

Systematic Review of Atypical Hemolytic Uremic Syndrome Biomarkers



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Introduction

- Atypical hemolytic uremic syndrome (aHUS) is a chronic and life-threatening disease arising from genetic and acquired abnormalities which result in uncontrolled activation of the complement system’s alternative pathway (AP).
- Diagnosing aHUS is challenging due to its poor penetrance and ambiguous clinical presentations similar to various forms of thrombotic microangiopathy (TMAs) and other causes of HUS.
- Genetic variants in complement regulatory proteins account for 50-60% of all patients with aHUS, with approximately 30-50% not having an identifiable mutation
- A gold standard test for the diagnosis of aHUS has yet to be found as there is large variability among affected patients which makes the interpretation of the causality of the identified variants complex.

Methodology

- **Design:** This is a systematic review of aHUS biomarkers directly or indirectly related to dysregulation of the AP pathway, coagulation, and renal injury.
- **Study Population:** All individuals diagnosed with aHUS who had a biomarker serum levels measured pre-intervention.
- **Study Variables:** Demographic information (age, gender), sample size, and study design as well as prospective biomarkers including: C3, C4, C4d, C5a, C5b-9, factors B, I or H, CH50, AH50, clusterin, cystatin-C, D-dimer, tumor necrosis factor receptor, β2-microglobulin, L-fatty acid binding protein, and VCAM-1.
- **Data Collection:** Patient populations were pooled with respect to the biomarker(s) they were tested for. The results are reported descriptively using mean (SD), median (IQR), range (min - max) or proportions for the different biomarkers sought then compared to the reference ranges observed in the healthy subjects.

References

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Objectives

- Establish a set of predefined biomarkers to quickly identify and/or confirm complement contribution in the TMA spectrum to document treatment efficacy.

Results

- The data for the 13 biomarkers are reported in Table 1 (N=855, 57 studies).
- Biomarker means/averages:
 - **Lower** among aHUS subjects as compared to the reference range (RR):
 - C3 [73.8 vs. RR: 75 - 175 mg/dL]
 - CH50 [28.3, vs. RR 30-75 U/ml]
 - AH50 [27.6%, vs. RR ≥46%];
 - FB [13.5 (6.8), vs. RR 15.2-42.3 mg/dL]
 - **Within** the RR among aHUS subjects:
 - C4 [21.0 vs. RR: 14 - 40 mg/dL]
 - C4d [4.2 vs. RR ≤9.8 ug/ml]
 - FH [38.9 vs. RR 23.6 - 43.1 mg/dL]
 - FI [96.8% vs. RR 70 - 130%]
 - **Higher** among aHUS subjects as compared to the RR:
 - C5a [62.1 vs. RR 10.6-26.3 mg/dL]
 - C5b-9 [426.6 vs. RR ≤250 ng/ml];
 - Bb [2.5 vs. RR ≤1.6 ug/ml] and
 - D-dimer [368.3 vs. RR <2.2 ng/ml]

Discussion

Biomarkers of Complement AP Dysregulation

- The AP system from C3 activation to downstream MAC formation, is controlled by different regulatory proteins with multiple points of regulation.
- Mutational variants in C3, C5a, C5b-9, Bb, and CFB all contribute to increased activation of the AP and consequently low C3 levels by cleavage and “consumption” of key regulatory proteins

Discussion

Renal injury biomarkers

- Kidneys are vulnerable to vascular endothelial injury, induction of the coagulation pathway, thrombus formation, and obstruction.⁴⁹⁻⁵³ However, relatively little to no clinical data was observed the following biomarkers during the acute episode, remission phase, or any follow-up:
 - Tumor necrosis factor receptor 1(TNFR1), clusterin, cystatin C, β2-microglobulin, liver-type fatty-acid-binding protein (L-FABP1), and vascular cell adhesion molecule-1 (VCAM1).

Mutational Markers

- Pathogenetic variants in one or more associated genes can also be used to identify aHUS
 - C3, CD46 (MCP), CFB, CFH, CFHR1, CFHR3, CFHR4, CFI, DGKE, and THBD
- Reported autoantibodies found in aHUS typically target proteins encoded by complement genes such as CFH and CFI.⁶⁵
 - Homozygous deletions in CFHR1, and CFHR3 have been associated with anti-FH antibodies positivity in 90% of cases.^{12,30,41}
- A multigene panel test is typically considered in individuals over the age of one who may have aHUS and typically tests for C3, CD46, CFB, CFH, CFI, DGKE, and THBD.^{64,65}
- Patients with autoantibodies may also be screened for CFH/CFHR1 and CFHR1/CFH hybrid alleles and deletions of CHFR1/CHFR4 and CHFR3/CHFR1.^{64,65}

Conclusion

- If a comprehensive complement profile were built using our data, aHUS would be identified by low levels of C3, CH50, AH50, and CFB along with increased levels of C5a, C5b-9, Bb, anti-CFH autoantibodies, and D-Dimer.

Biomarker	Unit	aHUS subjects					Reference range^
		Sample size	No. of studies	Mean (SD)	Median (IQR)	Range (Min - Max)	
C3*	mg/dl	774	40	73.75 (33.49)	70 (50.68 - 96)	13 - 221.3	75 - 175
C4\$	mg/dl	366	33	21.04 (9.7)	21 (14 - 28)	2 - 45	14 - 40
C4d\$	µg/ml	108	5	4.15 (3.86)	3.05 (1.9 - 5.43)	1.18 - 15.99	≤9.8
C5a+	mg/dl	117	6	62.11 (31.05)	51.3 (46.25 - 72.84)	19.9 - 148.7	10.6 - 26.3
C5b-9	ng/ml	200	17	426.6 (373.34)	297 (176 - 511.5)	24.4 - 1,840	≤250
CH50*	U/ml	63	9	28.25 (32.09)	24.25 (3.5 - 53.25)	3 - 154	30-75
AH50*	%	23	2	27.61% (30.24%)	10% (10% - 38.5%)	10% - 93%	≥46%
Bb+	µg/ml	83	6	2.52 (2.06)	1.9 (1.11 - 3)	0.26 - 7.39	≤1.6
CFB*	mg/dl	40	7	13.49 (6.79)	11.95 (7.88 - 17.55)	5 - 29.2	15.2 - 42.3
CFH\$	mg/dl	180	13	38.94 (83.81)	35.7 (24.15 - 52.75)	7.28 - 467	23.6 - 43.1
CFI\$	NA	28	7	6.71 (2.59)	6.4 (5.43 - 7.34)	3.7 - 18.1	NA
	%	12	4	96.83% (24.73%)	93.5% (85.75% - 106.75%)	62% - 145%	70 - 130%
D-Dimer+	ng/ml	5	4	368.32 (131.52)	349.6 (292 - 500)	200 - 500	<2.2

Table 1: Descriptive analysis of different biomarkers among aHUS subjects across included studies and reference range of normal healthy subjects. (*Lower among aHUS subjects as compared with the reference range; +Higher among aHUS subjects as compared with the reference range; \$Within reference range among aHUS subjects)