Membranous Nephropathy: 
Physiopathology and Natural History

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Membranous Nephropathy

- 1.3 cases/100,000
- >50 years old, 2/3 men
- Major cause of nephrotic syndrome
- Histological diagnosis by biopsy with the presence of immune complexes
- Podocyte dysfunction leading to proteinuria
- Idiopathic 85%
- Secondary 15%

Urine → Proteinuria

Podocyte autoantigens?
Recurrence after graft: 30-40%

+35% Nephrotic Syndrome

 owing to the small number of patients and the large standard error of the estimate.

**DISCUSSION**

The results of this study demonstrate that patients with idiopathic membranous nephropathy who receive only symptomatic treatment have a relatively benign course. The probability that end-stage renal disease would not develop was 88 percent after five years of follow-up and 73 percent after eight years. The results of this study and the few other published reports that allow the calculation of survival curves in untreated patients indicate that the probability of maintaining renal function five years after the onset of the disease ranges from 70 to 80 percent. 12-14,26-28

+20% ESKD

100 consecutive MN patients only treated with symptomatic therapy

2.3 g per deciliter, and the patient had variable edema. Proteinuria was defined as urinary protein excretion ≥2.5 g per 24 hours. Partial remission was defined as urinary protein excretion between 0.2 and 2.0 g per 24 hours and concentration ≥2.5 g per deciliter, and complete remission as urinary protein excretion ≤0.2 g per 24 hours. Kidney failure was defined as the development of end-stage renal disease, as eGFR <30 ml per minute per 1.73 m^2 of surface area. Hypertension was defined as systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥95 mm Hg.

**Results**

Complete remissions increased as a function of time (Fig. 4). Thirteen (35 percent) of the 37 patients followed for five years continued to have the nephrotic syndrome or sustained proteinuria. Among these patients, six had the nephrotic syndrome throughout the follow-up period, and seven relapsed after a partial remission. Of the 13 patients who had urinary protein excretion >2.0 but <3.5 g per 24 hours at base line, 7 had partial remissions; their protein excretion de...
Perform appropriate investigations to exclude secondary causes

Symptomatic Therapy

Urinary protein excretion persistently >4g/g
or
Complication related to the Nephrotic Syndrome
or
SCr >30% from the time of diagnosis

Immunosuppressive Therapy
- Corticosteroids + Cyclophosphamide
- CNI
Mecanisms of Immuns Complex Formation

Antigens identification?

Physiopathology

Antigens identification
1. Heymann Nephritis: Megalin

Immunization with crude renal extracts → IgG deposits → Podocytes → Complement Activation → Proteinuria

Formation *in situ* of immune complex

Antigen: Megalin
Human antigen?

*Heymann et al., PNAS 1959*

*Saito et al. PNAS 1994,*
2. Neutral Endopeptidase

Antenatal MN

New-born proteinuria J0

First human antigen

Mother NEP -

Baby NEP +

Alloimmunization in mother NEP-:
Ab anti-NEP

Debiec et al, NEJM 2002
3. PLA2R1 first antigen in Idiopathic MN

- Anti-PLA2R1 Ab: 70% Idiopathic MN
- Pathogenic role?

Beck et al., NEJM 2009
Correlation with disease activity

1st test anti-PLA2R1

WB
IIFT

Hofstra et al CJASN 2011
Hoxha et al., NDT 2011
ELISA PLA2R1 humain

IgG4

IgG tot

Other Antigen in Human Glomerular Extract?

Beck, Seminars in Nephrology 2010
4. THSD7A in Idiopathic MN

- Screening 500 MN patients
- 10% MN PLA2R1-
- PLA2R1+ or THSD7A+ but no double positive
- No clinical difference between PLA2R1+ and THSD7A+

_Tomas*, Beck* et al., NEJM_

_Brevet déposé_
Anti-THSD7A Ab

HEK THSD7A+

HEK THSD7A-

IIFT

WB

ELISA
Pathogenic role of Anti-THSD7A Ab

Tomas et al., JCI 2016
Anti-THSD7A Ab and Cancer

A Clinical Course

Chemotherapy: gemcitabine + cisplatin

Renal biopsy → CT scan 1 → Surgery → CT scan 2

THSD7A Antibody Level (serum dilution)

Protein-to-Creatinine Ratio

Week

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28

B THSD7A Staining in Kidney

C THSD7A Staining in Gallbladder Carcinoma

D THSD7A Staining in Lymph Node

E THSD7A, CD21, and DNA Staining in Lymph Node

Hoxha NEJM 2016
Four Podocyte Antigens

Megalin, 600 kDa, 4655 aa

THSD7A, 250 kDa, 1657 aa

PLA2R1, 180 kDa, 1463 aa

NEP, 90 kDa, 750 aa

- Complement-type repeat
- EGF-type repeat
- YWTD spacer region
- CysR
- FNII
- TSP1-like
- CTLD
- RGD-like
- Active site

Figure 2. Molecular features of antigens involved in alloimmune and autoimmune membranous nephropathy.
Pronosis Factor

MN PLA2R1+
Anti-PLA2R1 titer predict MN prognosis

Cohorte n=82

Cohorte n=66

Kanigicherla et al., KI 2013

Hoxha et al., JASN 2014
PLA2R1epitope? Disease Activity?

- 180 kDa
- ≈1500 aa
- 10 domains
- Conformational epitope
Identification of immunodominant Epitope in PLA2R1

Kao et al., JASN 2014

Fresquet et al., JASN 2014
Epitopes in PLA2R1: 50 sera – 3 epitope profiles

Expression of each mutants

Screening of 50 sera

3 Antibodies?

Seitz-Polksi et al., JASN 2016
Epitopes PLA2R1: 3 Epitopes - 3 domaines

Seitz-Polski et al., JASN 2016
Epitopes in PLA2R1: 69 patients - 3 groups

3 ELISA
CysR
CTLD1
CTLD7

3 groups
Immunisation against CysR: 100%
Immunisation against CTLD1/CTLD7: 46-60%

Prevalence:
68 + 42 + 32 +

Seitz-Polski et al., JASN, 2016
## Disease Activity

### 3 groups

<table>
<thead>
<tr>
<th></th>
<th>CysR</th>
<th>CysRC1</th>
<th>CysRC1C7</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>48±12</td>
<td>54±16</td>
<td>61±15</td>
<td>0.008</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>19M/4F</td>
<td>10M/4F</td>
<td>25M/7F</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Ac anti-PLA2R1 titer</strong></td>
<td>1369 (273-6717)</td>
<td>3873 (448-8237)</td>
<td>4732 (148-7766)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Proteinuria</strong></td>
<td>3.0* (0.3-7.8)</td>
<td>3.0* (0.8-12.0)</td>
<td>5.5* (0.3-24.0)</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>sCreatininemria</strong></td>
<td>92 (45-120)</td>
<td>109 (43-329)</td>
<td>106 (59-600)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Follow-up</strong></td>
<td>36 (12-190)</td>
<td>44 (18-120)</td>
<td>33 (12-216)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td>12/23 (25%)</td>
<td>8/14 (18%)</td>
<td>21/32 (4%)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Spontaneous remission</strong></td>
<td>10/23* (43%)</td>
<td>4/14 (29%)</td>
<td>4/32* (12%)</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Poor prognosis</strong></td>
<td>3/23* (43%)</td>
<td>7/14 (29%)</td>
<td>21/32* (12%)</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Hemodialysis</strong></td>
<td>0/23</td>
<td>2/14</td>
<td>10/32</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Follow-up 36 months (12-216)

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*Seitz-Polski et al., JASN 2016*
## Prognosis Factors

<table>
<thead>
<tr>
<th></th>
<th>Good Prognosis n=38</th>
<th>Bad Prognosis n=31</th>
<th>p value univariate</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td>9F/29M</td>
<td>6F/25M</td>
<td>ns</td>
</tr>
<tr>
<td>Age</td>
<td>50 +/- 2.2</td>
<td>62 +/- 2.6</td>
<td>0.0006</td>
</tr>
<tr>
<td>Proteinuria at diagnosis (g/g)</td>
<td>4.45</td>
<td>5.20</td>
<td>ns</td>
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<tr>
<td>Creatinemia at diagnosis (μmol/L)</td>
<td>88</td>
<td>113</td>
<td>0.0002</td>
</tr>
<tr>
<td>Proteinuria at test (g/g)</td>
<td>3.16</td>
<td>5.0</td>
<td>0.0082</td>
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<tr>
<td>Creatinemia at test (μmol/L)</td>
<td>88</td>
<td>112.5</td>
<td>0.003</td>
</tr>
<tr>
<td>CysR Group</td>
<td>20</td>
<td>3</td>
<td>0.0002</td>
</tr>
<tr>
<td>CysRC1 Group</td>
<td>7</td>
<td>7</td>
<td>ns</td>
</tr>
<tr>
<td>CysRC1C7 Group</td>
<td>11</td>
<td>21</td>
<td>0.0013</td>
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<tr>
<td>Immunosuppressive treatment</td>
<td>21</td>
<td>20</td>
<td>ns</td>
</tr>
<tr>
<td>PLA2R1 Titer</td>
<td>2594</td>
<td>5947</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Multivariate Cox regression Analysis

Seitz-Polski et al., JASN, 2016
Evolution during Follow-up

Seitz-Polski et al., JASN 2016
Mild disease  
Spontaneous remission

Seitz-Polski et al., JASN 2015

Severe disease

Epitope Spreading: Natural History

First event: anti-CysR reactivity

Second event: Intramolecular epitope spreading

Patient C  
CysR to CysRC1C7  
R → A  
(May 2011) → (Nov 2013)
Peptide CysR - Mimétisme Moléculaire ?

<table>
<thead>
<tr>
<th>Peptide ID</th>
<th>Sequences of Identified Peptides (Residue Numbers)</th>
<th>Sequence Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GIIFIQQESLKKC (39–51)</td>
<td>Ricin domain</td>
</tr>
<tr>
<td>2</td>
<td>SVTLLENCK (57–65)</td>
<td>Ricin domain</td>
</tr>
<tr>
<td>3</td>
<td>EDDLLWCATTSSR (198–209)</td>
<td>Fibronectin type II domain</td>
</tr>
<tr>
<td>4</td>
<td>YLNHDHEIVEKDAWK (357–372)</td>
<td>Linker between CTLD1 and CTLD2</td>
</tr>
<tr>
<td>5</td>
<td>YYATHCEPGWNPYNR (373–387)</td>
<td>Linker between CTLD1 and CTLD2</td>
</tr>
<tr>
<td>6</td>
<td>TWHEALR (399–405)</td>
<td>CTLD2</td>
</tr>
<tr>
<td>7</td>
<td>AGHMLSDESGCQEGWER (504–521)</td>
<td>Linker between CTLD2 and CTLD3</td>
</tr>
<tr>
<td>8</td>
<td>YSGGCCVAMRGR (613–623)</td>
<td>CTLD3</td>
</tr>
</tbody>
</table>

Fresquet et al., JASN 2014
Complete homology with the sequence LTLENCK which is a part of the bacterial cell wall enzyme D-alanyl-D alamine carboxypeptidase common to Clostridium species

Fresquet et al., JASN 2014
Epitope Spreading in Heymann Nephritis

Peptide L6

Ab + Active disease

Shah et al., JASN 2007
Epitope Spreading in THSD7A?

Link with disease Activity?

Beck ASN 2015
Epitope Spreading: Cohort confirmation

82 patients GEM entre 1978 - 2007

- 34 patients Isupp (42%)
- 38 Kidney Failure (46%)
- 25 ESKD (30%)

2 THSD7A-Ab + (2%)
- 2 patients Isupp (100%)
- 1 Kidney Failure
- 1 ESKD

42 PLA2R1-Ab + (52%)
- 24 patients Isupp (57%)
- 18 Kidney Failure (41%)
- 12 ESKD (28%)

38 double negative (46%)
- 9 patients Isupp (23%)
- 19 Kidney Failure (50%)
- 12 ESKD (32%)

Group CysR n=15
- 8 patients Isupp
- 1 Kidney Failure
- 0 ESKD

Group CysRC1 n=10
- 16 patients Isupp
- 17 Kidney Failure
- 12 ESKD

Group CysRC1C7 n=17

Seitz-Polski B. et al. in preparation
Epitope Spreading : a Bad prognosis factor

Seitz-Polski B. et al. in preparation
GEM-Ritux

75 MN PLA2R1+

38 Symptomatic therapy + Placébo

37 Symptomatic therapy + Rituximab

- Primary endpoint: Remission at M6 p>0.05
- Secondary endpoint:
  - Proteinuria<50% from baseline p<0.05
  - Remission at M12 p<0.05

- Tolerance : OK
- ≈Immunosuppressive Therapy

K. Dahan et al., JASN 2016
Cohorte GEM-Ritux

- 75 MN
  - 37 RTX+
    - 10 PLA2R1-
      - 8 Remission at M6
    - 27 PLA2R1+
      - 19 Active disease at M6
  - 38 RTX-
    - 10 PLA2R1-
      - 6 Remission at M6
    - 28 PLA2R1+
      - 27 PLA2R1+
        - 1 ech non testé?
      - 21 Active disease M6
38 RTX-

10 PLA2R1-

28 PLA2R1+

1 sample NA

27 PLA2R1+

10 CysR (37%)

Rem M6 n=5/10 50%

Active M6 n=5/10 50%

5 CysRC1 (18%)

Rem M6 n=0

Active M6 n=5/5 (100%)

12 CysRC7 ou CysRC1C7 (44%)

Rem M6 n=1/12 (8%)

Active M6 n=11/12 (92%)

<table>
<thead>
<tr>
<th></th>
<th>Spontaneous Remission n=6</th>
<th>Active Disease n=22</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spreading vs No Spreading M0</td>
<td>1/5</td>
<td>16/6</td>
<td>0.021*</td>
</tr>
</tbody>
</table>
En analyse multivariée seul le Spreading est associé à la rémission à M6 et au dernier suivi contrairement au titre d’Ac anti-PLA2R1 et au recours au Rituximab.
In development...
Conclusion

New anti-PLA2R1 test based upon epitope specificity:

• To stratify patients at risk of ESKD
• To treat earlier this group