Membranous nephropathy: from bench to bedside

HKSN Glomerulonephritis symposium
8th July 2017

Peter Mathieson
Bench → Bedside

Laboratory experiments
Animal models
Genetic analyses

Clinical experiments
Clinical trials
Treatment outcomes
The example of membranous nephropathy

Bedside ➔ Bench ➔ Bedside

Hypothesis: this part of the currently happening too fast
MN: the phenotype
Idiopathic MN: natural history

1. Dogma is that \( \frac{1}{3} \) of patients get better spontaneously, \( \frac{1}{3} \) stay the same and \( \frac{1}{3} \) get worse. The latter \( \frac{1}{3} \) have a high risk of reaching end-stage renal failure.

2. Very heavy proteinuria carries a worse prognosis, BUT....

3....even very severely affected patients can enter spontaneous long-term remission.

Therefore, controlled trials are essential.
MN: the situation prior to 2009

1. Very similar phenotypes in so-called “primary/idiopathic” and “secondary” MN necessitating a search for underlying causes

2. Genetics incompletely understood

3. Autoimmune aetiology assumed by extrapolation from animal models, but no direct evidence

4. Immunosuppressive treatments empirical

5. Reliance on clinical outcome parameters
good but can be expected to be better if the delivery rate for
the demand dose were more physiological than that obtained
with the rectangular bolus, and if the basal rate took into
account the circadian variation. The frequency with which
hypoglycaemia occurred—with or without symptoms—was
also in the range reported elsewhere.\textsuperscript{11,12} There was also no
appreciable formation of anti-insulin antibodies during
the course of the study. High-performance liquid
chromatography showed that the insulin was essentially
unchanged; in particular there was no increase in high
molecular weight derivatives.

Implantable, remote-controlled insulin pumps show
promise of becoming an excellent therapeutic tool for
unstable type I diabetics. The availability of implantable
glucose sensors will open the field for closed-loop automatic
feedback control. The present system already offers several
advantages over external insulin pumps—for example, the
hygienic and cosmetic advantages of an implantable device,
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low peripheral insulin levels) or intravenous (acute
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There are some theoretical advantages of an
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\textbf{PREDNISOLONE AND CHLORAMBUCIL
TREATMENT IN IDIOPATHIC MEMBRANOUS
NEPHРОPATHY WITH DETERIORATING
RENAL FUNCTION}

P. W. Mathieson\textsuperscript{1} A. N. Turner\textsuperscript{1}
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A. J. Rees\textsuperscript{1}

Departments of Medicine\textsuperscript{2} and Histopathology,\textsuperscript{2} Royal Postgraduate
Medical School, Hammersmith Hospital, London W12

\textbf{Summary} Eight patients with idiopathic
membranous nephropathy whose renal
function was deteriorating were given a 6-month course of
alternating monthly cycles of prednisolone and
chlorambucil. Proteinuria was reduced in all eight, from a
mean (SD) of 15.3 (5.9) g/24 h at the start of treatment to 2.1
(1.5) g/24 h at follow-up (p<0.05). Creatinine clearance
increased in six, and the rate of decline was reduced in the
other two (group mean 51.6 [17.8] ml/min at the start of
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patients.
MN: the situation prior to 2009

i.e. all “Bedside” and not much “Bench” except by extrapolation
So what happened in 2009?

1. An American group published a landmark study

2. A European group, chaired by a certain P. Mathieson, missed an opportunity....
So what happened in 2009?

1. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy.


M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy.


Author information

Abstract

BACKGROUND: Idiopathic membranous nephropathy, a common form of the nephrotic syndrome, is an antibody-mediated autoimmune disease. Serologic diagnosis has been elusive because the target antigen is unknown.

METHODS: We performed Western blotting of protein extracts from normal human glomeruli with serum samples from patients with idiopathic membranous nephropathy or other proteinuric or autoimmune diseases and from normal controls. We used mass spectrometry to identify the reactive protein bands and confirmed the identity and location of the target antigen with a monospecific antibody.

RESULTS: Serum samples from 26 of 37 patients (70%) with idiopathic but not secondary membranous nephropathy specifically identified the monospecific glycoprotein in nonreduced glomerular extract. Mass spectrometry of the reactive protein band detected the M-type phospholipase A2 (2R) receptor. Reactive serum specimens recognized recombinant PLA(2)R and bound the same 185-kD glomerular protein as did the monospecific PLA(2)R antibody. Anti-PLA(2)R autoantibodies in serum samples from patients with membranous nephropathy were mainly IgG4, the predominant immunoglobulin subclass in glomerular deposits. PLA(2)R was expressed in podocytes in normal human glomeruli and colocalized with immune deposits in glomeruli of patients with membranous nephropathy. IgG eluted from such deposits in patients with idiopathic membranous nephropathy, but not in those with lupus membranous or IgA nephropathy, recognized PLA(2)R.

CONCLUSIONS: A majority of patients with idiopathic membranous nephropathy have antibodies against a conformation-dependent epitope on PLA(2)R. PLA(2)R is present in normal podocytes and in immune deposits in patients with idiopathic membranous nephropathy, indicating that this major antigen is in this disease.

2009 Massachusetts Medical Society
So what happened in 2009?

2. Genome-wide association study in 3 European populations with membranous nephropathy
GWAS chromosome 2

Plotted SNPs

Position on chr2 (Mb)

Recombination rate (cM/Mb)

rs4664308

Log10(p-value)

r²

0.2

0.4

0.6

0.8

1.0
Table 3. Odds Ratios for Idiopathic Membranous Nephropathy, According to Single-Nucleotide Polymorphism and Genotype Combinations.\(^*\)

<table>
<thead>
<tr>
<th>SNP rs2187668 (HLA-DQA1)</th>
<th>SNP rs4664308 (PLA(_2)R1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
</tr>
<tr>
<td></td>
<td>14/354</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>1.00</td>
</tr>
<tr>
<td>GG</td>
<td>23/115</td>
</tr>
<tr>
<td>No. of cases/total no. of subjects</td>
<td>6.07 (3.01–12.27)</td>
</tr>
<tr>
<td>Odds ratio (95% CI)</td>
<td>5/11</td>
</tr>
<tr>
<td>AA</td>
<td>20.24 (5.51–74.38)</td>
</tr>
</tbody>
</table>

* Persons who were homozygous for the low-risk allele (GG) constituted the reference category. Numbers of cases and total numbers of subjects are from the joint analysis. OR denotes odds ratio, and SNP single-nucleotide polymorphism.
Pooled sequence from 335 individuals
961 variants

Batch0_ALL_hg19_2012_EUR

Plotted SNPs

\( r^2 \)

\(-\log_10(p\text{-value})\)

Recombination rate (cM/Mb)

Position on chr2 (Mb)

With thanks to Robert Kleta et al (UCL)
Phospholipase A2 Receptor (PLA2R1) Sequence Variants in Idiopathic Membranous Nephropathy


Abstract

The M-type receptor for phospholipase A2 (PLA2R1) is the major target antigen in idiopathic membranous nephropathy (iMN). Our recent genome-wide association study showed that genetic variants in an HLA-DQA1 and phospholipase A2 receptor (PLA2R1) allele associate most significantly with biopsy-proven iMN, suggesting that rare genetic variants within the coding region of the PLA2R1 gene may contribute to iMN.
GWAS chromosome 6
Pooled sequence from 335 individuals, chromosome 6 (less stringent) 7911 variants

With thanks to Robert Kleta et al (UCL)
Pooled sequence from 335 individuals, chromosome 6 (more stringent) 4968 variants

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Immunosuppression for progressive membranous nephropathy: a UK randomised controlled trial

Andrew Howman, Tracey L. Chapman, Maria M. Langdon, Caroline Ferguson, Dwomoa Adu, John Feehally, Gillian J. Gaskin, David R. W. Jayne, Donal O’Donoghue, Michael Boulton-Jones, Peter W. Mathieson

Summary

Background Membranous nephropathy leads to end-stage renal disease in more than 20% of patients. Although immunosuppressive therapy benefits some patients, trial evidence for the subset of patients with declining renal function is not available. We aimed to assess whether immunosuppression preserves renal function in patients with idiopathic membranous nephropathy with declining renal function.

Methods This randomised controlled trial was undertaken in 37 renal units across the UK. We recruited patients (18–75 years) with biopsy-proven idiopathic membranous nephropathy, a plasma creatinine concentration of less than 300 μmol/L, and at least a 20% decline in excretory renal function measured in the 2 years before study entry based on at least three measurements over a period of 3 months or longer. Patients were randomly assigned (1:1:1) by a random number table to receive supportive treatment only, supportive treatment plus 6 months of alternating cycles of prednisolone and chlorambucil, or supportive treatment plus 12 months of ciclosporin. The primary outcome was a further 20% decline in renal function from baseline, analysed by intention to treat. The trial is registered as an International Standard Randomised Controlled Trial, number 99959692.

Findings We randomly assigned 108 patients, 33 of whom received prednisolone and chlorambucil, 37 ciclosporin and 38 supportive therapy alone. Two patients (one who received ciclosporin and one who received supportive therapy) were ineligible, so were not included in the intention-to-treat analysis, and 45 patients deviated from the protocol before study end, mostly as a result of minor dose adjustments. Follow up was until primary endpoint for minimum of 3 years if primary endpoint was not reached. Risk of further 20% decline in renal function was significantly lower in the prednisolone and chlorambucil group than in the supportive care group (19 [58%] of 33 vs 27 [56%] of 48; P = 0.79).
Kaplan-Meier curves showing rate of development of further 20% decline in renal function

Comparisons: Hazard Ratio (95% CI) and p-value
Cyclosporin vs Supportive care. HR 1.17 (0.7, 1.96), 2p=0.5
Prednisolone / Chlorambucil vs Supportive care. HR 0.43 (0.25, 0.76), 2p=0.004
MN: situation by 2013
1. Genetics much better understood: two genes predispose, HLA-DRB1 & PLA2R (mechanisms remain unclear)
2. RCT data to suggest that CNI should not be used, pred + alkylating agent can still be effective in advanced disease
3. Increasing evidence that anti-PLA2R Abs associate with active disease, relapse, and ?with treatment response
4. Growing enthusiasm for rituximab….
So what about 2017?

Primary Membranous Nephropathy

William G. Couser

Abstract
Membranous nephropathy (MN) is a unique glomerular lesion that is the most common cause of idiopathic nephrotic syndrome in nondiabetic white adults. About 80% of cases are renal limited (primary MN, PMN) and 20% are associated with other systemic diseases or exposures (secondary MN). This review focuses only on PMN. Most cases of PMN have circulating IgG4 autoantibody to the podocyte membrane antigen PLA2R (70%), biopsy evidence PLA2R staining indicating recent immunologic disease activity despite negative serum antibody levels (15%), or serum anti-THSD7A (3%–5%). The remaining 10% without demonstrable anti-PLA2R/THSD7A antibody or antigen likely have PMN probably secondary to a different, still unidentified, anti-podocyte antibody. Considerable clinical and experimental data now suggests these antibodies are pathogenic. Clinically, 80% of patients with PMN present with nephrotic syndrome and 20% with non-nephrotic proteinuria. Untreated, about one third undergo spontaneous remission, especially those with absent or low anti-PLA2R levels, one-third progress to ESRD over 10 years, and the remainder develop nonprogressive CKD. Proteinuria can persist for months after circulating anti-PLA2R/THSD7A antibody is no longer detectable (immunologic remission). All patients with PMN should be treated with supportive care from the time of diagnosis to minimize protein excretion. Patients with elevated anti-PLA2R/THSD7A levels and proteinuria >3.5 g/d at diagnosis, and those who fail to reduce proteinuria to <3.5 g after 6 months of supportive care or have complications of nephrotic syndrome, should be considered for immunosuppressive therapy. Accepted regimens include steroids/cyclophosphamide, calcineurin inhibitors, and B cell depletion. With proper management, only 10% or less will develop ESRD over the subsequent 10 years.

Idiopathic MN: why do I say that bench to bedside is going too fast?
1. Anti-PLA2R (and anti-THSD7A) Abs have not yet been shown to be pathogenic
2. Rituximab has not been subjected to a good controlled trial (& the one in progress MENTOR is making the wrong comparison, Ritux vs CNI because CNIs are discredited)
3. Remember the variable natural history:
   JASN 2010; 21: 697-704. Spontaneous remissions in Memb 26% if proteinuria 8-12g/24h, 22% if >12g/24h, relapse rare.
Is rituximab the new wonder drug?

1. Uncontrolled case series suggest efficacy in IMN (also anecdotes in MCN/FSGS)

2. B cell depletion in an autoantibody-mediated disease seems logical (note that rituximab also has podocyte-protective actions)

3. BUT: it is very expensive and…. 

4. …..is not entirely safe, long-term effects are not known (eg increased PML risk in RA and lymphoma patients)
Idiopathic MN: way forward

1. The phenotype is easy to define (biopsy); serological tests have undoubtedly helped but renal biopsy remains essential.

2. The role of anti-PLA2R in stratification for treatment remains unproven.

3. Whilst I applaud efforts to minimise treatment toxicity, the enthusiasm for rituximab is not evidence-based. 

Controlled trials remain essential.
Take home messages

1. Understanding of membranous nephropathy has advanced very rapidly in recent years.

2. Tests for autoantibodies esp anti-PLA2R assist diagnosis, prognosis and management but have not yet in my view reached the point where they make renal biopsy unnecessary or can be used to determine treatment choice.

3. To those of us old enough to remember early years of ANA & ANCA, this is all v. familiar!

4. Nephrologists need to get better at RCTs