National study of membranoproliferative glomerulonephritis

Protocol

Purpose

The development of the UK Registry for Rare Kidney Disease (eponym RaDaR) permits the identification and recruitment of a sufficient number of patients with Membranoproliferative glomerulonephritis and Dense Deposit Disease to enable meaningful translational research. The registry is able to capture detailed clinical and laboratory data longitudinally. We propose to investigate phenotype-genotype correlations, evaluate certain biomarkers that relate closely to the immunological pathogenesis and pathology, and to identify cohorts of well characterised patients that would be suitable for recruitment into future clinical trials.

Background

Membranoproliferative glomerulonephritis (MPGN) and Dense Deposit Disease (DDD) are rare conditions. It has been difficult to improve our understanding of these diseases because of the small numbers of patients and their heterogeneity. Although they have come to be regarded as separate disease entities¹, MPGN and DDD have a number of clinical features in common, and there is an overlap in some patients. The natural history of these disorders is similar, and both can progress to end stage kidney failure over approximately a decade from the time of diagnosis. Treatment is unsatisfactory²,³,⁴,⁵.

MPGN and DDD are primarily diseases of the glomeruli of the kidney. Glomeruli are the specialised microscopic blood vessels that filter blood plasma as a first step in the process of regulating the salt and water balance of the body, and the elimination of waste products. The diagnosis is made on a kidney biopsy and the diagnostic terms reflect the pathological findings. In brief, there are changes in the capillary wall of the glomeruli. In MPGN there is deposition of immune complexes of immunoglobulins and complement proteins. These proteins normally participate in the defence of the body against infection. They do not normally deposit in the glomeruli. The distribution of these immune complexes can occur at various levels in the capillary wall; subendothelial, intramembranous or sub-epithelial. To some extent the distribution correlates with clinical and serological features of the disease. In DDD, in its pure form, there is deposition of complement proteins in the basement membrane without associated immunoglobulins, and a distinctive electron microscopic appearance. In both disorders the capillary wall becomes thickened, the mesangial supportive structure of the glomerulus proliferates and lays down increased matrix thereby damaging the normal glomerular structure. The term mesangiocapillary glomerulonephritis, preferred by some, is interchangeable with MPGN. DDD is often referred to as MPGN type 2. Some pathologists subdivide MPGN type 1 on the grounds of the distribution of immune complexes, and those with prominent sub-epithelial complexes are described as MPGN type 3.

MPGN and DDD usually present with nephrotic syndrome (oedema and very heavy proteinuria), nephritic syndrome (bloody urine with or without renal impairment), or a mixture of the two⁶,⁷. Patients with primary forms of these disorders are usually older children or young adults. Secondary forms of MPGN occur as a complication of chronic infection or leukaemia, mostly in later life. Disease activity is typically indolent and persistent in MPGN. In DDD it fluctuates with some periods of remission. However, in both types the disease progresses to end-stage kidney failure over a decade on average. Various clinical complications occur, segregated to the disease sub-type. Some patients with DDD develop retinal macular drusen or partial lipodystrophy. Patients with MPGN may develop urticaria. Anaemia is typical of both and is disproportional to the level of kidney function. Recurrence of the primary disease after transplantation is reported, more often in DDD⁸. The latter
feature indicates that the cause lies with immunological regulation in the patient, not in the kidney itself.

Usually the concentration of complement C3 in the plasma is reduced, and split products of C3 such as C3d are elevated, suggesting a problem with complement regulation and high complement turnover. Complement C9 is universally present in the capillary wall or mesangium on biopsies, supporting the idea that the terminal complement cascade operates at these sites. Auto-antibodies may be found but their specificity is questioned. C3 nephritic factor is an autoantibody against C3 which stabilises or activates the alternate complement pathway in vitro and thus may be pathogenic. Recently we have found anti-C1q autoantibody in MPGN type 1 in a pilot study.

Rare patients with a genetic mutation in the complement regulator factor H have been reported. A mouse knock out of murine factor H provides a model of MPGN. Atypical haemolytic uraemic syndrome, which has similar pathology to MPGN, is associated with a wide range of mutations in complement proteins that are predicted to cause loss of regulation of the alternative complement pathway. Therefore it is reasonable to explore the possibility that other mutations in complement underlie the pathogenesis of some cases of MPGN/DDD. Together these data suggest that defects in complement regulation, inherited and acquired, are central to the pathogenesis of MPGN and DDD.

Historically, patients with these disorders have been treated with high doses of corticosteroids over prolonged periods. While some marginal benefit has been shown in a single study, patients do not generally entered full remission with steroids, and steroid side-effects are often unacceptable. Radical new concepts of treatment include the interruption of terminal complement pathways by monoclonal antibodies targeting complement C5. Such treatments have become accepted in another disorder of complement regulation, paroxysmal nocturnal haemoglobinuria, and are under evaluation in atypical haemolytic uraemic syndrome which has close histological similarity with MPGN. In order to move towards a position in which novel therapies can be properly tested, there is a need for cohorts of patients with clearly defined subgroup diagnosis and disease staging, based on pathology and biomarkers. This study aims to characterise and identify such cohorts.

The Study

1. Identification of patients and recruitment.

Using the infrastructure of the National Registry for Rare Kidney Disease (RaDaR), it will be possible to identify all children and most adults with MPGN and DDD in the UK on an ongoing basis. RaDaR is a secure web-based disease registry capable of capturing longitudinal and detailed clinical information. Patients will have previously consented to participate in the registry. In doing so they will have agreed that they can be contacted by the MPGN/DDD Research Group. The generic REC agreement that governs participation in the registry, "National Registry of Rare Kidney Disease", has been approved by North Somerset and South Bristol Research Ethics Committee, reference number 09/H0106/72, dated 12th November 2009. In brief, participants will have been approached, recruited and their consent obtained by their nephrologist (local investigator). Clinical information will then be entered into the RaDaR database online by the nephrologist or his or her research assistant. Patients will have their own password so as to access their own data and to receive patient information relevant to their own condition. Participants may withdraw from the registry at any time if they wish by notifying the registry.

In order to participate in further research as outlined below, a second level of consent is required. Patients will again be approached and recruited by their nephrologist using the patient information sheets and letter of invitation from the MPGN/DDD Research Group. Written consent will be obtained by the nephrologist. Notification that consent has been obtained will be indicated via the
RaDaR website by the nephrologist, with paper copies of the consent form held in the patient's record and by the patient.

2. Obtaining biological samples.

a) Kidney biopsies. Pathology review

Every patient will have undergone a kidney biopsy as a routine diagnostic procedure, upon which the diagnosis of MPGN or DDD will have been made. The pathology department that undertook the original biopsy will be contacted by the study group via the local nephrologist and asked to release the slides and/or the original block for review by the MPGN/DDD study group pathologists. In brief, the slides and block will be link-anonymised using a unique patient identifier of the RaDaR registry, and sent to Birmingham Children's Hospital. Here the slides will be converted to electronic images under the direction of Dr Roger Malcomson. These will then be reviewed by the pathology group and their findings recorded on the RaDaR database. Where necessary the block will be sectioned to provide further unstained sections for special immunohistochemical studies or to repeat any conventional staining thought to be necessary for analysis. Ultra structure images will be requested likewise. The original slides and block will be returned to the pathology department with a turnaround time not to exceed six weeks. At the end of the study any re-cut sections used for special immunohistochemical studies will be destroyed.

b) Blood and urine samples. Biomarker development

An additional amount of blood will be taken during routine venesection. This will consist of 10mL placed into EDTA, and 10mL into plain tubes. The samples will be urgently separated and the plasma and serum stored as 1mL aliquots, labelled with the unique patient identifier. The cellular deposit from the EDTA sample will be used for DNA extraction. The plasma samples will be urgently frozen and stored at -80°C pending transport to the central specimen reception for the study in the Wellcome Clinical Research Facility at University Hospital Birmingham. Here all samples will be catalogued. The cellular deposit from the EDTA samples will be used for DNA extraction. Samples will be batched and distributed to the collaborating research departments, including the biorepository of the Department of Renal Immunobiology University of Birmingham. No human cellular material will be stored.

The participating laboratories are as follows.

DNA analysis for complement regulator genes will be undertaken in the Department of Human Genetics Newcastle University under the direction of Professor Tim Goodship. This laboratory is accredited for diagnostic investigation of complement regulatory genes.

Complement C1q autoantibody will be screened on serum samples. Positive specimens will be further examined for target specificity in Cardiff University under the direction of Professor Paul Morgan and Dr Claire Harris. C3-NeF will be analysed by Dr Danielle Paixao-Cavalcante and Dr Claire Harris in Cardiff University.

Complement terminal attack complex C5b-9 will be measured by Professor Morgan, Cardiff University.

Complement split products, plasma CFHR5 and CFH levels will be measured by Dr Matthew Pickering, Imperial College London.

Patients will provide further plasma samples at six months and one year from diagnosis, and at yearly intervals thereafter until such time as they reach end-stage kidney failure. DNA will only be extracted on one occasion unless there is a particularly low yield in which case it may need to be repeated. At the end of the study any unused samples will be destroyed.
Patients will be asked to produce an aliquot of urine on three consecutive mornings (the first urine voided on rising in the morning) for analysis of albumin, total protein and creatinine concentration. These will be assayed in their local laboratory. The mean urine protein/creatinine and albumin/creatinine ratios will be used to indicate disease activity.

A checklist of routine investigations regarded as good practice in the diagnosis and follow up of MPGN patients will appear on the RaDaR website. Results from those investigations will be entered into the registry and will be used in analysis by the MPGN/DDD Research Group.

Results from research laboratories will be entered into the RaDaR site to match to the clinical data. Here they will accessed only by the research team. However, where a research investigation is an accredited laboratory investigation, and as long as the result of the research is published, the data will become accessible to the local investigator and the patient.

3. Phenotype/Genotype analysis

The Department of Human Genetics Newcastle University has an existing project to investigate complement regulatory genomics in idiopathic MPGN/DDD. The structured collection of clinical and laboratory in RaDaR will be used to undertake genotype-phenotype correlations. Correlates will include: age of onset of disease; pathological subtype; pathological staging in which the degree of interstitial scarring is scored; disease activity indicated by the severity of proteinuria; ongoing routine laboratory and clinical parameters; specific biomarkers such as complement C5b-9 and anti-complement auto-antibodies.

4. Cohort development.

The database will be interrogated to find patients with active disease who have not yet developed extensive interstitial scarring who may be considered for novel therapeutic studies. Such cohorts will be characterised according to the pathological subgroup and the presence or absence of biomarkers relevant to the pathogenesis. In the first instance the study group will investigate whether there are sufficient patients with MPGN type 1 who have clear evidence of complement activation and who have active but early disease to permit the design and development of phase 2 and phase 3 clinical trials with novel complement inhibitory agents. Standard statistical methods of cluster and correspondence analysis will be applied.

5. Supplementary investigation

It is expected that in a few of the research participants there will be an anti-complement auto-antibody that is deemed to require further detailed analysis of its functional effects on complement activity in vitro. This is to explore the possible pathogenic mechanisms of such auto-antibodies. Selected patients will be approached for supplementary investigation via the local investigator, and asked to consent separately for a single further blood sample to be obtained at the same time as a routine blood test. This will be transferred urgently to Cardiff University. B cells from the sample will be transformed with EBV to establish antibody generating clones to produce consistent supplies of the antibody for experimentation. This is superior to the alternative of repeated blood sampling of an index patient and will yield more informative and accurate data regarding pathogenesis. If the antibody produced using this technique is particularly informative, DNA encoding the variable region of the antibody will be purified from the B cell cones and used to generate a recombinant version of the antibody (‘single chain Fv’). This small antibody fragment will be used in biophysical/structural techniques to understand at the molecular level how the initial pathogenic antibody causes disease. At the end of the experiment the B cell clones will be destroyed.
Governance

The clinical data will be held by the RaDaR database. This database has secured full ethical approval for data collection and for patients to be approached for studies. The research team will be able to access the data through the website in a link-anonymised form for the period of the study. Analysis of the data will be undertaken by the MPGN/DDD Research Group in collaboration with RaDaR.

RaDaR is a development of the Renal Association, and is operated by the UK Renal Registry (UKRR). Governance of RaDaR will be undertaken under the authority of the Renal Association of Great Britain, the professional body for nephrologists in the UK, via its Clinical Affairs Board.

The business aspects and strategic direction of UKRR are overseen by the UKRR Management Board, comprising the Trustees of the Renal Association together with the Director and General Manager of the UKRR. The Management Board is chaired by the immediate past President of the Renal Association. The UKRR Management Board meets face to face annually. Additional virtual meetings of the Management Board are held as deemed necessary by the Chair throughout the year by phone conference or email.

The RaDaR Committee is a subcommittee of UKRR Committee, and will be responsible for all operational aspects of the rare disease registry. The committee will consist of a Chairperson, an honorary secretary and one other member all of whom are members of the Renal Association and appointed by it. In addition the RaDaR Committee will have representatives from participating Disease Specific Research Groups (the MPGN/DDD research group being one of the) or their deputies, two user representatives (patients or carers), a doctor training in nephrology or paediatric nephrology, and a renal health professional with no medical qualification. The RaDaR Committee will report to both the UKRR Committee and to the Research Committee of the Renal Association.

Ownership of data

There are two levels of ownership.
Part 1). Data collected from the local investigator and/or the patient directly are the property and responsibility of RaDaR. This includes knowledge of the patient's identity. This is known as Part 1 Information.
Part 2). Data that are generated by the research group or individuals within the research group, including results that are work in progress, will be held within RaDaR in link-anonymised form. They remain the property of the research group or responsible researcher within the research group. RaDaR guarantees security of access to the responsible research individual or group. At the end of a study Part 2 information is stored by RaDaR for a minimum of 5 years on behalf of the research group.

Ethics

The patient, or in the case of children their parents or guardians, will be informed about the study by letter of invitation from the MPGN/DDD Research Group sent via the nephrologist responsible for the patient’s care. They will be provided with the relevant patient information sheet. Information will be offered in the patient’s first language, either using translation services provided locally within the NHS, or pre-prepared in the case of major languages such as Welsh. Information for children will also be provided. Written consent will be obtained. A copy of it will be held in the patient’s hospital medical record, and a copy sent to the patient’s general practitioner. The referring nephrologist will be required to confirm that consent has been obtained. Failure to do so will prevent the patient’s data and samples from being entered into the study.

For children, the period of consent will be capped at 18 years, and at 16 years of age an automatic reminder will be issued to the nephrologist that consent will need to be given independently by the patient. This is designed to give adequate time for the research subject to formulate an independent opinion about their participation as adults. Teenage patients can electively consent for themselves
before 18 years of age if they wish. If a research subject reaches 18 years of age and has neither consented for him or herself nor withdrawn, the registry record will be frozen, and the local nephrologist informed. The patient and the clinician will no longer be able to access the information on that patient.

Patients may withdraw from the study at any time by notifying the registry or their lead clinician in writing. As data is held by the registry under separate ethical approval, this will not be affected unless they withdraw from the registry as well. Samples from that patient that have already taken during this study will be disposed of. They will be notified in writing that this has been done via the RaDaR database.

Generic approval for participation in the registry has been approved by the North Somerset and South Bristol Research Ethics Committee, reference number 09/H0106/72. A further application to the same REC is outstanding for the project herein.

**Patient Information**

Information for patients will be hosted on the RaDaR database. This includes both generic and disease specific information, and can be accessed by patients and their local investigator. It will not include personal data.

Information about a specific patient, including patient identifier and personal data (Part 1 Information) can only be accessed by the patient and the local investigator using a password. Data that is research in progress, Part 2 Information, will not be visible to the patient or the local investigator. The research group can move data from Part 2 to Part 1 if it relates to accredited laboratory results that are considered to have an impact on patient care.

Part 1 Information about patients in the study will only be accessible to the MPGN/DDD Study Group with the written agreement of RaDaR, and only in link-anonymised form. The Study group will be able to set up web pages to carry Part 2 research information that is the property of the research group, and which is not disclosed to patients or local investigators. RaDaR guarantees the security of this data. At the end of the study, the data will remain with RaDaR. Any future research group wishing to undertake research on existing Part 1 Information will need permission of RaDaR.

**References**

1. Dense deposit disease is not a membranoproliferative glomerulonephritis. Walker PD. Mod Pathol 2007, 20(6) 605-16


