Supplemental Methods

Details of excluded patients

5 of 22 children from the UK and Ireland presenting with aHUS and anti-FH autoantibodies at a titre above the international consensus positive threshold of 100 RU were excluded from this study because: 1 child had Shiga toxin associated HUS and the low titre autoantibody was not felt to be significant; 1 individual presented aged 7 years with aHUS in 1992, but did not have serum tested for autoantibodies until 22 years later (the clinical course was of multiple relapses in childhood, treated with plasma exchange (PEX) and intravenous immunoglobulin (IVIG), and peritoneal dialysis for year before recovery of renal function – which has been stable since); 1 individual had serum tested for autoantibodies 10 years after presenting with aHUS; in 2 individuals the initial assay was positive but no samples were available to confirm positivity using the international consensus assay.

eGFR calculation

The estimated glomerular filtration rate (eGFR) was calculated: for children (<18 years), the Schwartz formula was used: eGFR (mL/min/1.73 m^2) = [0.55 x height (centimetres) x K (constant)] / serum creatinine (µmol/L) x 0.0113 (correction factor for mg/dL); in first year of life, for pre-term babies K=0.33 and for full-term infants K=0.45; for infants and children between ages of 1 and 12 years, K=0.55; adolescent boys, K=0.7. For adults the abbreviated MDRD equation was used: 186 x (Creat / 88.4)$^{-1.154}$ x (Age)$^{-0.203}$ x (0.742 if female) x (1.210 if black).

Factor H autoantibody assay

The consensus assay was performed as previously described[1]. A 96 well Maxisorb^{tm} ELISA plate (Nunc) was coated with 50µl/well of purified factor H (CompTech, Tyler, Texas, USA) at 5µg/ml or molar equivalents of factor H fragments (short consensus repeats [SCRs] 1-7 (generated in house) 8-15, 19-20)[2, 3] or factor H–related protein 1 fragment (SCRs 4-5)[4] in Dulbecco's PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) and incubated overnight at 4°C. Alternatively, for detection of circulating immune complexes (CIC) plates were coated with OX24 (2µg/ml) in 0.1M carbonate buffer, pH 9.6. Plates were washed once with PBS, then blocked with 200µl PBS/0.1% Tween (PBS-T) per well for 1hr at room temperature (for CIC, plates were blocked with 1% BSA/PBS-T). A second (replicate) plate was incubated with block only. Plates were washed 3 times with PBS/0.1% Tween. Individual test samples were diluted then 1/50 in PBS-T and 50 µl applied in triplicate to each plate, including a positive, negative and 'no serum' control. A standard curve was established using positive sample applied in doubling dilution from 1/25 to 1/3200. Samples and controls were incubated for exactly 1hr at room temperature followed by 3 washes with PBS-T. A 1/20,000 dilution of HRP conjugated goat anti-human IgG (Stratech Scientific Ltd, UK) in PBS-T (50µl/well) was then applied for exactly 1 hour. Both test and control plates were washed 3 times with PBS-T, 50µl/well of TMB substrate was applied (readymade,

Leico Technologies) and plates developed for 5-10min. The reaction was stopped using 50µl/well 10% H_2SO_4 and OD450nm measurements taken using an EL-800 plate reader (Biotek, UK). The readings from the 'block only' plate were then subtracted from the factor H coated plate. An ELISA was considered valid when the range between positive and negative control was greater than 1.0 (OD450). Readings from the background subtracted positive control standard curve were assigned 4000 relative units (RU) for a 1/25 dilution of Newcastle positive serum. These were plotted using PrismGraph 3 software, which allowed automatic interpolation of sample RU from a curve fit based on four-parameter logistic non-linear regression. Samples were analysed on three individual experiments and the mean of these used in the figures herein.

Western blotting confirmation of factor H autoantibody.

Purified complement factor H (Comptech) was diluted in solubilizing buffer (non-reducing) and loaded onto a 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) preparative gel. After transfer to nitrocellulose and blocking as described previously, the nitrocellulose was cut into 0.5- to 1-cm wide strips. These strips were then incubated with individual sera samples (1/100 in 5% dried milk/PBS) for 1 to 2 hours at room temperature. After washing as described previously, bound autoantibody was detected by the use of goat anti-human IgG-HRP incubated for 1 to 2 hours at room temperature. Blots were then washed twice with PBS/0.01% Tween 20 and with PBS only. All blots were developed by the use of an enhanced chemiluminescence (ECL) substrate (Pierce) according to the manufacturer's specifications.

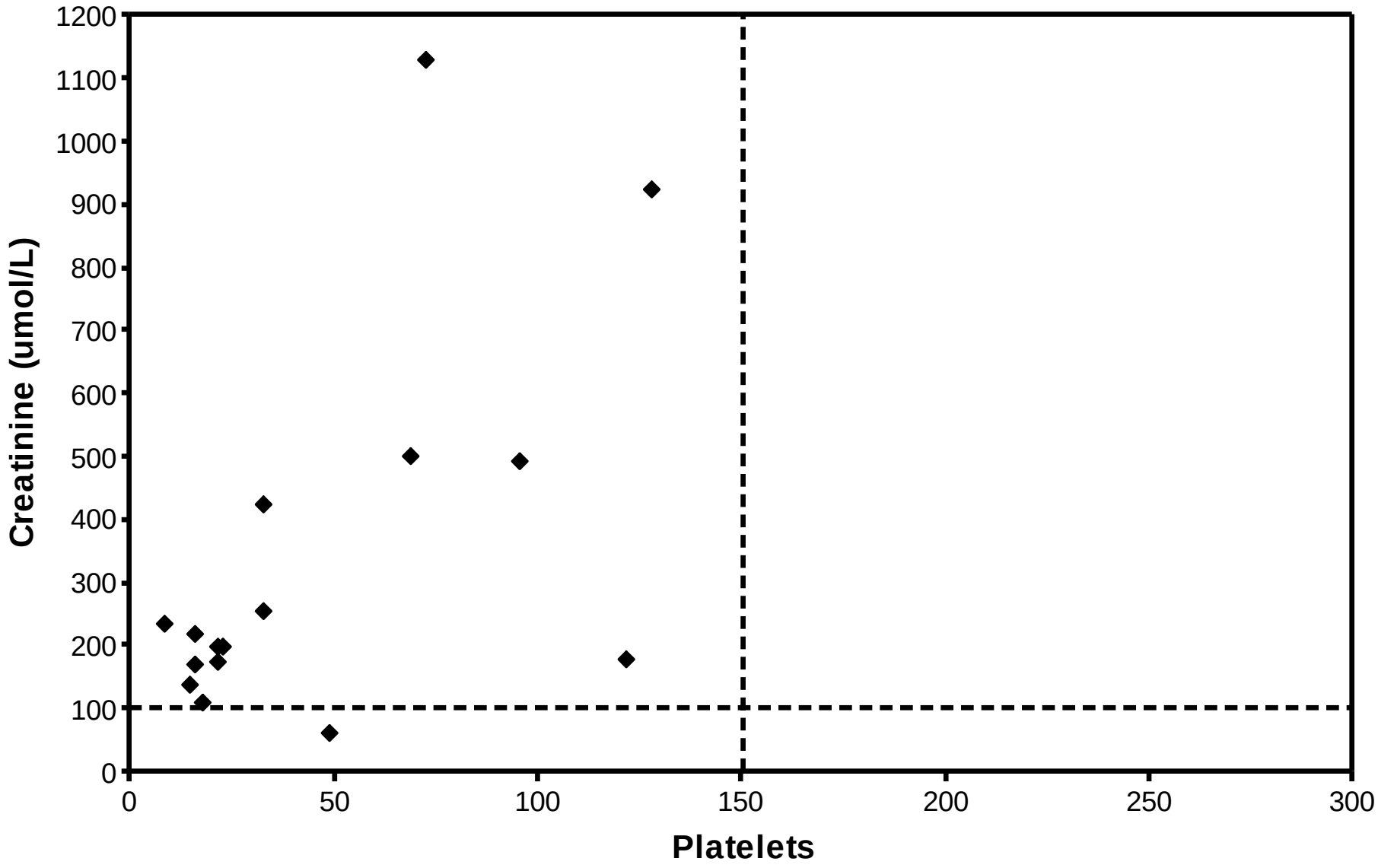
1. Watson R, Lindner S, Bordereau P*, et al.* Standardisation of the factor H autoantibody assay. *Immunobiology* 2014; **219:** 9-16.

2. Hocking HG, Herbert AP, Kavanagh D*, et al.* Structure of the N-terminal region of complement factor H and conformational implications of disease-linked sequence variations. *The Journal of biological chemistry* 2008; **283:** 9475-9487.

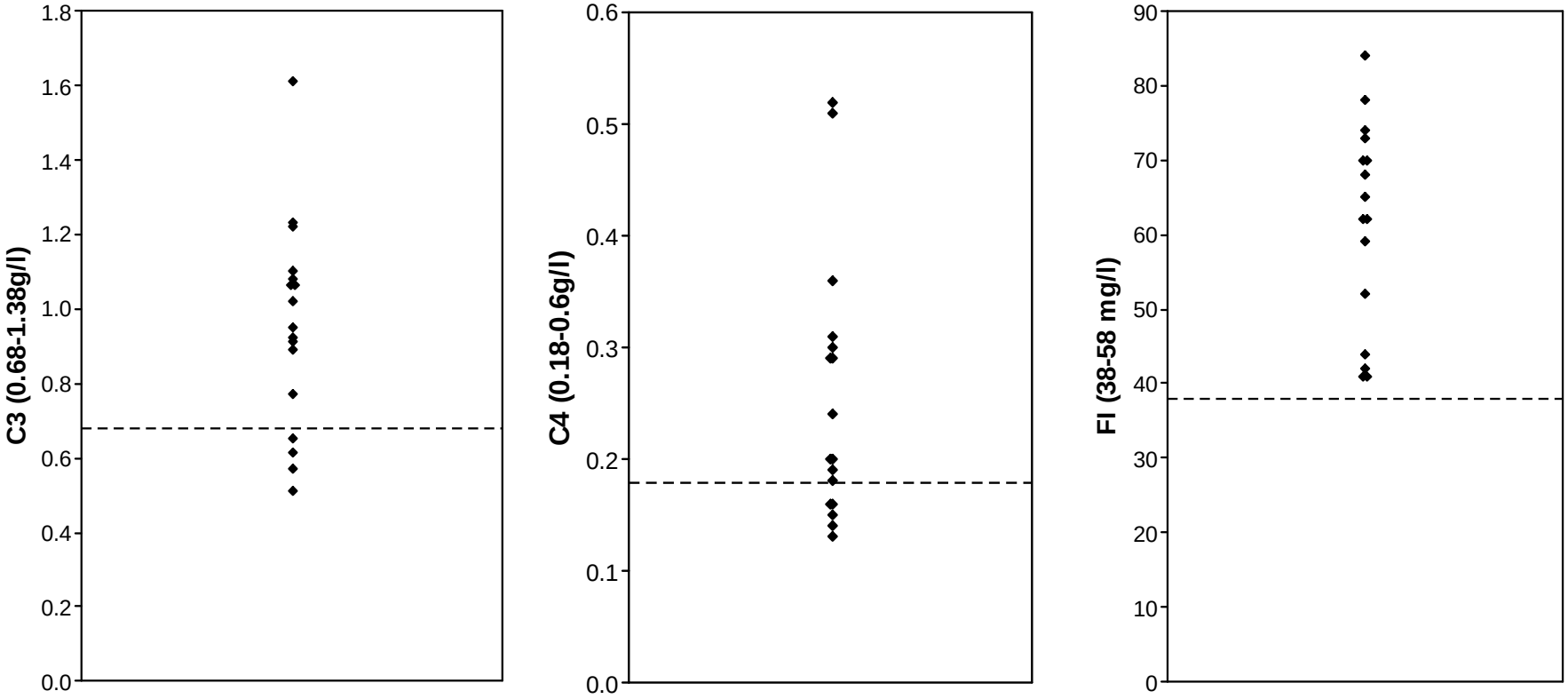
3. Schmidt CQ, Herbert AP, Kavanagh D*, et al.* A new map of glycosaminoglycan and C3b binding sites on factor H. *Journal of immunology* 2008; **181:** 2610-2619.

4. Ferreira VP, Herbert AP, Cortes C*, et al.* The binding of factor H to a complex of physiological polyanions and C3b on cells is impaired in atypical hemolytic uremic syndrome. *Journal of immunology* 2009; **182:** 7009-7018.

Supplemental Figure 1: Serum creatinine and platelet values at presentation

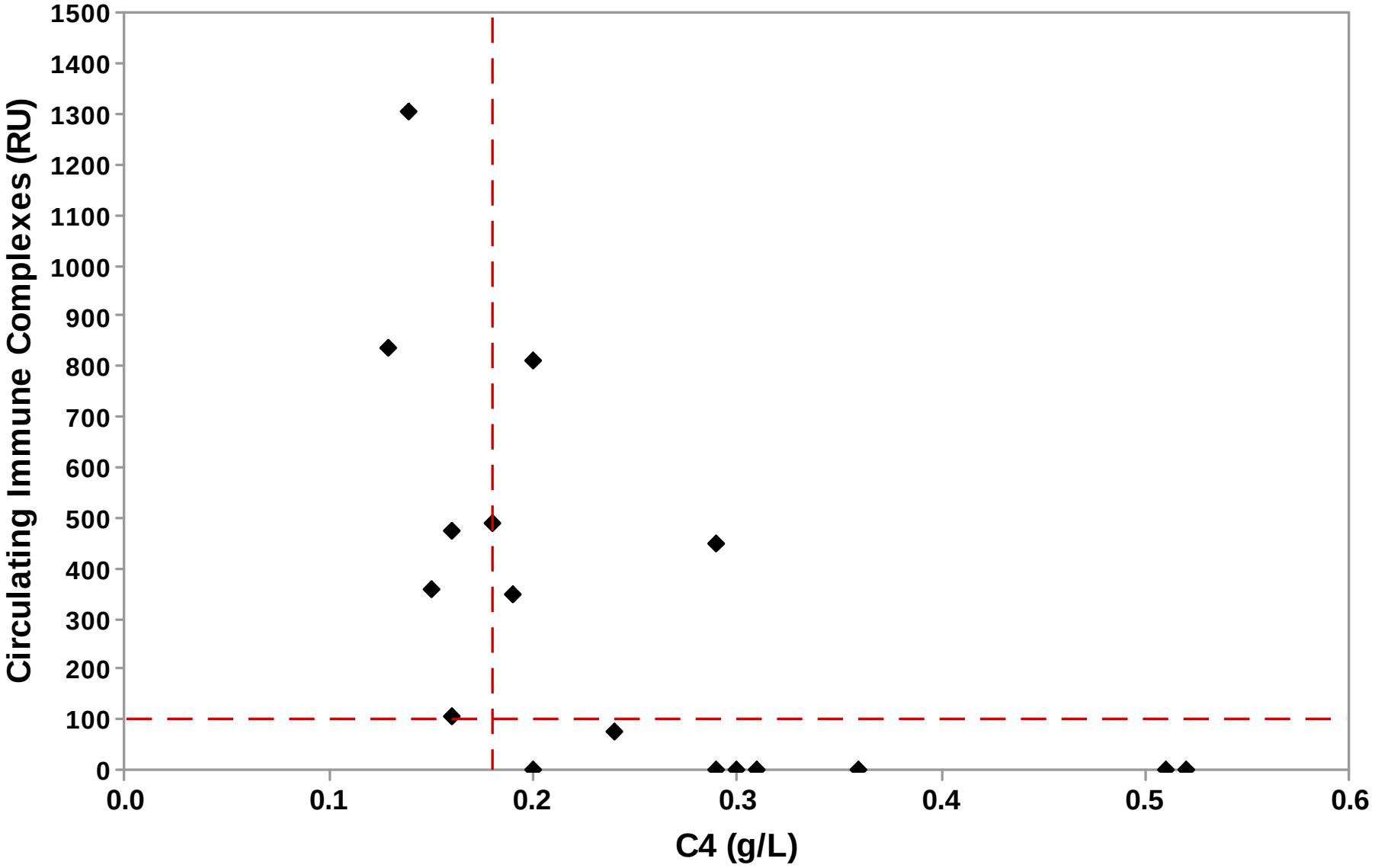


Supplemental Figure 2: Initial complement antigenic levels. A. C3. Normal in 76% of patients. **B**. C4. Normal in 71% of patients. **C**. Factor I. Normal in all patients.
The dashed lines represent the lower limit of the normal ranges.



Supplemental Figure 3: C4 level and FH/autoantibody circulating immune complexes

Abbreviations: RU, relative units



Supplemental Table 1: Autoantibody reactivity with short factor H fragments.

CFHR1 copy number and autoantibody binding to factor H fragments (SCRs 1-7, 8-15, 16-18 and 19-20) (positive threshold >100RU).

Abbreviations: SCR = Short Consensus Repeat.

Patient	*CFHR1* copy number	SCRs 1-7	SCRs 8-15	SCRs 16-18	SCRs 19-20
2	0	130	0	24	613
4	0	7	0	0	2171
5	0	235	3	57	144
6	2	3126	9	615	0
10	0	0	0	0	1570
12	0	41	450	0	377
14	0	33	60	0	2365
15	0	7	0	81	2245
16	0	0	0	0	239
17	0	1	3	0	2370
18	0	42	0	0	981
19	2	5	0	0	410
20	0	46	0	0	219
21	0	496	76	0	2714
22	2	327	0	13	1
23	0	0	0	0	1050
24	0	0	0	104	3243

Supplemental Table 2: Initial titres of factor H autoantibody (aFH), circulating immune complexes (CiC), and autoantibody reactivity with factor H–related proteins 1-5 (positive threshold >100RU)

Abbreviations: aFH, factor H autoantibody; CiC, circulating immune complexes; FHR, factor H related proteins; RU, relative units.

Patient	aFH	CiC	FHR1	FHR2	FHR3	FHR4	FHR5
2	630	357	694	74	0	0	0
4	1249	0	792	0	0	0	21
5	573	812	0	352	147	169	43
6	2017	0	0	0	0	0	0
10	3432	840	612	0	0	0	0
12	812	350	0	0	0	0	0
14	722	0	132	0	0	29	3
15	4000	450	1380	65	39	0	0
16	4000	0	1350	0	0	0	45
17	2194	75	600	0	0	0	41
18	2130	490	900	22	0	0	0
19	2319	0	500	0	0	0	0
20	1594	106	0	0	0	0	0
21	971	0	700	0	0	0	0
22	277	0	0	0	37	30	21
23	1350	474	279	0	359	468	341
24	3396	1307	2645	0	0	20	0

Supplemental Figure 4: Outcome according to dialysis requirement at presentation.

75% of patients who required dialysis within one week of presentation fully recovered renal function.

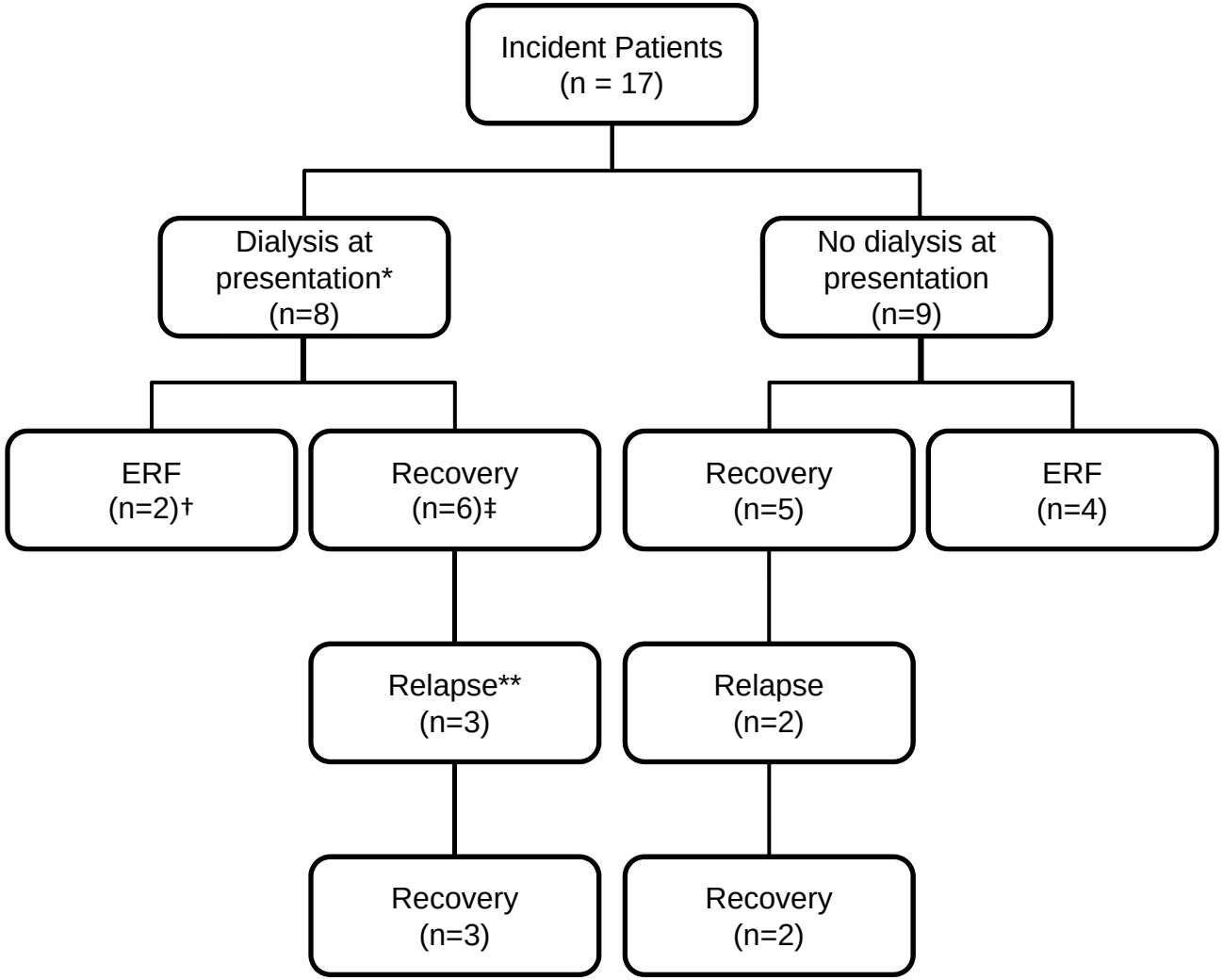
*Defined as dialysis within the first week of presentation

**Defined as recurrence >1 month after presentation and >15 days after disease remission

† Management: supportive:1, PEX:1

‡ Management: PEX:4, eculizumab: 2

Abbreviations: ERF, established renal failure; PEX, plasma exchange.



Supplemental Table 3: Treatment modality and resultant renal function

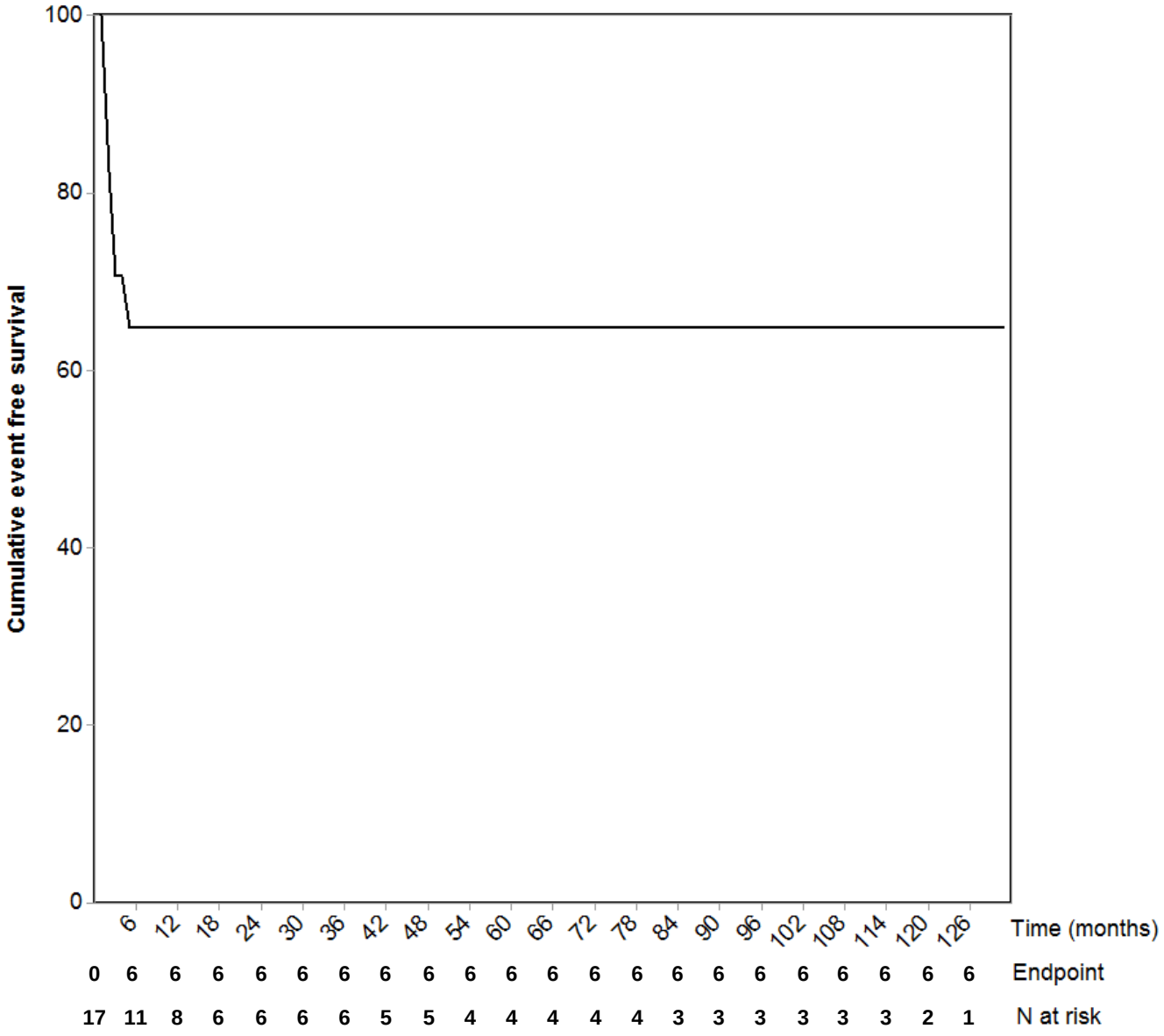
Patient	Treatment at first presentation	Dialysis (duration if recovery)	ERF	Relapse	Renal Transplant	Subsequent treatment	Duration of follow up (months)	eGFR (mL/min/1.73m^2) at most recent follow up
2	FFP, PEX	Yes (5 weeks)	No	Yes	n/a	PEX at relapse	163	>60†
4	FFP, PEX, IVIG	Yes	Yes	No	Yes aHUS recurrence	ECU post-transplant	75	47.6*
5	PEX	Yes	Yes	No	Yes Graft failure (AMR)		151	ERF
6	PEX	Yes	Yes	No	No. Ineligible		130	ERF
10	PEX, CS	No	No	Multiple	n/a	Regular PEX, withdrawn 2012	117	>60*
12	FFP, PEX, IVIG	Yes	Yes	No	Yes		98	>60*
14	FFP, ECU	No	No	No	n/a		7	>60*
15	ECU	Yes (5 days)	No	No	n/a		11	>60*
16	PEX	Yes (3 days)	No	Yes	n/a	ECU at relapse	81	>53*
17	PEX	No	No	No	n/a		108	>60*
18	FFP, PEX	No	No	Yes	n/a	PEX at relapse	123	>60*
19	SUP	Yes	Yes	No	Yes		64	48.4*
20	ECU	No	No	No	n/a		62	>60*
21	FFP, PEX	Yes (6 days)	No	Yes	n/a	PEX, RTX at relapse	9	>60†
22	SUP	Yes	Yes	No	Yes		84	>60*
23	PEX	Yes (5 days)	No	No	n/a		12	>60*
24	ECU	Yes (5 weeks)	No	No	n/a		22	52.3*

eGFR: by Schwartz formula for patients <18 years at last follow up (*) and by abbreviated MDRD equation for patients >18 at last follow up (†)

Abbreviations: AMR, antibody mediated rejection; CS, corticosteroids; ECU, eculizumab; eGFR: estimated glomerular filtration rate; ERF, established renal failure; FFP, fresh frozen plasm; IVIG, intravenous immunoglobulin; MMF, mycophenolate mofetil; n/a, not applicable; PEX, plasma exchange; RTX, rituximab; SUP, supportive management.

Supplemental Figure 5: Kaplan Meier survival curve showing probability of renal survival

35% of patients developed ERF, and in all cases this occurred at the first presentation of aHUS. There were no deaths.



Supplemental Table 4: Comparison with other cohorts

		Dragon-Durey *et al.*[22]	Sana *et al.*[28]	Sinha *et al.*[6]	Lee *et al.*[24]	Geerdink *et al.*[23]	Noris *et al.*[25]	Abarrategui-Garrido *et al.*[19]	Hofer *et al.*[15]	Moore *et al.*[9]	UK and Ireland
# Patients		45 (38 children)	4 children	138 children	15 children	6 children	8 (6 children)	7 children	25 children	13 children	17 children
Age (years)		Median 8.5 (children only)	Mean 6	Mean 8.4	Mean 8	1-7 years at onset in 83.3%	N/A	Mean 5.2	Mean 7.9	Median 8	Median 8
% Male		66% (children only)	N/A	72.5%	27%	50%	50%	N/A	N/A	46%	65%
Gastrointestinal symptoms		84% (diarrhoea 53%)	25%	Diarrhoea 9.4%	Diarrhoea 13%	83%	14.3%	N/A	87%	N/A	75% (diarrhoea 50%)
Extra-renal manifestations		50% hepatitis 23% pancreatitis 23.5% seizures	N/A	57.3% hepatic 40.6% seizures	7% hepatitis 7% pancreatitis 7% CNS	N/A	14.3% CNS	N/A	11% CNS 58% other	N/A	19% seizures 13% hepatitis 6% pancreatitis
Concomitant rare genetic variants		0/26	0	N/A	0/15	2/6 (33%): *CFI, C3*	2/8 (25%): *CFH* x2	0/7	1/8 (12.5%): *CFI*	5/13 (38%): *CFH, CFI, CD46, C3* x2	7/17 (41%): *CFH, CFI* x4, *CD46, C3*
Complement analysis	**Low C3**	58%	50%	62%	67%	N/A	43%	57%	41%	27%	24%
	Low C4	0	N/A	N/A	N/A	N/A	14%	14%	15%	27%	29%
	Low FH	22%	N/A	N/A	N/A	N/A	29%	0	N/A	9%	12%
Treatment and evolution of first episode											
Supportive		6 (13% of total): ERF 1 (17%); relapses 2 (33%); TR 1 (17%); CKD 1 (17%)	0	N/A	N/A	2 (33%)	N/A	N/A	N/A	10 (77%)	2 (12% of total): ERF 2 (100%)
PI		6 (13% of total): relapses 5 (83%), TR 1 (17%)	0	N/A	91%	N/A	N/A	N/A	N/A	N/A	n/a
PEX		15 (33% of total): ERF 1 (7%); death 1 (7%); CKD 3 (20%); TR 3 (20%); relapses 6 (40%)	0	105 (76%) in total	69%	N/A	N/A	N/A	N/A	3 (23%)	11 (65% of total): ERF 4 (36%); relapses 5 (45%)
PEX or PI		N/A	0	N/A	N/A	4/6 (67%) (chronic in 3)	7/8 (87.5%)	5/7 (71%)	18/19 (95%)	N/A	n/a
PEX + Corticosteroids		0	0	N/A	N/A	N/A	3/8 (37.5%): remission in 2/3 (67%)	1	N/A	N/A	1
PEX + Immunosuppression		3 (6.7% of total)	4 (100%)	87 (63%) IS	7 (54%) steroids +/- IS	0	3/8 (37.5%)	2 (29%)	0	0	0
	Azathioprine	0	0	N/A	N/A	n/a	0	1	n/a	n/a	n/a
	Steroids + cyclophosphamide	2: no renal sequelae	4 (at relapse in patient treated with rituximab)	49	N/A	n/a	0	0	n/a	n/a	n/a
	Steroids + rituximab	0	(rituximab initially in 1)	18	N/A	n/a	0	0	n/a	n/a	n/a
	Steroids + MMF	1: no renal sequelae	0	N/A	N/A	n/a	0	0	n/a	n/a	n/a
Eculizumab		0	0	0	0	0	0	0	0	0	4 (24% of total): 100% sustained remission
Maintenance Immunosuppression		N/A	n/a	47 (34%): steroids alone 21; plus azathioprine 8; plus MMF 18	N/A	n/a	N/A	N/A	n/a	n/a	0
Long term outcomes											
Sustained remission		N/A (25% no renal sequelae)	100% after cyclophosphamide	N/A	62% no sequelae	0	12% complete remission	N/A	N/A	N/A	n/a
Relapse		58%	25% (following rituximab in 1)	14 (11.5%); (4 whilst on IS)	31%	50%	37.5%	N/A	N/A	23%	29% overall
ERF		27%	0	32.8%	0	0	63%	43%	N/A	46%	35% overall
CKD (not including ERF)		39%	0	N/A	15%	N/A	N/A	N/A	N/A	N/A	CKD stage 3: 1 (6%)
Death		4 (8.9%)	0	20 (16.4%)	0	1 (16.7%)	0	1 (14.3%)	N/A	0	0
		Deaths: unknown; sudden death post dialysis; pulmonary hypertension; cardiac insufficiency	n/a	Deaths: complications of renal failure 16; sepsis 4	n/a	N/A	n/a	Death: myocarditis	N/A	n/a	n/a
Outcome of transplantation											
Recurrence		3 without specific management	n/a	0	n/a	0	1	0	N/A	0	1 early recurrence (*CFI* mutation) treated with Eculizumab
No recurrence		3 with specific management	n/a	3 with specific management (PEX/IVIG/rituximab)	n/a	0	0	2	N/A	3	4 without specific management (1 graft loss rejection)

Abbreviations: CKD, chronic kidney disease; ERF, established renal failure; FH, factor H; IS, immunosuppression; IVIG, intravenous immunoglobulin; N/A, not available; n/a, not applicable; PEX, plasma exchange; PI, plasma infusion; TR, treatment resistance.