Proteomic analysis of glomeruli from patients with IgA nephropathy reveals complement activation as important prognostic marker

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AIM

The clinical course of IgA nephropathy (IgAN) is variable and complement activation may predict prognosis. IgAN patients with medium risk of progression (based on proteinuria and eGFR) were divided into 2 groups (progressive vs non progressive) and complement related proteins from their

MATHERIAL AND METHODS

glomeruli were compared, aiming to detect potential markers of progression.

Based on data from the Norwegian Kidney Biopsy Registry and the Norwegian Renal Registry, 2 patient groups were selected:

- 9 patients with progressive IgAN with eGFR >45 ml/min/1.73m2 who developed ESRD during 10 year follow-up
- 16 patients with stable IgAN with eGFR >45 ml/min/1.73m2 who did not develop ESRD during 10 year follow-up

100 glomerular cross sections were microdissected for each patient. Samples were analyzed by liquid chromatography-tandem mass spectrometry and relative abundances of complement-related proteins were compared between groups.

RESULTS

| Table 1: Patients characteristics (* p < 0.05) | | | | | |
|--|--------------------------|-----------------------|--|--|--|
| | IgAN without progression | IgAN with progression | | | |
| N | 16 | 9 | | | |
| Year of diagnosis | 1996 ± 3.4 | 1998 ± 5.7 | | | |
| Proportion female | 12.5% | 33.3% | | | |
| Age (years) | 31.4 ± 13.4 | 31.2 ± 15.8 | | | |
| Serum creatinine (mol/l) | 91.5 ± 21.5 | 105.8 ±25.6* | | | |
| Estimated glomerular filtration rate (ml/min/1.73m²) | 92.5 ± 23.3 | 74.1 ± 21.9* | | | |
| Systolic blood pressure (mmHg) | 127.2 ± 14.3 | 135.4 ± 25.8 | | | |
| Urinary protein (grams/24h) | 1.76 ± 1 | 2.0 ± 1.98 * | | | |
| No of years of follow-up | 16.3 ± 3.4 | | | | |
| No of years from biopsy to ESRD | | 5.8 ± 2.5 | | | |

- A total of 2018 were identified with 2 or more unique peptides and were used in quantitative analyses.
- In progressive vs non-progressive IgAN, 196 proteins displayed a significant differential abundance, among these, 19 proteins were related to the complement system.

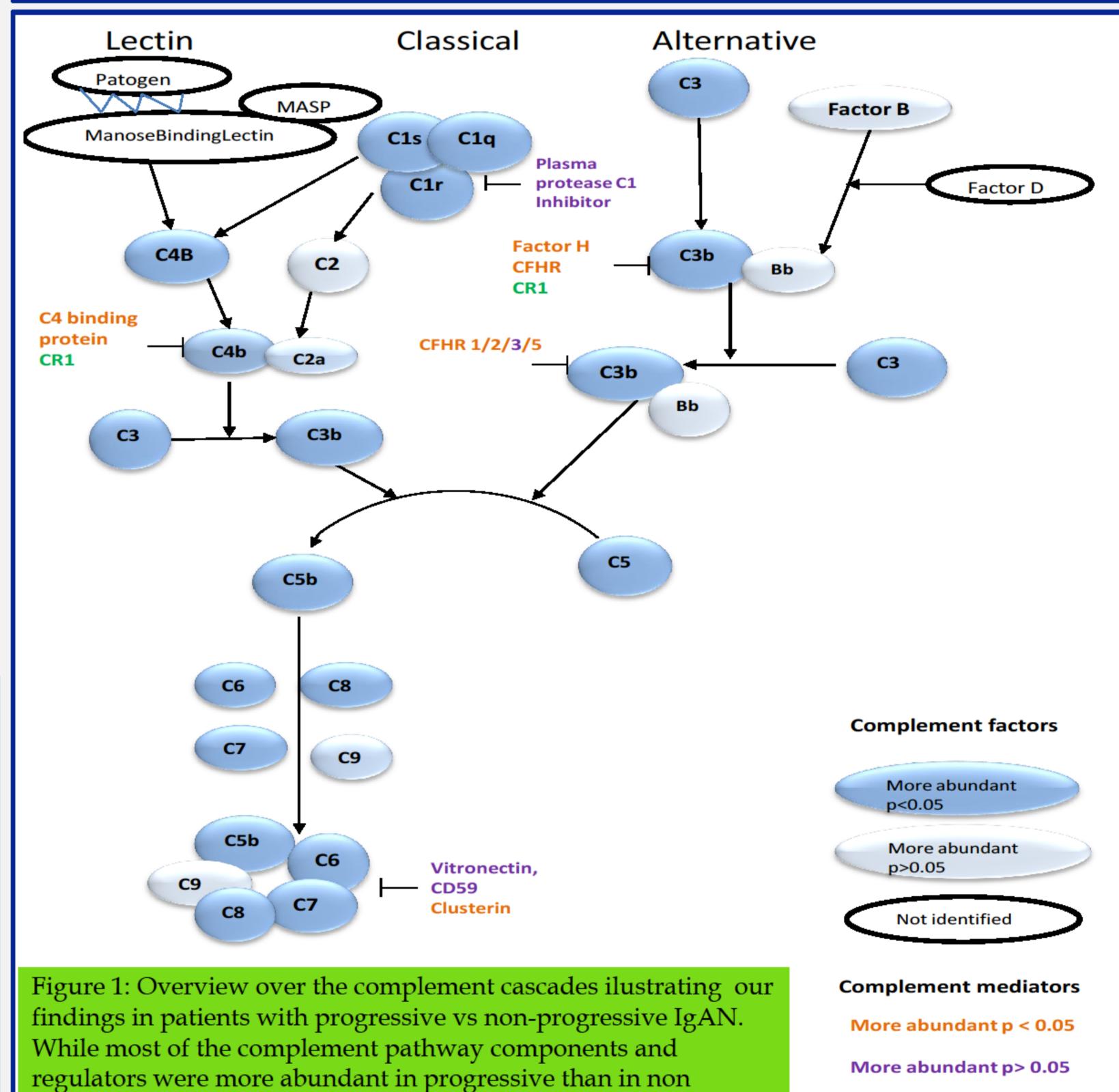
Associations with variables at time of biopsy

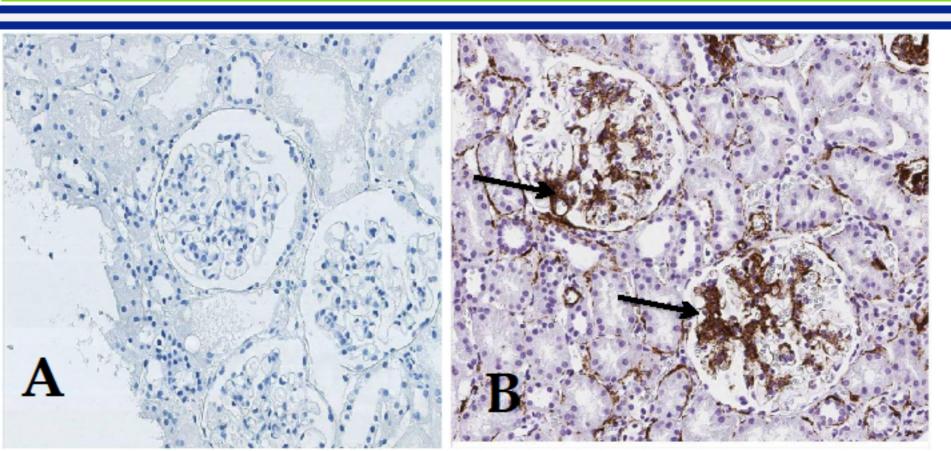
Associations between the complement related proteins and clinical markers were investigated. These analyses showed that C1r, C1s, C5, C6, C8, C9 and Clusterin had higher abundance with lower eGFR. There was no significant associations with urinary protein excretion but there was increased abundances of the proteins C1r, C1s, C4, C5, C8, C9, Complement factor H, Complement factor H-related protein 3, and C4b binding protein alpha with increasing systolic blood pressure.

RESULTS

Table 2: Change in abundance (fold change) of quantified complement and complement related proteins in progressive vs non-progressive IgAN UniProt Fold Unique

| Accession | Peptides | Description | Change | T test |
|------------------------------|----------|---------------------------------------|--------|--------|
| Complement system components | | | | |
| P02746 | 4 | Complement C1q subcomponent subunit B | 1.15 | 0.48 |
| P02747 | 4 | Complement C1q subcomponent subunit C | 1.89 | 0.04 |
| P00736 | 2 | Complement C1r subcomponent | 2.17 | 0.0003 |
| P09871 | 2 | Complement C1s subcomponent | 1,50 | 0.03 |
| P00751 | 17 | Complement factor B | 1.18 | 0.43 |
| P06681 | 2 | Complement C2 | 2.73 | 0.14 |
| P01024 | 105 | Complement C3 | 1.39 | 0.01 |
| P0C0L4 | 44 | Complement C4-A | 1.27 | 0.35 |
| P0C0L5 | 5 | Complement C4-B | 1.73 | 0.03 |
| P01031 | 39 | Complement C5 | 1.64 | 0.0046 |
| P13671 | 16 | Complement component C6 | 2.11 | 0.0002 |
| P10643 | 14 | Complement component C7 | 2.15 | 0.0002 |
| P07360 | 10 | Complement component C8 gamma chain | 1.62 | 0.0003 |
| P07357 | 19 | Complement component C8 alpha chain | 1.88 | 0.0004 |
| P07358 | 14 | Complement component C8 beta chain | 1.74 | 0.01 |
| P02748 | 25 | Complement component C9 | 1.46 | 0.05 |
| Complement system regulators | | | | |
| P13987 | 2 | CD59 glycoprotein | 1.29 | 0.11 |
| P10909 | 14 | Clusterin | 1.75 | 0.0003 |
| P08603 | 38 | Complement factor H | 1.43 | 0.02 |
| Q03591 | 9 | Complement factor H-related protein 1 | 1.63 | 0.03 |
| P36980 | 3 | Complement factor H-related protein 2 | 2.33 | 0.01 |
| Q02985 | 4 | Complement factor H-related protein 3 | 1.97 | 0.08 |
| Q9BXR6 | 22 | Complement factor H-related protein 5 | 1.73 | 0.0029 |
| P17927 | 13 | Complement receptor type 1 | 0.62 | 0.025 |
| P04003 | 14 | C4b-binding protein alpha chain | 2.48 | 0.02 |
| P04004 | 18 | Vitronectin | 1.14 | 0.29 |
| P05155 | 4 | Plasma protease C1 inhibitor | 1.27 | 0.22 |





progressive IgAN, CR1 was less abundant. Analysis of

complement subcomponents was not possible.

Figure 2 : Immunohistochemical staining for MAC (C5b-9). Biopsy from patient with non progressive IgAN (A) shows no positivity for MAC staining while staining in progressive IgAN (B) shows glomerular, tubular and vascular positivity for MAC. The arrows indicate the positively stained mesangial areas.

Less abundant, p<0.05

CONCLUSION

Higher glomerular abundance of complement related proteins were associated with a progressive clinical course in IgAN

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