Supplemental Information

Manuscript Title: The predictive value of PNH clones, 6p CN-LOH, and clonal TCR gene rearrangement for aplastic anemia diagnosis

Running Head: Improved diagnostic algorithm for AA

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Data Sharing Statement

Detailed data tables with aggregated frequencies of clinical findings denoted by diagnostic group are available in the Supplemental Data.

Supplemental Tables

Supplemental Table S1. Demographics of 454 consecutively enrolled patients in the BMF registry

Diagnostic Group		Patients	Sex		Race					Ethnicity			6p CN-LOH Median Age at Testing, Years (Range)	PNH Median Age at Testing, Years (Range)	TCR Median Age at Testing, Years (Range)
		n=454	Male n (%)	Female n (%)	African American n (%)	Asian n (%)	White n(%)	Interracial n (%)	Unknown n (%)	Hispanic n (%)	Not Hispanic n (%)	Unknown n (%)			
Aplastic Anemia		170	91 (54%)	79 (46%)	18 (11%)	9 (5%)	132 (78%)	6 (4%)	5 (3%)	4 (2%)	161 (95%)	5 (3%)	15 (0.5-80)	20 (1-81)	19.5 (2-80)
PNH Disease		42	16 (38%)	26 (62%)	10 (24%)	1 (2%)	29 (69%)	1 (2%)	1 (2%)	2 (5%)	39 (93%)	1 (2%)	27 (14-80)	28 (14-80)	28 (15-68)
Classical IBMFS	**SDS**	11	4 (36%)	7 (64%)	0 (0%)	0 (0%)	11 (100%)	0 (0%)	0 (0%)	2 (18%)	9 (82%)	0 (0%)	8.5 (2-50)	N/A	12 (7-50)
	DC	34	26 (76%)	8 (24%)	1 (3%)	1 (3%)	31 (91%)	0 (0%)	1 (3%)	2 (6%)	31 (91%)	1 (3%)	15 (1-60)	14 (13-62)	22 (3-51)
	DBA	35	20 (57%)	15 (43%)	6 (17%)	1 (3%)	28 (80%)	0 (0%)	0 (0%)	2 (6%)	33 (94%)	0 (0%)	9.5 (0.5-36)	15 (N/A)	19.5 (1-35)
	FA	19	11 (58%)	8 (42%)	4 (21%)	1 (5%)	14 (74%)	0 (0%)	0 (0%)	2 (11%)	17 (89%)	0 (0%)	7.5 (4-37)	25 (20-30)	13.5 (4-35)
	SCN	10	3 (30%)	7 (70%)	1 (10%)	0 (0%)	9 (90%)	0 (0%)	0 (0%)	2 (20%)	8 (80%)	0 (0%)	9.5 (0.5-23)	N/A	22 (N/A)
Other Inherited		38	21 (55%)	17 (45%)	5 (13%)	3 (8%)	28 (74%)	2 (5%)	0 (0%)	10 (26%)	28 (74%)	0 (0%)	6 (0.5-38)	18 (14-22)	6 (0.5-18)
BMF NOS		14	8 (57%)	6 (43%)	1 (7%)	0 (0%)	12 (86%)	0 (0%)	1 (7%)	0 (0%)	13 (93%)	1 (7%)	7 (0.5-44)	N/A	5 (0.5-10)
Neutropenia NOS		42	21 (50%)	21 (50%)	5 (12%)	1 (2%)	33 (79%)	1 (2%)	2 (5%)	3 (7%)	37 (88%)	2 (5%)	7 (0.5-47)	35 (N/A)	24 (17-47)
MDS		14	3 (21%)	11 (79%)	1 (7%)	2 (14%)	11 (79%)	0 (0%)	0 (0%)	0 (0%)	14 (100%)	0 (0%)	17 (6-52)	22 (14-52)	18 (11-52)
Other		25	15 (60%)	10 (40%)	3 (12%)	2 (8%)	20 (80%)	0 (0%)	0 (0%)	0 (0%)	25 (100%)	0 (0%)	9 (1-58)	18 (4-58)	18 (5-58)

Supplemental Table S2. Genetic diagnoses for classical IBMFS patients

		Total Count n= 109	Mutated Gene (Affected Patients)
Classical IBMFS	**SDS**	11	*SBDS* (11)
	DC	34	*DKC1* (6)
			TERC (7)
			TERT (6)
			RTEL1 (4)
			Unknown* (11)
	DBA	35	*RPS19* (8)
			RPL11 (4)
			RPL5 (4)
			RPS26 (2)
			RPL26 (1)
			RPL35a (1)
			RPS17 (1)
			RPS24 (1)
			Unknown* (13)
	FA	19	*FANCA* (9)
			FANCC (2)
			BRCA2 (3)
			Unknown* (5)
	SCN	10	*ELANE* (8)
			Unknown* (2)

*Patients without a confirmed genetic diagnosis were diagnosed clinically using syndrome-specific functional tests and established diagnostic criteria[1].

Supplemental Table S3. Prevalence and size of PNH granulocyte clones by diagnostic group

Diagnostic Group		PNH Granulocyte Clone Size[a]					
		Total n (%)	**Subclinical n (%)**	**Small n (%)**	**Moderate n (%)**	**Large n (%)**	**Unknown n (%)**
Aplastic Anemia		58 (46%)	24 (41%)	18 (31%)	7 (12%)	N/A[b]	9 (16%)
PNH Disease		42 (100%)	0 (0%)	1 (2%)[c]	0 (0%)	29 (69%)	12 (29%)
Classical IBMFS	**SDS**	N/A	N/A	N/A	N/A	N/A	N/A
	DC	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	DBA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	FA	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	SCN	N/A	N/A	N/A	N/A	N/A	N/A
Other Inherited		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Neutropenia NOS		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
MDS		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
BMF NOS		N/A	N/A	N/A	N/A	N/A	N/A
Other		0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

a. Subclinical <1%, Small 1-9.99%, Moderate 10-30%, Large >30%; b. AA patients with Large clones were classified under the PNH Disease diagnostic group; c. One PNH patient has undergone spontaneous remission, as previously described[2].

Supplemental Table S4. Breakpoint coordinates of acquired 6p CN-LOH in 16 AA patients

Patient ID	6p CN-LOH Region	Clone Size	HLA Class I Genes Included in 6p CN-LOH Region	Age at Array, years
54.01	6pterp21.31	10%	A, B, C	21
	6pterp21.1	10%	A, B, C	
56.01	6pterp21.31	N/A	A, B, C	14
	6pterp21.1	N/A	A, B, C	
	6pterp12.1	N/A	A, B, C	
	6pterp11.1	N/A	A, B, C	
281.01	6pterp21.33	50%	A, B, C	9
	6pterp21.31	10%	A, B, C	
284.01	6pterp21.32	35%	A, B, C	48
	6pterp11.1	15%	A, B, C	
348.01	6pterp21.31	N/A	A, B, C	34
390.01	6 WC	20%	A, B, C	62
471.01	6pterp21.32	30%	A, B, C	16
	6pterp21.31	45%	A, B, C	
	6pterp21.2	15%	A, B, C	
	6pterp12.1	10%	A, B, C	
505.01	6pterp21.31	5%	A, B, C	8
	6pterp12.1	5%	A, B, C	
564.01	6pterp12.1	<5%	A, B, C	12
575.01	6pterp21.31	10%	A, B, C	19
597.01	6pterp21.31	10%	A, B, C	15
	6pterp21.31	10%	A, B, C	
602.01	6pterp21.32	N/A	A, B, C	63
626.01	6pterp21.1	15%	A, B, C	54
	6pterp12.1	15%	A, B, C	
658.01	6pterp21.2	10%	A, B, C	3
672.01	6pterp21.31	10%	A, B, C	17
675.01	6pterp12.1	20%	A, B, C	80
	6 WC	10%	A, B, C	

Supplemental Table S5. Prevalence of cytogenetic abnormalities by diagnostic group

		Aplastic Anemia	PNH Disease	Classical IBMFS					Other Inherited	Neutropenia NOS	MDS	BMF NOS	Other
				SDS	DC	DBA	FA	SCN					
No Acquired Cytogenetic Abnormalities[a]		149 (98%)	23 (92%)	8 (80%)	27 (87.1%)	24 (100%)	15 (88.2%)	8 (100%)	23 (74.2%)	32 (100%)	4 (40%)	11 (84.6%)	12 (63.2%)
Acquired Cytogenetic Abnormalities Present		3 (1.9%)	2 (8%)	2 (20%)	4 (12.9%)	0 (0%)	2 (11.8%)	0 (0%)	8 (25.8%)	0 (0%)	6 (60%)	2 (15.4%)	7 (36.8%)
Acquired Abnormality	-7/ del(7q)	0	0	1	0	0	0	0	3	0	3	0	1
	i(7q)	0	0	0	1	0	0	0	0	0	0	0	0
	+8	1	1	0	0	0	0	0	0	0	1	1	0
	del(13q)	1	0	0	0	0	0	0	0	0	0	0	0
	-17/ del(17p)	0	0	0	1	0	0	0	0	0	0	0	0
	del(20q)	0	0	0	0	0	0	0	1	0	2	0	0
	Other Single Or Double Abnormalities	1	1	0	1	0	2	0	4	0	0	1	4
	Complex	0	0	1	1	0	0	0	0	0	0	0	2
Cytogenetic Data N/A		18[b]	17	1	3	11	2	2	7	10	4	1	6
Total Cohort		**170**	**42**	**11**	**34**	**35**	**19**	**10**	**38**	**42**	**14**	**14**	**25**

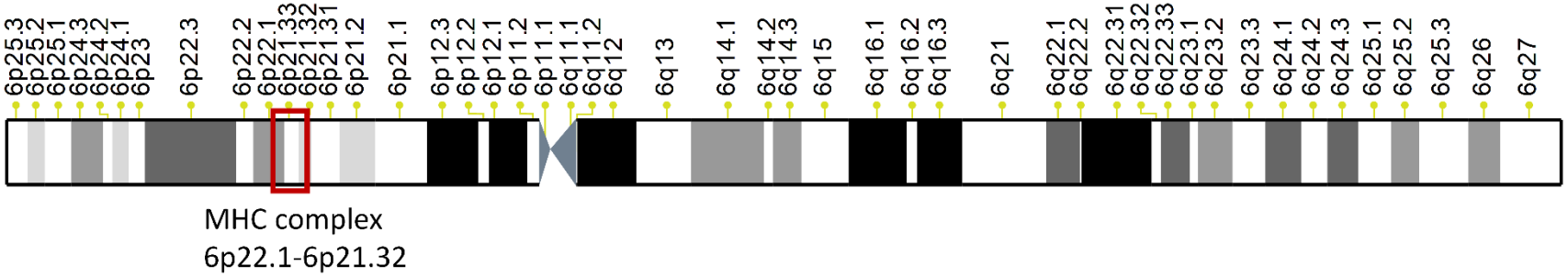
a. Constitutional cytogenetic abnormalities were identified in 1 AA, 3 DC, and 1 DBA patients; b. 5 of 18 AA patients without available cytogenetic data had SNP-A or MDS FISH panel results that indicated no abnormalities.

Supplemental Table S6. Associations between PNH clones, 6p CN-LOH, and clonal *TRG* rearrangement and clinical characteristics of AA patients

	PNH^{gran} Positive vs. Negative	**Acquired 6p CN-LOH^{MHC} Positive vs. Negative**	**Clonal *TRG* Rearrangement Positive vs. Negative**
Age at diagnosis (pediatric vs. adult*)	Pediatric-onset AA: 63.7% (35 of 55 PNH^{gran}-positive) vs. 66.7% (40 of 60 PNH^{gran}-negative); OR 0.88; p=0.845	Pediatric-onset AA: 62.5% (10 of 16 6p CN-LOH^{MHC}-positive) vs. 73.2% (79 of 108 6p CN-LOH^{MHC}-negative); OR 0.61; p=0.384	**Pediatric-onset AA: 28.6% (4 of 14 patients with clonal *TRG* rearrangement) vs. 67.2% (41 of 61 patients with negative *TRG* clonality testing); OR 0.19; p=0.0137**
Age at Testing (pediatric vs. adult*)	Pediatric age at testing: 58.2% (32 of 55 PNH^{gran}-positive) vs. 61.7% (37 of 60 PNH^{gran}-negative); OR 0.86; p=0.708	Pediatric age at testing: 56.3% (9 of 16 6p CN-LOH^{MHC}-positive) vs. 75.0% (81 of 108 6p CN-LOH^{MHC}-negative); OR 0.43; p=0.137	**Pediatric age at testing: 28.6% (4 of 14 patients with clonal *TRG* rearrangement) vs. 62.3% (38 of 61 patients with negative *TRG* clonality testing); OR 0.24; p=0.0350**
Disease duration at testing (<1 year vs. >1 year)	AA duration <1yr: 38.5% (20 of 52 PNH^{gran}-positive) vs. 40.7% (24 of 59 PNH^{gran}-negative); OR 0.91; p=0.848	AA duration <1yr: 43.8% (7 of 16 6p CN-LOH^{MHC}-positive) vs. 53.7% (51 of 95 6p CN-LOH^{MHC}-negative); OR 0.67; p=0.591	AA duration <1yr: 38.5% (10 of 14 patients with clonal *TRG* rearrangement) vs. 73.3% (44 of 60 patients with negative *TRG* clonality testing); OR 0.91; p=1.000
AA severity	NSAA n=18, SAA n=25, VSAA n=8 in PNH^{gran}-positive vs. NSAA n=20, SAA n=22, VSAA n=17 in PNH^{gran}-negative; p=0.229	NSAA n=4, SAA n=9, VSAA n=2 in 6p CN-LOH^{MHC}-positive vs. NSAA n=36, SAA n=42, VSAA n=25 in 6p CN-LOH^{MHC}-negative; p=0.429	NSAA n=4, SAA n=8, VSAA n=1 in patients with clonal *TRG* rearrangement vs. NSAA n=11, SAA n=29, VSAA n=19 in patients with negative *TRG* clonality testing; p=0.166
BM cellularity at AA diagnosis	**BM cellularity ≤ 5%: 27.8% (15 of 54 PNH^{gran}-positive) vs. 51.7% (31 of 60 PNH^{gran}-negative); OR 0.360; p=0.0128**	BM cellularity ≤ 5%: 50.0% (8 of 16 of 6p CN-LOH^{MHC}-positive) vs. 38.7% (41 of 106 of 6p CN-LOH^{MHC}-negative); OR 1.59; p=0.429	BM cellularity ≤ 5%: 50.0% (4 of 8 patients with clonal *TRG* rearrangement) vs. 38.7% (23 of 59 patients with negative *TRG* clonality testing); OR 1.57; p=0.705

*Pediatric age was defined according to the American Academy of Pediatrics as 21 years or younger.

Supplemental Figures



Supplemental Figure S1. Chromosome 6 ideogram showing the location of the MHC region
HLA genes are located within the MHC region of the short arm of chromosome 6. HLA-A is located at 6p22.1 while HLA-B and HLA-C are located at 6p22.33.

Supplemental References

1. Wilson DB, Link DC, Mason PJ, Bessler M. Inherited bone marrow failure syndromes in adolescents and young adults. *Ann Med*. 2014;46(6):353-363.
2. Babushok DV, Stanley N, Xie HM, et al. Clonal Replacement Underlies Spontaneous Remission in Paroxysmal Nocturnal Haemoglobinuria. *Br J Haematol*. 2017;176(3):487-490.