Acquired Drivers of Disease
C3 glomerulopathy
C3 nephritic factor and other autoantibodies

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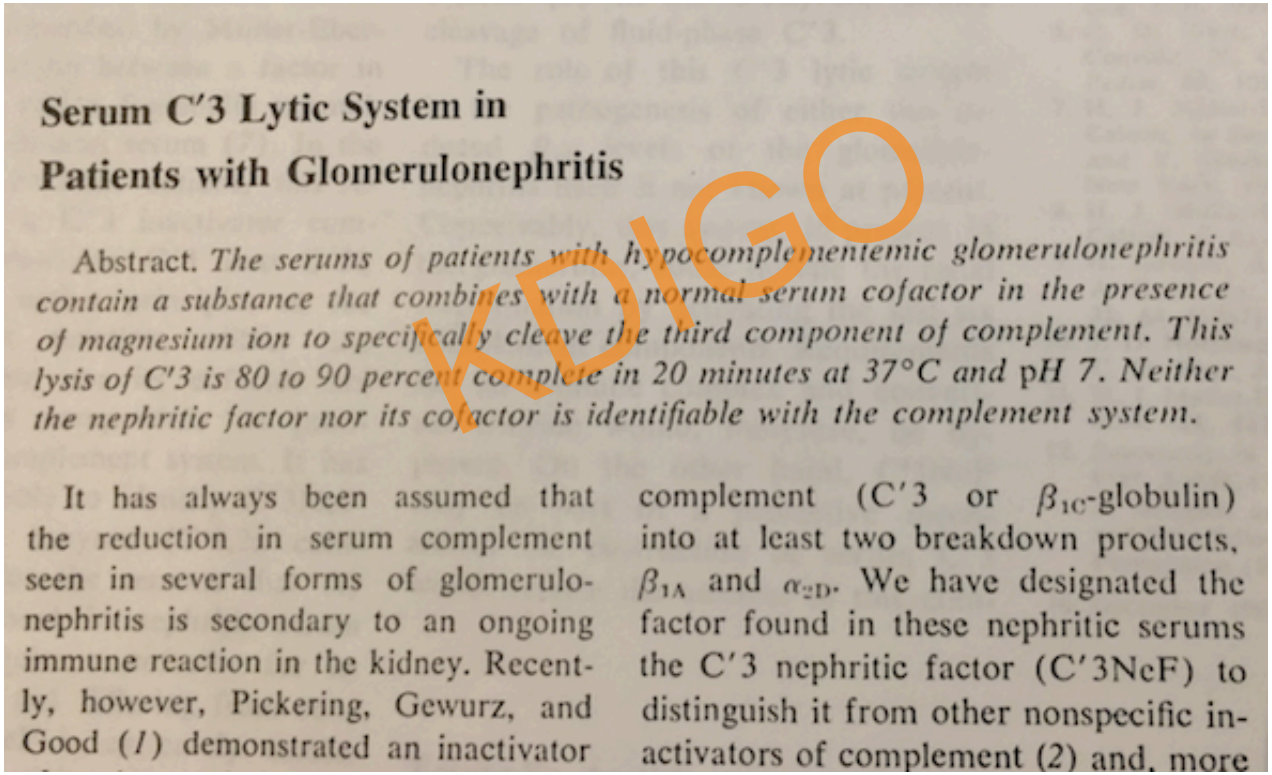
Disclosure of Interests

- Alexion – Funding for pre-clinical studies
- Achillion – Consultancy

C3 nephritic factors

Autoantibodies that prolong the half-life of the alternative pathway C3 convertase (denoted C3NeF)[1]



Serum C′3 Lytic System in Patients with Glomerulonephritis

Abstract. *The serums of patients with hypocomplementemic glomerulonephritis contain a substance that combines with a normal serum cofactor in the presence of magnesium ion to specifically cleave the third component of complement. This lysis of C′3 is 80 to 90 percent complete in 20 minutes at 37°C and pH 7. Neither the nephritic factor nor its cofactor is identifiable with the complement system.*

It has always been assumed that the reduction in serum complement seen in several forms of glomerulonephritis is secondary to an ongoing immune reaction in the kidney. Recently, however, Pickering, Gewurz, and Good (*1*) demonstrated an inactivator complement (C′3 or β_{1C}-globulin) into at least two breakdown products, β_{1A} and α_{2D}. We have designated the factor found in these nephritic serums the C′3 nephritic factor (C′3NeF) to distinguish it from other nonspecific inactivators of complement (*2*) and, more

[1] Spitzer et al, Science 1969

C3 nephritic factors

Autoantibodies that prolong the half-life of the alternative pathway C3 convertase (denoted C3NeF)

- Required Mg but not Ca
- Extremely stable
- was an IgG molecule[2-4]
- shown to bind to C3bBb[1]
- heavily glycosylated and removal of carbohydrate groups was associated with loss of activity[5]
- ?acquired antibody – discordant occurrence in identical twins[6]

[1] Daha et al., J. Immunol 1977; [2] Davis AE et al, PNAS 1977; [3] Scott DM et al Clin. Exp. Immunol 1978; [4] Daha et al., J. Immunol 1979; [5] Scott DM et al Clin. Exp. Immunol 1981; [6] Reichel et al., Klein. Wochenschr 1976

C3 nephritic factors

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Robust association with C3 glomerulopathy

e.g. Dense deposit disease 86%[1]; C3 glomerulonephritis 54%[1]

Table 4 | Complement component assessment according to histological type

	All	MPGN 1	DDD	GNC3
N	115	41	22	53
C3[a] (660 to 1250 mg/l)	621.91 ± 339.5	583.1 ± 360.7	492.8 ± 337.7	705.4 ± 305.2
Low C3 (<660 mg/l)	53 (46.1%)	19 (46.3%)	13 (59.1%)	21 (39.6%)
C4[a] (93 to 380 mg/l)	227.9 ± 86.3	198.4 ± 65.7	204.8 ± 88.9	260.8 ± 89.3
Low C4 (<93 mg/l)	2 (1.7%)	1 (2.4%)	1 (4.5%)	0
Factor B[a] (90 to 320 mg/l)	116.4 ± 49.3	110.9 ± 42.2	112.6 ± 39.9	122.2 ± 57.7
Low factor B (<90 mg/l)	34 (29.6%)	14 (34.1%)	6 (27.3%)	14 (26.4%)
Low factor H (<338 mg/l)	8 (6.9%)	2 (4.9%)	4 (18.2%)	2 (3.8%)
Low factor I (<42 mg/l)	3 (2.6%)	3 (7.3%)	0	0
C3NeF	65 (58.6%)[b]	22 (53.6%)	19 (86.4%)	24 (45.3%)
Unexplained C3 <660 mg/l	6 (5.2%)	1 (2.4%)	0	5 (9.4%)

Abbreviations: C3NeF, C3 nephritic factor; DDD, dense deposit disease; GNC3, glomerulonephritis with isolated C3 deposits; MPGN, membranoproliferative glomerulonephritis.
[a]Normal values are indicated in brackets.
[b]C3NeF determination was not available in four patients.
Patients under immunosuppressive therapy at the time of complement assessment were excluded from this analysis (N=19). Mean ± s.d., number (percentage).

[1] Servais et al Kidney Int 2012

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[1] Servais et al Kidney Int 2012; [2] Fremeaux-Bacchi et al Nephrol. Dial. Transplantation 1994; [3] Niel et al Paed Nephrol 2015

C3 nephritic factors

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Heterogeneous...

C3NeF of the amplification loop

C3 low, normal C5
'MPGN2', Partial lipodystrophy

C3NeF of the terminal pathway

C3 and C5 activation
Properdin-dependent (low properdin levels)
'MPGN3', 'MPGN1' (some)

[1] Clardy et al., Clin. Immunol. Immunopathol. 1989; [2] Mollness et al., Clin. Exp. Immunol. 1986

C3 nephritic factors

Autoantibodies that prolong the half-life of the alternative pathway C3 convertase (denoted C3NeF)

Heterogeneous...

Patient	Sex/Age	*In vitro* C3 conversion (%)	C3 60–135%	C3dg 20–45 AU/ml	C5 80–130%	TCC 2·2–6·6 AU/ml	TCC/C5 <8·2	Diagnosis
A	M 56	Neg	21	261	56	13·0	23·2	AGN†
		Neg	24	146	49	11·5	23·4	
		Neg	21	121	52	5·9	11·3	
		Neg	30	69	114	4·0	3·5	
		Neg	80	28	56	3·3	5·8	
1	F 21	75	13	250	155	2·8	1·8	MPGN‡
		95	<10	350	100	3·6	3·6	
2	F 36	70	23	275	100	6·0	6·0	PLD§
3	F 20	30	70	51	104	6·0	5·7	MPGN
		40	68	112	80	17·3	21·6	
		60	40	299	120	6·3	5·2	
4	M 10	20*	<10	107	25	5·2	20·8	MPGN
		30*	<10	120	23	5·9	25·6	
		25*	11	105	12	5·2	43·3	
5	F 33	100*	<10	222	32	6·7	20·9	MPGN
		65*	<10	195	54	6·3	11·6	
6	F 6	60*	<10	240	5	10·3	206·0	MPGN

* 3 h incubation (slow conversion).
† Acute glomerulonephritis (post-streptococcal).
‡ Membranoproliferative glomerulonephritis.
§ Partial lipodystrophy.

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Properdin-*independent* C3NeF have no effect on C5 activation; increase convertase half-life >50-fold[4]

Properdin-*dependent* C3NeF enhance C5 activation and terminal pathway activation; increase convertase half-life 10-20-fold[4]

[1] Servais et al Kidney Int 2012; [2] Fremeaux-Bacchi et al Nephrol. Dial. Transplantation 1994; [3] Niel et al Paed Nephrol 2015; [4] Paixao-Cavalcante et al Kidney International 2012

C3 nephritic factors

Autoantibodies that prolong the half-life of the alternative pathway C3 convertase (denoted C3NeF)

Relationship to renal disease:

- Systemic hypocomplementaemia that is associated with C3NeF predisposes to nephritis
- C3NeF acts locally to cause tissue injury
- Abnormal immune complex clearance due to both hypocomplementaemia and molecular interactions of C3NeF with regulators

C3 nephritic factors

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Relationship to renal disease:

Conflicting associations between:

- serum C3 and C3NeF levels
- presence of C3NeF and rate of progression of nephritis
- C3Nef and recurrence of disease in allograft

C3 nephritic factors

Several assays that utilise convertase binding and stabilisation[1-3]

Techniques include:

- Fluid-phase C3 activation
- C3 convertase stabilisation
- C3 convertase stabilisation with properdin
- C3Nef IgG-binding to pre-formed convertase on cells[3] or ELISA[4] plates
- Hemolytic assay

[1] Fremeaux-Bacchi et al Nephrol. Dial. Transplantation 1994; [2] Paixao-Cavalcante et al Kidney International 2012; [3] Zhang et al CJASN 2014; [4] Enzyme-linked immunosorbent assay

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Other autoantibodies:

- Anti-factor B autoantibodies[5], anti-factor H antibodies[6]
- Combined anti-C3 and anti-factor B autoantibodies[7]

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Breakout group - Questions

3. How should C3 nephritic factors be measured in the clinical laboratory? (Discussion needs to include relevance of quality controls, reliable quantitation, standardization of tests)

5. At what time and at which frequency should acquired factors be screened?

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1. What are the clinically important acquired factors that should be screened for in patients with C3 glomerulopathy?

2. What are the clinically important acquired factors that should be screened for in patients with aHUS?

4. How should anti-factor H autoantibodies be measured and quantified? (Discussion needs to include relevance of quality controls, reliable quantitation, standardization of tests)

5. At what time and at which frequency should acquired factors be screened?

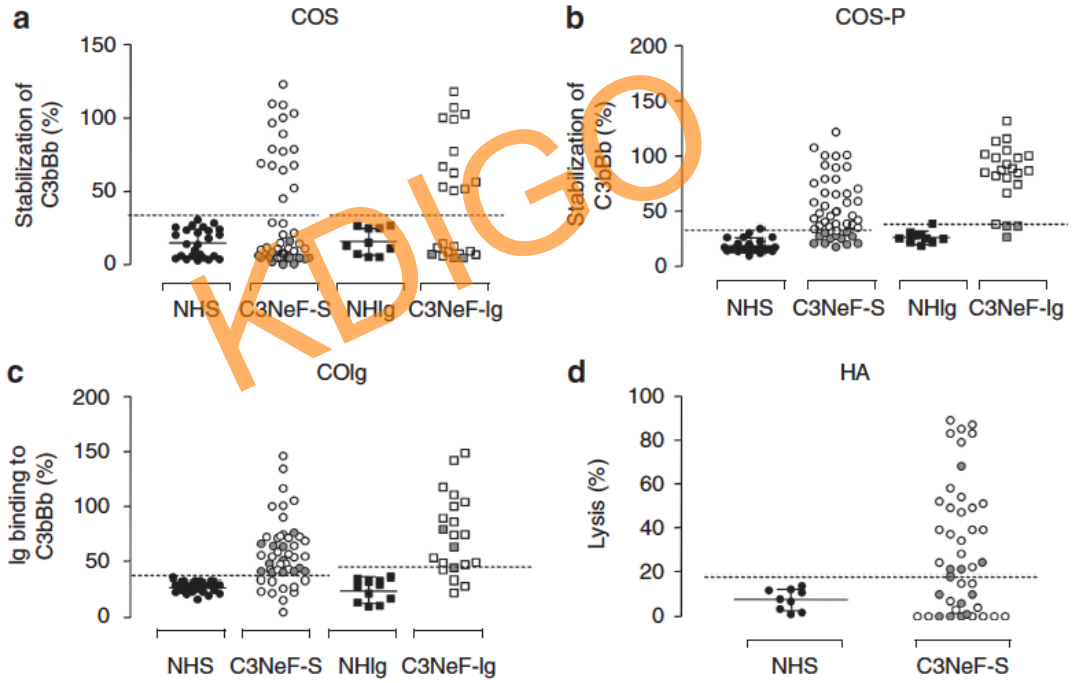
6. Should we include the screening of other biomarkers or genetic predisposition for acquired disease?

7. Can acquired drivers of disease be used to define subtypes of C3G?

C3 nephritic factors

Several assays that utilise convertase binding and stabilisation

Example[1]:



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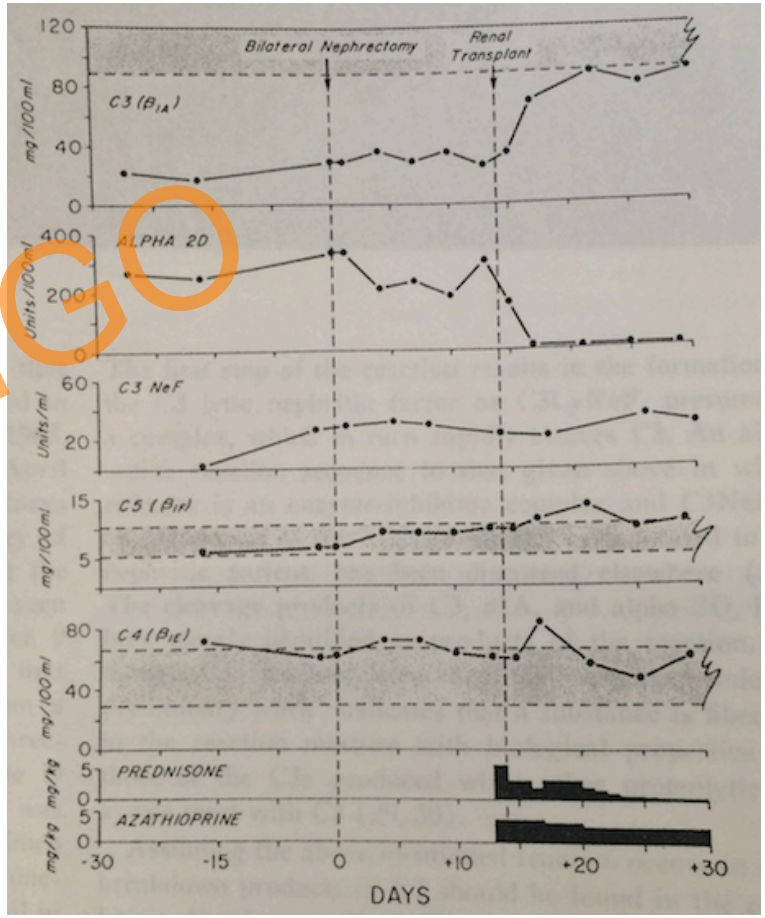

[1] Vallota et al J. Clin. Invest. 1971