Measuring Complement Biomarkers in the Urine of Patients with C3 Glomerulopathy

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Introduction

- C3 Glomerulopathy (C3G) is a rare complement-mediated renal disease characterized by abnormally high activity of the alternative pathway (AP) of complement that results in chronic glomerulonephritis.
- Previous studies have shown elevated urinary complement protein levels in patients with many other renal diseases, including IgA nephropathy[1], diabetic nephropathy[2] and membranous nephropathy[3]. However, to the best of our knowledge, urinary biomarkers for C3G have not yet been reported.
- In C3G, systemic AP activity can be identified and quantitated by measuring plasma biomarkers like C3, C4, C3c, factor B, Ba, Bb, C5 and soluble C5b-9 (sC5b-9). In contrast, local AP dysregulation in the kidney is harder to quantify. We hypothesized that renal complement dysregulation can be measured by quantitating complement biomarkers in the urine in patients with C3G.
- We conducted a study measuring urinary complement biomarkers in a cohort of patients some of whom were on Eculizumab. Corresponding plasma biomarkers were measured for comparison. Data were compared to normal controls

Methods

- Complement plasma biomarkers were measured by ELISA or RID from Quidel and The Binding Site, respectively. Classical and alternative pathway activities were assessed using Quidel and Wieslab kits, respectively.
- Urinary biomarkers were measured by ELISAs from Abcam (C3, C4, C5, factor B), Quidel (C3a, sC5b-9, Ba, Bb), or Bethyl Labs (Albumin).
- Serum and urine creatinine levels were measured by the Jaffe method using a kit from BioAssays.
- Second urination of the day was collected and spun down. Supernatant was transferred to clean tubes in small aliquots and immediately frozen and stored at -80°C.
- Hema-Combistix strips (Siemens) were used to measure hematuria, proteinuria and pH of the urine samples.

Patients and Controls

Table 1. Patient and control data

	Patients w/o Ecu	Patients with Ecu	Controls
Age in years (IQR)	25 (18-35)	24 (13-46)	46 (32-50)
Gender M/F	12/4	3/9	14/11
Ethnicity[a]	1 A, 1 L, 14 W	12 W	4 A, 21 W
Proteinuria (g/gCr) (IQR)	0.77 (0.19-1.5)	0.25 (0.06-1.5)	N/A
Hematuria	None-+++	None->+++	N/A
eGFR (IQR)	99.0 (59.8-129)	66.5 (38.5-108)	66.0 (59.5-86.8)
CKD stage	1-5	1-4	1-2

Serum, plasma and urine were collected from 16 patients with C3G who had not received Eculizumab (Ecu) and 12 patients who were on Ecu. Additionally, serum and plasma from 12 controls were collected and analyzed, along with urine from 25 controls. (N/A: not applicable) (a, A=Asian, L=Latino, W=White/European)

Results

1) Plasma Biomarkers

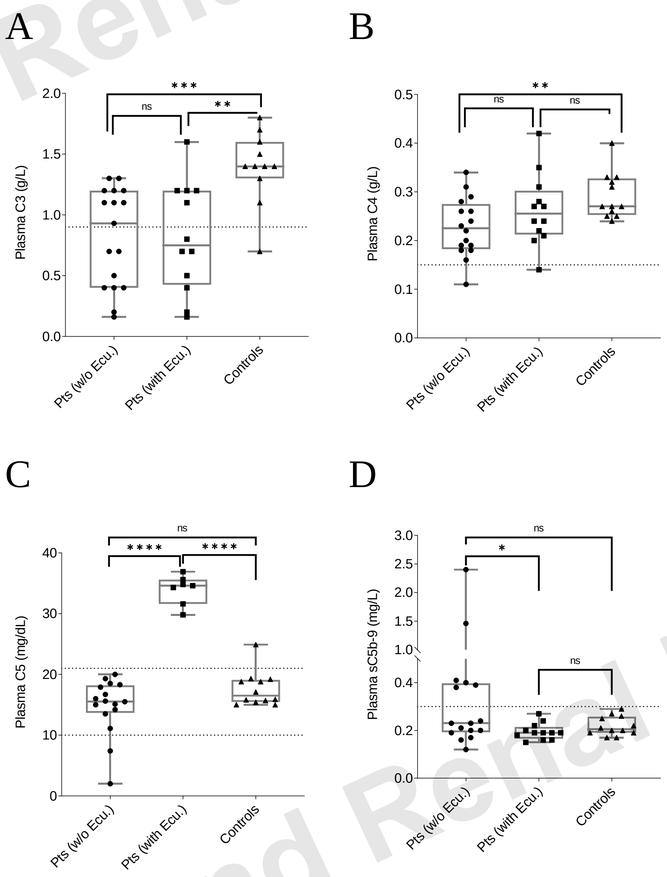

Figure 1. Plasma protein levels. A) C3. Reduced C3 in patients indicates ongoing systemic complement activation; B) C4. C4 is normal in most patients; C) C5. C5 is high in patients on Ecu, consistent with effective complement blockade; D) sC5b-9 is elevated in patients not on Ecu but normalized in patient on Ecu. These data are indicative of ongoing AP and terminal pathway dysregulation.

2) Urine Biomarkers

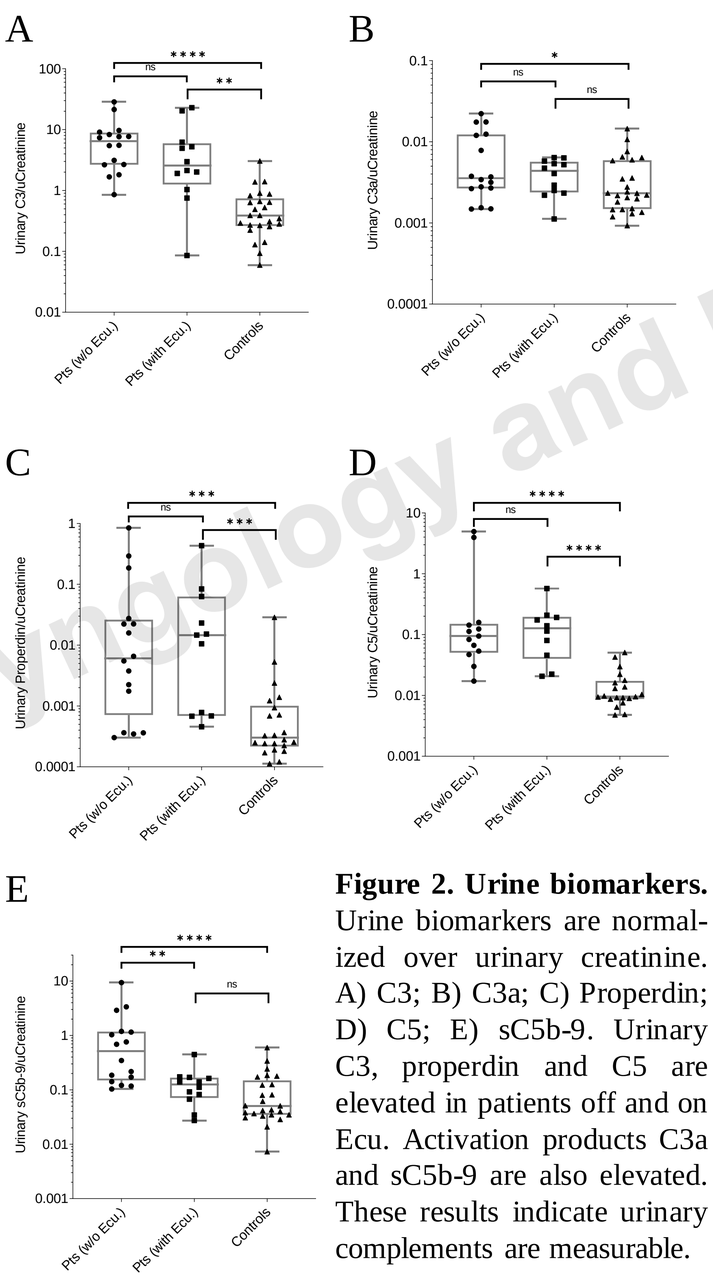

Figure 2. Urine biomarkers. Urine biomarkers are normalized over urinary creatinine. A) C3; B) C3a; C) Properdin; D) C5; E) sC5b-9. Urinary C3, properdin and C5 are elevated in patients off and on Ecu. Activation products C3a and sC5b-9 are also elevated. These results indicate urinary complements are measurable.

3) Plasma and Urinary Protein Correlation

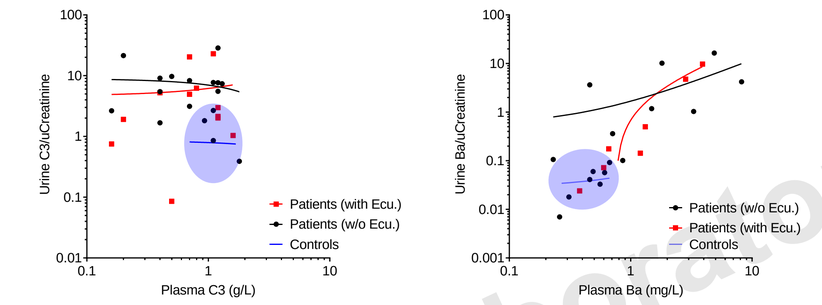

Figure 3. Correlation of plasma and urinary complement proteins. (A) C3. No correlation is observed in C3, C4, C5, properdin, Bb, C3a, C3c and sC5b-9. (B) Ba. Plasma and urinary levels of Ba are correlated in both patient groups but not controls. This correlation is consistent with progression to chronic renal disease in patients as Ba increases as renal function decreases. Control data points are located in the shaded areas.

4) Urine Biomarkers and Proteinuria

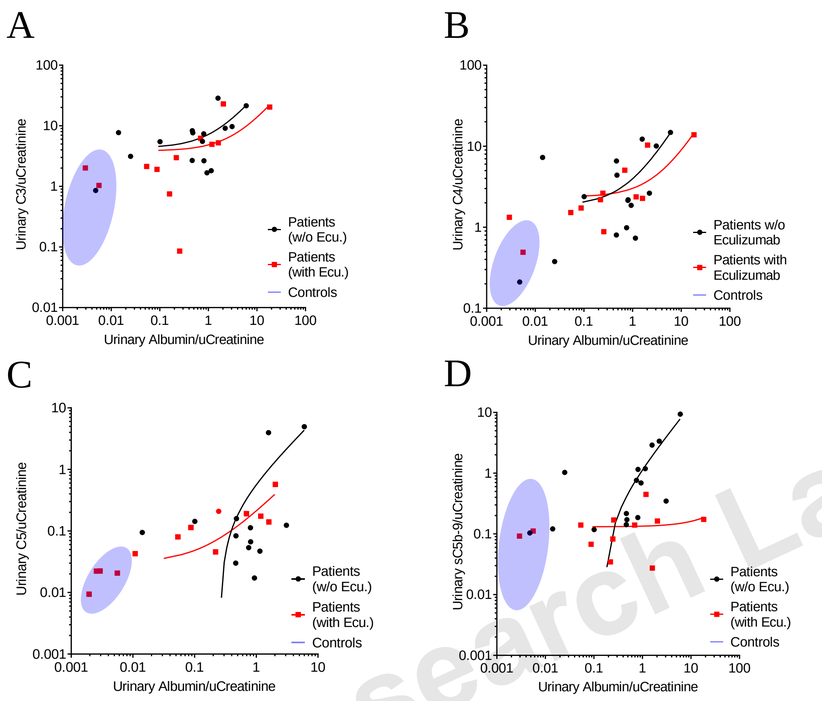

Figure 4. Selected urinary protein levels. Urine protein levels are plotted against urine albumin as a proxy for proteinuria. A) C3, B) C4, and C) C5, respectively. Urinary C3, C4, and C5 is highly correlated with proteinuria in patients with and without Ecu. D) sC5b-9. sC5b-9 is highly correlated with proteinuria in the patient without Ecu but not in patient on Ecu or in controls. Control data points are located in the shaded areas.

Discussion

- Our data demonstrate that quantifying complement biomarkers in urine is possible. Remarkable elevations are observed in urinary C3, C4, FB, properdin and C5 in patients, and these increases are independent of Eculizumab treatment.
- The significance of these data is impacted by the high correlation with proteinuria, suggesting that urinary levels of these proteins are confounded by serum proteins "leaking" through damaged glomeruli.
- Complement activation products are also highly elevated. The clinical values of these data can be compromised by local complement activation when "leaked" proteins reach on the tubular lumen, especially when patients are not on Eculizumab.
- Normalized urinary sC5b-9 in patients on Eculizumab reflects the efficacy of medication as renal complement activation is prevented. Therefore, monitoring urinary sC5b-9 may be useful to evaluate complement activity in the kidney.

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References

1. Onda K, Ohsawa I, Ohi H, Tamano M, Mano S, Wakabayashi M, Toki A, Horikoshi S, Fujita T, Tomino Y. Excretion of complement proteins and its activation marker C5b-9 in IgA nephropathy in relation to renal function. *BMC Nephrol*. (2011) Nov 23;12:64. doi: 10.1186/1471-2369-12-64.
2. Morita Y, Ikeguchi H, Nakamura J, Hotta N, Yuzawa Y, Matsuo S. Complement activation products in the urine from proteinuric patients. *J Am Soc Nephrol.* (2000) Apr;11(4):700-7.
3. Ogrodowski JL, Hebert LA, Sedmak D, Cosio FG, Tamerius J, Kolb W. Measurement of SC5b-9 in urine in patients with the nephrotic syndrome. *Kidney Int.*(1991) Dec;40(6):1141-7.