CLINICAL, PROGNOSTIC AND PATHOGENETIC ROLE OF ANTIPLA$_2$R ANTIBODIES IN MEMBRANOUS NEPHROPATHY-ASSOCIATED PODOCYTE DYSFUNCTION

Mella A, Cantaluppi V, Burdese M, Medica D, Cortazzi S, Colla L, Besso L, Biancone L

Renal Transplantation Unit "A. Vercellone", Division of Nephrology Dialysis and Transplantation, Department of Medical Sciences, Città della Salute e della Scienza Hospital and University of Turin, Turin, Italy

OBJECTIVES

- **Membranous nephropathy** (MN) is the most **common cause of nephrotic syndrome** in adults[1]
- Outcome and treatment response are **variable**[2]
- The identification of type M phospholipase A_2 receptor (PLA_2R) as the target antigens of circulating and deposited antibodies (**Ab antiPLA$_2$R**), positive in almost **70% of adult 'idiopathic'** MN patients[3], paved the way to some clinical and experimental studies focusing about the role of this antibodies in disease activity and treatment response
- In addition, **plasma of MN** patients **downregulate** the expression of the **slit diaphragm protein** nephrin on podocyte cell surface with a consequent alteration of permeselectivity[4,5]
- The aims of this study are to **evaluate the clinical and prognostic role of Ab antiPLAR** in our single-center case series of MN patients and to **evaluate in vitro** their **potential role** in the **alteration of podocyte function**

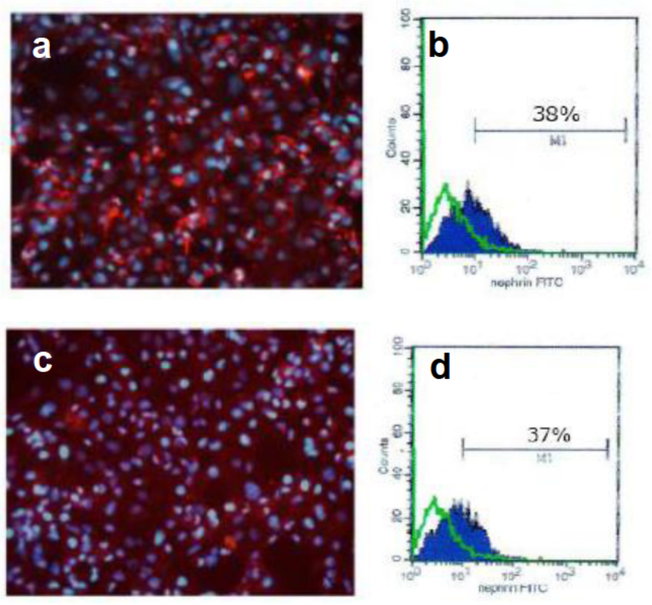

Figure 1. Nephrin expression (qRTPCR and FACS).on cultured podocyte incubated with sera from Ab antiPLA2R positive (a,b) and negative (c,d) patients

METHODS

- Retrospective analysis among the **80 idiopathic MN patients** treated and followed in Our Center between **01/01/2004-06/30/2013**
- Ab antiPLA$_2$R were measured with **ELISA test** (EUROIMMUN)
- Sera obtained from **positive Ab antiPLA$_2$R** patients were **tested in vitro** on cultured human podocytes evaluating **cytoxicity** (XTT assay), **apoptosis** (TUNEL and caspase ELISA) and **expression of nephrin** (qRTPCR and FACS)

RESULTS

Overall patients characteristics

- Ratio M/F 1.9:1; age at diagnosis 58.3±16.8 years; serum creatinine (sCr) and proteinuria (Pt) at diagnosis 1.22±0.68 mg/dL and 7.05±4.39 gr/day respectively
- In **20/80** available serum before treatment (**Group A**)
  → **positivity for Ab antiPLA$_2$R** in **13/20** (65%)

Group A characteristics

- Ratio M/F 1.8:1; age at diagnosis 64.9±14.6 years; sCr and Pt at diagnosis 1.42±0.76 mg/dL and 7.23±4.85 gr/day respectively
- Treatment: "Ponticelli" regimen 6/20; Cyclosporine 8/20; Rituximab 3/20; Mycophenolate Mofetil 2/10; Adrenocorticotrophic hormone 1/20
- Outcomes: f/up 2.75±1.55 years, no progression to ESRD; 1 patient died for a pulmonary carcinoma; **9/20 experienced a clinical remission** (4/9 complete, 5/9 partial)
- In **12/19** second serum sample available→7/12 pre-treatment positive→**1/7 remained** Ab antiPLA$_2$R **positive** despite **clinical remission**

Ab antiPLA$_2$R concentrations and correlations

- Median concentration **1020,25 RU/mL** (min 24,01max 7388,24); **no statistical difference** between positive and negative Ab antiPLA$_2$R patients in **remission rates**
- **High** Ab antiPLA$_2$R **concentration** correlates with a **low rate of clinical remission** ($p<0.05$)
  → **cutoff value** (ROC curve) **400 RU/ml**

In vitro assays on human podocytes

- Sera from MN patients vs nonaffected sujbects exerted **deleterious effects** on podocyte function
- Sera induced a **dose-dependent cytotoxicity**, triggered **apoptosis** and significantly **decreased the surface expression of nephrin** (figure 1), but **no difference between** sera obtained from **positive vs negative** Ab antiPLA$_2$R patients was observed

CONCLUSIONS

- **Ab antiPLA$_2$R** have **modified** the **current managment** of MN
- The availability of **ELISA test** offers a new opportunity for a **better undestanding of disease activity**
- Our study did **not demonstrate** a **specific injurious effect** of **Ab antiPLA$_2$R** on podocytes, suggesting the presence of other antibodies or of triggering of alternative pathways involved in glomerular injury

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