1H ,9F10.5)
      RETURN
      END
      SUBROUTINE HESS2(N,XC,HESLC,LH,HESDC)
      INTEGER N,LH,JJ,KK
      DOUBLE PRECISION XC(N),HESLC(LH),HESDC(N),D(150,10),NR(150,10),
     1 HES1(9),HES2(9),PS1(150),PS2(150)
      COMMON JJ,KK,D,NR
C     TO CALCULATE THE HESSIAN MATRIX OF THE FUNCTION
C     AT THE CURRENT POINT XC
C     THE DIAGONAL ELEMENTS ARE PUT INTO HESDC
C     AND THE LOWER TRIANGULAR PART OF THE MATRIX
C     (ACROSS ROWS) ARE PUT INTO HESLC
      DO 5 J=1,JJ
      PS1(J)=0.0D0
      PS2(J)=0.0D0
    5 CONTINUE
      DO 6 K=1,N
```

```
      HES1(K)=0.0D0
      HES2(K)=0.0D0
    6 CONTINUE
      DO 20 J=1,JJ
      PS1(J)=D(J,1)
      PS2(J)=NR(J,1)
      DO 10 K=2,KK
      PS1(J)=PS1(J)+D(J,K)
      PS2(J)=PS2(J)+NR(J,K)*DEXP(XC(K-1))
   10 CONTINUE
      DO 15 K3=1,N
      HES1(K3)=HES1(K3)+PS1(J)*NR(J,K3+1)/PS2(J)
      HES2(K3)=HES2(K3)+PS1(J)*NR(J,K3+1)*NR(J,K3+1)
     1 /(PS2(J)*PS2(J))
   15 CONTINUE
   20 CONTINUE
      DO 25 K4=1,N
      HESDC(K4)=DEXP(XC(K4))*(HES1(K4)-HES2(K4)*DEXP(XC(K4)))
   25 CONTINUE
      M=0
      DO 40 I=2,N
      DO 35 J=1,I-1
      M=M+1
      HESLC(M)=0.0D0
      DO 30 J1=1,JJ
      HESLC(M)=HESLC(M)+PS1(J1)*NR(J1,I+1)*NR(J1,J+1)
     1 /(PS2(J1)*PS2(J1))
   30 CONTINUE
      HESLC(M)=-DEXP(XC(I))*DEXP(XC(J))*HESLC(M)
   35 CONTINUE
   40 CONTINUE
C     MONITOR CONVERGENCE
      WRITE(7,3000) (XC(K),K=1,N)
      WRITE(7,3000) (HESDC(K),K=1,N)
      M1=1
      M2=0
      DO 50 I=1,N-1
      M1=M1+I-1
      M2=M2+I
      WRITE(7,3000) (HESLC(K),K=M1,M2)
   50 CONTINUE
 3000 FORMAT(1H ,9F10.5)
      RETURN
      END
```

define the marker states; if no value was available then the marker state was set to 9 (missing).

Finally, the frequency distribution of patients was determined across each of the marker states at each ESRF time; these were the 'numbers at risk'. The marker state(s) for the patient(s) at ESRF was also noted; if this was 'missing', then the ESRF was not 'valid', and the data relating to this event time was excluded from the analysis.

Structure and formal parameters:

The following input/output files were defined:

Input	unit 3	GAIL1 DATA	(specially prepared file - see below)
Input	unit 9	RENBAS DATA	(Basic data file - see Appendix I)
Input	unit 8	REN2 DATA	(Follow-up cards 2.1/2.2 - see Appendix I)
Output	unit 7	GAIL2 DATA	(results for input to Program 1)

The following variables were read from the input file GAIL1 DATA:

Name:	Type:	Format:	Description:
CC	Integer	I5	Acceptable value of 'first manifestation' for inclusion (i.e. 2)
NS	Integer	I5	Number of strata (i.e. 2)
S(NS)	Integer array	5X,10I5	Codes taken by stratifying variance (i.e. 1,2)
NCD	Integer	I5	Number of codes for 'outcome' which indicated 'ESRF' (There were two - either dialysis/transplant or death due to renal failure)
CD(NCD)	Integer array	5X,10I5	The corresponding 'outcome' codes for ESRF (i.e. 2,3)

ND	Integer	I5	The number of distinct failure-times in this subgroup of patients

and, for each distinct failure-time:

DTIME(ND)	Real array	F5.0,I5	The time from onset in weeks ...
NUMD(ND)	Integer array		... and the number of failures occurring at this time
SNOD(ND, NUMD(ND))	Integer array	5X,10I5	and the series number of the patients concerned
IFLAG	Integer	I5	Option to override the stratification (i.e. no=1; yes=2)

The data file GAIL1 DATA which was used to obtain all the results described in this study is shown in Fig. XXXVIII.

For each patient, the following variables were read from RENBAS DATA (see Format 1000 in the program listing):

Name:	Type:	Description:
NO	Integer	Series number
STRAT	Integer	Stratifying variable ('sex')
MO,YO	Integer	Month and year of onset
COND	Integer	Condition for entry ('first manifestation')
DE,ME,YE	Integer	Day, month and year of 'outcome'
STATE	Integer	'Outcome'

For each patient whose condition code COND matched CC, the following variables were read from REN2 DATA (Format 2000) for each follow-up attendance:

Name:	Type:	Description:
PC	Real	Plasma creatinine concentration (100)
CCR	Real	Creatinine clearance
UP	Real	24h urinary protein loss (100)

FIG. XXXVIII

Input file GAIL1

```
2
2
        1     2
2
        2     3
41
44      1
      462
46      2
       99   429
53      1
      229
66      1
      400
67      1
       59
81      1
       14
106     1
      445
107     1
      366
109     1
      443
124     1
      466
126     1
      421
135     1
      449
137     2
       73    79
169     2
       22   342
180     1
      457
181     1
      417
182     1
       41
185     1
       56
186     1
      517
191     1
      483
201     1
       11
203     1
      149
208     1
      415
214     1
      446
222     1
      522
235     1
      464
240     1
      454
247     1
      383
263     1
      426
272     1
      536
295     1
       78
```

313 1
217
333 1
452
368 1
47
373 1
527
513 1
82
532 1
376
567 1
361
594 1
232
757 1
427
919 1
225
2

The subroutine PERCR was called to calculate the urinary protein/creatinine ratio (x100) from these variables. Missing data were coded 99.99.

The following variables were created for each patient:

Name:	Type:	Description:
N	Integer	The total number of follow-ups
DATA(N)	Real array	The urinary protein/creatinine ratios at each follow-up
WEEK(N)	Real array	The corresponding time in weeks from the patient's onset

The subroutine MARKER was called to calculate the marker states at each of the ND failure-times, thus creating:

Name:	Type:	Description:
MARK(ND)	Integer array	Marker status (missing data coded 9)

Finally, the following variables were used to accumulate information across all patients:

Name:	Type:	Description:
NR(ND, NS,10)	Integer array	The number of patients at risk, at each failure time, within each stratum, and for each of up to 10 marker states (Only states 0,1,2 were in fact used. State 9 contained 'missing' data)
D(ND,NS, 10)	Integer array	The number of failures at each failure time, within each stratum, and for each of the marker states

The latter two matrices were written to the output file, GAIL2 DATA. (State 9, containing missing data, was ignored).

Auxiliary Algorithms

(a) SUBROUTINE PERCR(PC,CA,CCR,UP)

This subroutine calculated the urinary protein/creatinine ratio from the plasma creatinine concentration, creatinine clearance and 24 hour urinary protein loss, as described above. the formal parameters were as follows:

Name:	Type:	Description:
PC	Real	Plasma creatinine concentration (x100)
CA	Real	Method of calculation of creatinine clearance.
CCR	Real	Creatinine clearance
UP	Real	24 hr urinary protein loss (x100) and the urinary protein/creatinine ratio returned by the subroutine

(b) SUBROUTINE MARKER (N,NO,STRAT,MO,YO,DE,ME,YE,STATE,DATA, WEEK,MARK)

This subroutine returned, for each patient, the marker states corresponding to each of the ND distinct failure-times. The marker states were initialised to '9', the missing value codes. A 'window' was defined containing all urinary protein/creatinine ratios within +/- eight weeks of the failure-time minus the 'lag' time; the result nearest to this time point was determined. The marker state was calculated using subroutine VALSTAT.

The total follow-up was calculated for each patient. Care was taken to ensure that patients were removed from the risk set for failure-times greater than this; since, for the lagged analyses, they may still have had a definable marker status.

The formal parameters were as follows:

Name:	Type:	Description:
N	Integer	Number of follow-ups
NO	Integer	Series number
STRAT	Integer	Stratifying variable ('sex')
MO,YO	Integer	Month and year of 'onset'
DE,ME,YE	Integer	Day, month and year of 'outcome' (ESRF, death or final assessment)
STATE	Integer	'Outcome'
DATA(N)	Double precision array	Values of the urinary protein/creatinine ratio
WEEK(N)	Double precision array	Corresponding time in weeks from the patient's onset
MARK(ND)	Integer array	Marker states at the times of each 'failure' (ESRF), returned by the subroutine

(c) SUBROUTINE VALSTAT(DA,MARD)

This subroutine returned the marker state for the current value of the urinary protein/creatinine ratio, as follows:

Ratio (g/g)	Marker state
<4	0
4-7.99	1
8 or more	2
missing	9

The formal parameters were:

Name:	Type:	Description:
DA	Integer	The 'current' value of the urinary protein/ratio
MARD	Integer	The marker state, returned by the subroutine

(d) SUBROUTINE DAYS(DD,MM,YY,IDAYS)

This subroutine was used by subroutine MARKER to calculate the total follow-up for each patient. (See Appendix III).

Listings:

The listings for this program and the associated subroutines are given in Fig. XXXIX.

FIG. XXXIX

Listing of program GAIL1C with associated subroutines

```
      PROGRAM GAIL1C
      REAL LAG,DTIME(150),MAX,DATA(250),WEEK(250),
     1 PC,CA,CCR,UP
      INTEGER NS,S(10),NCD,CD(8),CC,ND,SNOD(150,5),NUMD(150),
     1 NO,EXCL(22),STRAT,COND,SNO,DO,MO,YO,DE,ME,YE,STATE,
     2 MARK(150),NR(150,10,10),D(150,10,10),ISUM
      COMMON DO,NCD,CD,ND,SNOD,NUMD,LAG,DTIME,MAX
C
C     PATIENT EXCLUSIONS
      DATA EXCL/238,277,285,304,392,407,474,55,68,87,97,193,
     1 511,471,489,490,512,137,325,532,281,438/
C
C     PROGRAM TO CREATE A DATA SET TO CARRY
C     OUT GAILS ANALYSIS
C     LINDA HUNT MARCH 1986
C     MODIFIED TO RUN ON CMS SEPT 1988
C
C     MARKER STATES DEFINED BY CUPROT <4 4-7.99 8+
C     INITIALLY SET UP FOR PATIENTS WITH NS ONSET (MANIF=2)
C     AND (OPTIONAL) STRATIFICATION BY SEX
C
C     SET MAXIMUM VALUE OF DATA
C        EG FOR CUPROT*100, SET MAX=9990
      MAX=9900
C
C     USER CAN AMEND SUBROUTINE VALSTAT TO REDEFINE
C     STATES=0,1,2 ETC FOR A GIVEN INDIVIDUALS DATA
C     AT THE CORRESPONDING DEATH TIMES.
C     IF THE DATA IS ABOVE THE MAXIMUM VALUE THEN
C     THE STATE IS AUTOMATICALLY CODED 9
C
C     READ THE ALLOWABLE CONDITION CODE FOR ENTRY
C        EG FOR MANIF=FLUID RETENTION, SET CC=2
      READ(3,500) CC
C
C     READ THE NUMBER OF STRATA
C     (UP TO 10 INCLUDING MISSING VALUE)
C        EG FOR SEX, NS=2
      READ(3,500) NS
C
C     READ THE CODES USED IN THE BASIC DATA FILE
C     FOR THE CHOSEN STRATA
C        EG FOR SEX, CODES ARE 1,2
      READ(3,600) (S(K),K=1,NS)
C
C     READ ALSO THE NUMBER OF CODES USED IN THE BASIC DATA FILE
C     FOR FAILURE
C        EG FOR RENAL DEATH, CODES ARE 2 AND 3, THUS SET NCD=2
      READ(3,500) NCD
C
C     NOW READ THE CODES USED FOR FAILURE
      READ(3,600) (CD(K),K=1,NCD)
C
C     READ THE NUMBER OF DISTINCT DEATH TIMES
C     (MAXIMUM 150)
      READ(3,500) ND
C
C     FOR EACH DEATH TIME, READ THE DEATH TIME
C     AND THE NUMBER OF PATIENTS WHO DIED,
C     FINALLY THE SERIES NUMBERS OF THESE PATIENTS
C     (ASSUMED NOT MORE THAN 5)
      DO 1 J=1,ND
      READ(3,700) DTIME(J),NUMD(J)
      READ(3,600) (SNOD(J,K),K=1,NUMD(J))
    1 CONTINUE
C
C     READ OPTION TO OVERRIDE DEFINITIONS OF STRATA
C     AND THUS PUT ALL PATIENTS IN THE SAME STRATUM
```

```
C             SET IFLAG=1 TO LEAVE
C             SET IFLAG=2 TO OVERRIDE STRATA
      READ(3,500) IFLAG
      IF (IFLAG .EQ. 2) NS=1
C
C     READ LAG TIME IN WEEKS
      PRINT *,'TYPE THE LAG TIME REQUIRED IN WEEKS'
      PRINT *,'EG IF 4 MONTHS, TYPE 17.5'
      READ *, LAG
C
C     INITIALISATION OF D AND NR MATRICES
      DO 4 I=1,10
      DO 3 J=1,NS
      DO 2 K=1,150
      D(K,J,I)=0
      NR(K,J,I)=0
    2 CONTINUE
    3 CONTINUE
    4 CONTINUE
C
C     SET DAY OF ONSET AS 15TH OF THE MONTH
      DO=15
C
C     READ FROM BASIC FILE:
C     NO,STRATA=SEX,MO,YO,CONDITION=MANIF,DE,ME,YE,STATE (FORMAT 1000)
C     READ FROM FOLLOWUP FILE:
C     SNO,PCREAT*100,CALC,CRCLEAR,UPROT*100,WEEK          (FORMAT 2000)
    5 READ(8,1000,END=100)NO,STRAT,MO,YO,COND,DE,ME,YE,STATE
      IF (COND .NE. CC) GOTO 5
      DO 6 J=1,22
      IF (NO .EQ. EXCL(J)) GOTO 5
    6 CONTINUE
   10 READ(9,2000,END=100)SNO,PC,CA,CCR,UP,WEEK(1)
      IF (SNO .NE. NO) GOTO 10
   15 IF (WEEK(1) .EQ. 9999) GOTO 5
      CALL PERCR(PC,CA,CCR,UP)
      DATA(1)=UP
      N=1
   20 READ(9,2000,END=60)SNO,PC,CA,CCR,UP,WEEK(N+1)
      IF (SNO .NE. NO)GOTO 60
      CALL PERCR(PC,CA,CCR,UP)
      DATA(N+1)=UP
      N=N+1
      GOTO 20
   60 CALL MARKER(N,NO,STRAT,MO,YO,DE,ME,YE,STATE,DATA,WEEK,MARK)
      IF (IFLAG .NE. 2) GOTO 62
      JJ=1
      GOTO 70
   62 DO 65 J=1,NS
      IF (STRAT .NE. S(J)) GOTO 65
      JJ=J
      GOTO 70
   65 CONTINUE
   70 DO 85 I=1,ND
      DO 80 K=1,10
      IF (MARK(I) .NE. K-1) GOTO 80
      NR(I,JJ,K)=NR(I,JJ,K)+1
      DO 75 L=1,5
      IF (NO .EQ. SNOD(I,L)) D(I,JJ,K)=D(I,JJ,K)+1
   75 CONTINUE
      GO TO 85
   80 CONTINUE
   85 CONTINUE
   90 READ(8,1000,END=100)NO,STRAT,MO,YO,COND,DE,ME,YE,STATE
      IF (COND .NE. CC) GOTO 90
      DO 96 J=1,22
      IF (NO .EQ. EXCL(J)) GOTO 90
   96 CONTINUE
      IF (NO .NE. SNO) GOTO 10
      WEEK(1)=WEEK(N+1)
```

```
      GOTO 15
C
C     WRITE OUT RESULTS TO A FILE
  100 PRINT *, ' '
      PRINT *, 'RESULTS ARE WRITTEN TO A FILE, STRATUM BY STRATUM'
      PRINT *, ' '
      PRINT *, 'AT EACH TIME WHEN AN INFORMATIVE DEATH OCCURS,'
      PRINT *, 'THE NUMBERS OF DEATHS ARE GIVEN FOR EACH STATE 0,1 ETC'
      PRINT *, 'AND, UNDERNEATH THESE, THE NUMBERS AT RISK'
      PRINT *, ' '
      PRINT *, 'MISSING DATA (STATE 9) ARE IGNORED'
      DO 150 J=1,NS
      ISUM=0
      DO 130 I=1,ND
      DO 120 K=1,9
      IF (D(I,J,K) .GT. 0) GOTO 125
  120 CONTINUE
      GOTO 130
  125 ISUM=ISUM+1
      WRITE(7,5000) (D(I,J,K),K=1,9)
      WRITE(7,5000) (NR(I,J,K),K=1,9)
  130 CONTINUE
      PRINT *, ' '
      PRINT *, 'FOR STRATUM ',J,',',ISUM,' CASES WERE WRITTEN'
  150 CONTINUE
      PRINT *, 'REMINDER- LAG IS ',LAG,' WEEKS'
C
C     FORMAT STATEMENTS
  500 FORMAT(I5)
  600 FORMAT(5X,10I5)
  700 FORMAT(F5.0,I5)
 1000 FORMAT(I4,8X,I1,2X,2I2,2X,I1,11(/),6X,3I2,I1)
 2000 FORMAT(I4,17X,F3.0,13X,F1.0,F3.0,3X,F4.0,22X,F10.0/1X)
 5000 FORMAT(9I5)
      END
      SUBROUTINE PERCR(PC,CA,CCR,UP)
      REAL PC,CA,CCR,UP
C     SUBROUTINE TO CORRECT 24 HR URINE PROTEIN
C     FOR MGS OF URINARY CREATININE OUTPUT,
C     (IE CALCULATE CUPROT)
      IF (UP .GE. 6600 .OR. PC .EQ. 999 .OR. CA .NE. 1
     1 .OR. CCR .EQ. 0 .OR. CCR .EQ. 999) GOTO 10
      UP=(UP*613.9)/(PC*CCR)
      GOTO 20
   10 UP=9999
   20 RETURN
      END
      SUBROUTINE MARKER(N,NO,STRAT,MO,YO,DE,ME,YE,STATE,DATA,WEEK,MARK)
      REAL FWEEK,LAG,DTIME(150),MAX,DATA(N),WEEK(N),
     1 T,TA,TB,WWEEK(250),WDATA(250),TMIN,DA
      INTEGER NCD,CD(8),ND,SNOD(150,5),NUMD(150),
     1 ND,MARK(150),MARD,STRAT,
     2 DO,MO,YO,DE,ME,YE,STATE,DUM1,DUM2,IWEEK,NI
      COMMON DO,NCD,CD,ND,SNOD,NUMD,LAG,DTIME,MAX
C
C     INITIALISE STATE VALUES TO 9
      DO 20 I=1,ND
      MARK(I)=9
   20 CONTINUE
C
C     FIND TOTAL FOLLOW UP TIME FOR THIS PATIENT IN WEEKS
C     THEN ENSURE PATIENT IS REMOVED FROM THE RISK SETS AFTER THE
C     PREVIOUS FAILURE TIME IF CENSORED,
C     OTHERWISE AFTER THE RELEVENT FAILURE TIME
      CALL DAYS(DO,MO,YO,DUM1)
      CALL DAYS(DE,ME,YE,DUM2)
      FWEEK=(FLOAT(DUM2)-FLOAT(DUM1))/7.0
      IWEEK=ANINT(FWEEK)
      NI=ND
      DO 55 I=1,ND
```

```
      IF (IWEEK .GT. DTIME(I)) GOTO 55
C     CENSORED PATIENTS REMOVED AT PREVIOUS FAILURE TIME,
C     UNLESS CENSORING OCCURS AT A FAILURE TIME
      DO 25 K=1,NCD
      IF (STATE .EQ. CD(K)) GOTO 35
   25 CONTINUE
      IF (IWEEK .EQ. DTIME(I)) GOTO 30
      NI=I-1
      GOTO 60
   30 NI=I
      GOTO 60
C     FAILURES REMOVED AT CURRENT
   35 DO 40 K=1,NUMD(I)
      IF (NO .EQ. SNOD(I,K)) GOTO 50
   40 CONTINUE
      NI=I-1
      GOTO 60
   50 NI=I
      GOTO 60
   55 CONTINUE
C
C     NOW COMPUTE STATE VALUES FOR THIS PATIENT (0 TO 8)
C     FOR EACH DEATH TIME IF THERE IS A PROXIMAL VALUE OF DATA,
C     OTHERWISE LEAVE STATE AS 9
C     'PROXIMAL' MEANS AS CLOSE AS POSSIBLE TO
C     EACH TIME, AND WITHIN 8 WEEKS
C     (IF THERE ARE TWO EQUIDISTANT RESULTS ON EITHER SIDE
C     THEN THE FIRST IS TAKEN)
   60 DO 100 I=1,NI
C     IN THIS LOOP I REFERS TO THE ITH DEATH TIME
      T=DTIME(I)-LAG
      TMIN=8.
      TA=T-TMIN
      TB=T+TMIN
C     PUT ALL VALUES WITHIN 8 WEEKS FROM ITH TIME
C     IN A WINDOW
      J=0
      DO 70 K=1,N
      IF (WEEK(K) .LT. TA) GOTO 70
      IF (WEEK(K) .GT. TB) GOTO 70
      J=J+1
      WWEEK(J)=WEEK(K)
      WDATA(J)=DATA(K)
   70 CONTINUE
C     THEN FIND WHICH, IF ANY, IS THE NEAREST TO ITH TIME
      IF (J .EQ. 0) GOTO 100
      IF (J .GE. 2) GOTO 75
      DA=WDATA(1)
      GOTO 90
   75 DA=MAX+1.
      DO 85 K=1,J
      L=J-K+1
      IF (WDATA(L) .GT. MAX) GOTO 85
      IF (WWEEK(L) .LT. T) GOTO 80
      IF ((WWEEK(L)-T) .GT. TMIN) GOTO 85
      TMIN=(WWEEK(L)-T)
      DA=WDATA(L)
      GOTO 85
   80 IF ((T-WWEEK(L)) .GT. TMIN) GOTO 90
      DA=WDATA(L)
      GOTO 95
   85 CONTINUE
   90 IF (DA .GT. MAX) GOTO 100
   95 CALL VALSTAT(DA,MARD)
      MARK(I)=MARD
  100 CONTINUE
      RETURN
      END
      SUBROUTINE DAYS(DD,MM,YY,IDAYS)
      INTEGER DD,MM,YY,IDAYS,IX,IY,ISUM(10)
```

```
C      SUBROUTINE TO CALCULATE DAYS FROM 1-1-40 TO DD-MM-YY
C      NB OK FOR ONSET AND FOLLOW UP DATES SINCE
C         EARLIEST ONSET YEAR IS 1945
       DATA ISUM/59,90,120,151,181,212,243,273,304,334/
       M=0
       IX=YY-40
   10  IX=IX-4
       M=M+1
       IF (IX .GE. 4) GOTO 10
       IDAYS=M*1461
       IF (IX .NE. 0) IDAYS=IDAYS+365*IX+1
       IF (MM .GT. 2) GOTO 50
       IDAYS=IDAYS+(MM-1)*31+DD-1
       GOTO 100
   50  IF (IX) 60,55,60
   55  IDAYS=IDAYS+ISUM(MM-2)+DD
       GOTO 100
   60  IDAYS=IDAYS+ISUM(MM-2)+DD-1
  100  RETURN
       END
       SUBROUTINE VALSTAT(DA,MARD)
       REAL DA
       INTEGER MARD
C      USERS SUBROUTINE TO DEFINE STATES FOR THE INDIVIDUAL
C      ACCORDING TO THE VALUE OF THE DATA
C        0    CUPROT LT 4.00
C        1    CUPROT GE 4.00 AND LE 7.99
C        2    CUPROT GE 8.00
       IF (DA .GE. 400) GOTO 10
       MARD=0
       GOTO 100
   10  IF (DA .GE. 800) GOTO 20
       MARD=1
       GOTO 100
   20  MARD=2
  100  RETURN
       END
```

APPENDIX V

Program to prepare a data file for Kay's analysis

Introduction

For the 'screen' variables, which were measured at infrequent intervals during follow-up, Kay's method (Kay, 1986) was used to relate the variable with ESRF.

Two or more disease states were defined, depending on the observed value of the variable. For a 'screen variable', for example, one could define two states according to whether the level is below or above normal. Instantaneous transition rates were defined between the states and from each state to the 'death' state (i.e. death 'rates'):

$$\lambda_{ij} = \lim_{dt \to 0} \frac{P(\text{transition } i \text{ to } j \text{ in time interval } t \text{ to } t+dt \;/\; \text{state } i \text{ at time } t)}{dt}$$

for $i, j = 1,2 \quad i \neq j$

and,

death rate from state i:

$$\mu_{i3} = \lim_{dt \to 0} \frac{P(\text{transition } i \text{ to death state in time } t \text{ to } t+dt \;/\; \text{state } i \text{ at time } t)}{dt}$$

for $i = 1,2$

The rates were assumed to be the same for all patients and constant in time. Kay formulated the probability of moving from a state i to a state j (or the death rate) in a given time interval T, in terms of these transition rates, assuming that the process was Markov. He constructed a likelihood equation from these probabilities and the time intervals observed for the complete data set. The transition rates were estimated by maximum likelihood. For full details, the reader is referred to Kay's paper. The death rates

from each of the marker states could be compared, thus determining whether an increased or decreased level was associated with a worse outcome. Transition rates between the states could also be examined.

The author had access to Kay's program for the two-state analysis. The only remaining problem was extract the data from the follow-up files used in this study (see Appendix I) in a suitable form for input to Kay's program. The program described below was written to carry out the data extraction.

KDATA:

Language:

FORTRAN 77

Description:

The program was written to extract, for a given variable, the number of data values for each patient, the data values themselves (recorded '1' for state 1; '2' for state 2) and the associated times from onset when the values were measured. if the patient progressed to ESRF, then this was regarded as state 3, and the time from onset was calculated.

Structure and formal parameters:

PROGRAM KDATA

The following input/output files were defined:

Input	unit 3	RENBAS DATA	(Basic data file - see Appendix I)
Input	unit 9	REN5X DATA	(Follow-up cards 2.3/2.4/2.5 expanded to include 2.1 and 2.2 and weeks on onset - see Appendix I)
Output	unit 7	KAY DATA	(Output file)

The format statement 3000 (see listing) was amended according to the variable to be analysed. In conjunction with this, the following variables needed to be redefined:

Name:	Type:	Description:
EYEAR	Integer	Earliest year for inclusion (Follow-ups before this date were excluded)
MV	Real	Maximum value of the variable (Results greater than this were to be regarded as missing)

The following variables were requested from the keyboard at run-time:

Name:	Type:	Description:
CUT	Real	Cut-point for analysis
ALLOW	Integer	Allowable 'first manifestation' for inclusion (User to type 1 for asymptomatic proteinuria 2 for nephrotic syndrome etc or 0 for all patients, irrespective of onset)

For each patient, the following variables were read from the basic data file, RENBAS DATA (see format statement 4000):

Name:	Type:	Description:
NO	Integer	Series number
MO,YD	Integer	Month and year of onset
MANIF	Integer	First manifestation
DE,ME,YE	Integer	Day, month and year of 'outcome' (ESRF or death or final assessment)
STATE	Integer	'Outcome' status

Subject to satisfying the appropriate inclusion criteria, for each patient the non-missing values of the variable were accumulated,

together with the corresponding times from onset. The following variables were created for each patient:

Name:	Type:	Description:
N	Integer	Number of data values (maximum 50)
WEEK(N)	Integer array	The corresponding times from onset
VAR(N)	Real array	The data values
MARK(N)	Integer array	The marker states (1 or 2) defined from each data value

If the patient experienced ESRF (that is, STATE was coded 2 or 3), then the time from onset was calculated, using subroutine IDAYS. For this patient, the number of follow-ups, N, was increased by 1. The two new variables calculated were:

Name:	Type:	Description:
MARKN	Integer	Marker state for ESRF (that is, 3)
WEEKN	Integer	Time from onset

Providing that N was greater than 1, the values of N, the successive values of WEEK(N), MARK(N) and, finally (if applicable), the values of WEEKN and MARKN were written to the output file KAY DATA.

Auxilliary Algorithm:

SUBROUTINE DAYS(DD,MM,YY,IDAYS)

See Appendix III

Listing:

The listing for this program is given in Fig. XL.

FIG. XL

Listing of program KDATA with associated subroutines

```
      PROGRAM KDATA
      REAL MV,CUT,VAR(50),FWEEK
      INTEGER ALLOW,EYEAR,EXCL(22),
     1 SNO,YF,WEEK(50),WEEKN,
     2 NO,DO,MO,YO,MANIF,DE,ME,YE,STATE,MARK(50),
     3 DUM1,DUM2,MARKN
C
C          PROGRAM TO GENERATE A DATA FILE SUITABLE FOR
C          INPUT TO RICHARD KAY'S PROGRAM (CANCER5.FTN)
C                    LINDA HUNT OCT 1987
C                    REVISED DEC 1987
C                    MODIFIED FOR CMS JAN 1988/JUNE 1989
C          UP TO 50 VARIABLE VALUES PER PATIENT ARE ALLOWED
C          (ELSE CHANGE THE ARRAY DIMENSIONS ABOVE)
C
C     NOTE MARKER STATE 3 (THE DEATH STATE) IN THIS ANALYSIS
C     CORRESPONDS TO OUTCOME STATE 2 OR 3 (ESRF)
C     THE PROGRAM CAN BE MODIFIED TO INCLUDE OTHER DEATHS
C
      DATA EXCL/238,277,285,304,392,407,474,55,68,87,97,193,
     1 511,471,489,490,512,137,325,532,281,438/
C
C     ******************************************************
C     *    BEFORE RUNNING THIS PROGRAM PLEASE ALTER        *
C     *    FORMAT 3000 BELOW ACCORDING TO THE VARIABLE     *
C     *    TO BE ANALYSED                                  *
C     *    ALSO CHANGE THE 'MISSING' CODE....              *
C     *    ABOVE WHICH ALL VALUES ARE IGNORED              *
C     *      (EG FOR CHOLESTEROL, 99.8 COULD BE USED)      *
C     *    ALSO, IF THE METHOD HAS CHANGED, GIVE THE       *
C     *    EARLIEST YEAR THAT RESULTS ARE TO BE INCLUDED   *
C     *    (EG EYEAR=40)                                   *
C     *    (ANY RESULTS BEFORE THIS DATE ARE IGNORED)      *
C     ******************************************************
C     CURRENT SETTING FOR.....CHOL
 3000 FORMAT(I4,6X,I2,58X,I10,3(/),25X,F3.1,/,1X)
      MV=99.8
      EYEAR=40
C     ******************************************************
C
      PRINT *, 'PROGRAM TO GENERATE A DATA FILE FOR'
      PRINT *,'ANALYSIS BY RICHARD KAYS PROGRAM'
      PRINT *,' '
C
C     READ THE CUTPOINT TO DEFINE THE TWO VARIABLE STATES
C     EG IF VAR IS LESS THAN CUTPOINT, STATE=1
C        ELSE STATE=2
      PRINT *, 'TYPE IN THE CUT-POINT TO BE USED'
      READ *, CUT
C
C     THIS PROGRAM ASSUMES THAT THE PATIENTS
C     ARE TO BE ANALYSED ACCORDING TO FIRST MANIFESTATION
C     THEREFORE READ ALLOWABLE VALUE OF MANIF
C     EG IF NS ONSET, SET ALLOW=2
C     HOWEVER, IF ALL PATIENTS ARE TO BE ANALYSED,
C     IRRESPECTIVE OF FIRST MANIFESTATION, SET ALLOW=0.
      PRINT *,' '
      PRINT *,'GIVE ALLOWABLE VALUE OF MANIF (1 OR 2)'
      PRINT *,'(IF ALL PATIENTS ARE TO BE ANALYSED, TYPE 0)'
      READ *, ALLOW
      PRINT *,' '
      PRINT *,'RESULTS WILL BE WRITTEN TO FILE KAY DATA A1'
      PRINT *,'NB VALUES ABOVE',MV,'WILL BE IGNORED'
      PRINT *,'   SO ALSO WILL RESULTS BEFORE YEAR 19',EYEAR
      PRINT *,'  CUT-POINT=',CUT
      IF (ALLOW .EQ. 0) GOTO 48
      PRINT *,'   MANIF=',ALLOW
      GOTO 49
   48 PRINT *,'   ALL PATIENTS ANALYSED IRRESPECTIVE OF MANIF'
```

```
C
   49 K=0
      MARKN=3
      DO=15
      I=1
   50 READ(9,3000,END=6000) SNO,YF,WEEK(1),VAR(1)
   55 IF (VAR(1) .GT. MV .OR. YF .LT. EYEAR .OR.
     1 WEEK(1) .EQ. 9999) GOTO 50
   60 READ(3,4000,END=6000) NO,MO,YO,MANIF,DE,ME,YE,STATE
      IF (NO .NE. SNO) GOTO 60
      DO 62 J=1,22
      IF (NO .EQ. EXCL(J)) GOTO 65
   62 CONTINUE
      IF (MANIF .EQ. ALLOW) GOTO 70
      IF (ALLOW .EQ. 0) GOTO 70
   65 READ(9,3000,END=6000) SNO,YF,WEEK(1),VAR(1)
      IF (SNO .EQ. NO) GOTO 65
      GOTO 55
   70 READ(9,3000,END=100) SNO,YF,WEEK(I+1),VAR(I+1)
      IF (SNO .NE. NO) GOTO 75
      IF (VAR(I+1) .GT. MV .OR. YF .LT. EYEAR .OR.
     1 WEEK(I+1) .EQ. 9999) GOTO 70
      I=I+1
      GOTO 70
   75 IF (I .GT. 1 .OR.
     1 (I .EQ. 1 .AND. STATE .EQ. 2 .AND. MO .NE. 99) .OR.
     2 (I .EQ. 1 .AND. STATE .EQ. 3 .AND. MO .NE. 99))
     3 GOTO 80
      VAR(1)=VAR(2)
      WEEK(1)=WEEK(2)
      GOTO 55
   80 DO 85 J=1,I
      IF (VAR(J) .LT. CUT) MARK(J)=1
      IF (VAR(J) .GE. CUT) MARK(J)=2
   85 CONTINUE
      N=I
      IF (STATE .LT. 2 .OR. STATE .GT. 3) GOTO 90
      CALL DAYS(DO,MO,YO,DUM1)
      CALL DAYS(DE,ME,YE,DUM2)
      FWEEK=(FLOAT(DUM2)-FLOAT(DUM1))/7.0
      WEEKN=ANINT(FWEEK)
      N=N+1
   90 WRITE(7,5000) NO,N
      K=K+1
      DO 95 J=1,I
      WRITE(7,5500) WEEK(J),MARK(J)
   95 CONTINUE
      IF (N .GT. I) WRITE(7,5500) WEEKN,MARKN
      VAR(1)=VAR(I+1)
      WEEK(1)=WEEK(I+1)
      I=1
      GOTO 55
  100 IF (I .EQ. 1 .AND.
     1 (STATE .LT. 2 .OR. STATE .GT. 3 .OR. MO .EQ. 99))
     2 GOTO 6000
      DO 105 J=1,I
      IF (VAR(J) .LT. CUT) MARK(J)=1
      IF (VAR(J) .GE. CUT) MARK(J)=2
  105 CONTINUE
      N=I
      IF (STATE .LT. 2 .OR. STATE .GT. 3) GOTO 110
      CALL DAYS(DO,MO,YO,DUM1)
      CALL DAYS(DE,ME,YE,DUM2)
      FWEEK=(FLOAT(DUM2)-FLOAT(DUM1))/7.0
      WEEKN=ANINT(FWEEK)
      N=N+1
  110 WRITE(7,5000) NO,N
      K=K+1
      DO 115 J=1,N
      WRITE(7,5500) WEEK(J),MARK(J)
```

```
  115 CONTINUE
      IF (N .GT. I) WRITE(7,5500) WEEKN,MARKN
C
C     FORMAT STATEMENTS
 4000 FORMAT(I4,11X,2I2,2X,I1,11(/),6X,3I2,I1)
 5000 FORMAT(2I5)
 5500 FORMAT(5X,2I5)
 6000 PRINT *, ' '
      PRINT *, ' NUMBER OF PATIENTS FOR ANALYSIS=',K
      END
      SUBROUTINE DAYS(DD,MM,YY,IDAYS)
      INTEGER DD,MM,YY,IDAYS
C     SUBROUTINE TO CALCULATE DAYS FROM 1-1-40 TO DD-MM-YY
C     NB OK FOR ONSET AND FOLLOW UP DATES SINCE
C        EARLIEST ONSET YEAR IS 1945
      INTEGER ISUM(10)
      DATA ISUM/59,90,120,151,181,212,243,273,304,334/
      M=0
      IX=YY-40
   10 IX=IX-4
      M=M+1
      IF (IX .GE. 4) GOTO 10
      IDAYS=M*1461
      IF (IX .NE. 0) IDAYS=IDAYS+365*IX+1
      IF (MM .GT. 2) GOTO 50
      IDAYS=IDAYS+(MM-1)*31+DD-1
      GOTO 100
   50 IF (IX) 60,55,60
   55 IDAYS=IDAYS+ISUM(MM-2)+DD
      GOTO 100
   60 IDAYS=IDAYS+ISUM(MM-2)+DD-1
  100 RETURN
      END
```

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