



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 508.BONE MARROW FAILURE: ACQUIRED

**Distinct Immune and Molecular Profiles of Hepatitis-Associated Aplastic Anemia**

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**Background:** Hepatitis-associated aplastic anemia (HAAA) is characterized by the development of hematopoietic failure within months following an acute episode of non-infectious hepatitis. Although disease presentation with pancytopenia and a hypocellular marrow in HAAA is similar to non-hepatitis immune AA (non-HAAA), the immune and molecular profile in HAAA has not been completely elucidated. Here, we aimed to assess HAAA patients for cytokines and presence of clonal events linked to AA, the presence of paroxysmal nocturnal hemoglobinuria (PNH) clones and somatic mutations in peripheral blood mononuclear cells (PBMC) subpopulations, and compared to a non-HAAA cohort.

**Methods:** A cohort of HAAA patients (n=30), age and sex-matched non-HAAA patients (n=24) and healthy controls (n=23) seen at the National Institutes of Health since 2003 were retrospectively screened for a panel of inflammatory cytokines and growth factors by Luminex, and somatic mutations in sorted CD3<sup>-</sup>, CD3<sup>+</sup>CD4<sup>+</sup>, and CD3<sup>+</sup>CD8<sup>+</sup> PBMC fractions by error-corrected DNA sequencing. A customized panel with 42 myeloid-related and 49 autoimmunity-related genes at minimum limited of detection of 0.1% were used. All samples were before any immunosuppressive treatment (IST). Cytokine levels and frequencies of PNH clones and clonal hematopoiesis (CH) in different fractions were compared among groups.

**Results:** HAAA patients were in average young (median age=23); 37% were at age < 18 (Table 1). Although disease severity and blood counts were similar to the non-HAAA cohort, immune and molecular markers of immune AA were distinct among cohorts. The frequency of PNH clones was significantly lower in HAAA compared to the non-HAAA cohort (15% v 58%, p=0.0008). The median size of PNH clones in HAAA and non-HAAA was 1.4% and 7.4%, respectively (p=0.03). The cytokine profiles performed in the sera of 22 HAAA before IST were marked by significantly higher levels of IL-1ra (p=0.0008), CXCL-10 (p=0.0038), and VEGF (p=0.0023) in comparison to 31 non-HAAA assessed patients; IL-6 (p=0.0002) and CD40L (p=0.0001) levels were significantly lower than in non-HAAA. As reported in immune AA, HAAA patients had higher levels of TPO (p=0.0001), G-CSF (p=0.0001), and EPO (p=0.0001) than controls; lower levels of CCL-5 (p=0.0001) and CCL-11 (p=0.0025) were seen in the HAAA. FLT3-L levels were markedly elevated in both AA cohorts when compared to HC.

The clonal landscape was also strikingly different between HAAA and non-HAAA in all fractions evaluated (Figure 1). In HAAA, 3 out of 21 assessed patients (14%) had CH in the CD3<sup>-</sup> fraction at maximum variant allele frequency (VAF) of 1.8%; one patient had two *RUNX1* mutations while a *JAK2* and *DNMT3A* mutation were found in the other two patients. No mutations were found in CD4<sup>+</sup> and CD8<sup>+</sup> compartments in subjects with HAAA. In contrast, 13 of 24 non-HAAA patients (54%) had CH in the CD3<sup>-</sup> populations, which was dominated by *PIGA*, *BCOR*, and *DNMT3A* clones, a pattern consistent with the known clonal landscape of immune AA. The frequency of non-HAAA with CH in the CD4<sup>+</sup> and CD8<sup>+</sup> compartments was 7/24 (29%) and 8/24 (30%) patients, respectively; CH in these fractions was similar to those found in CD3<sup>-</sup> dominated by mutations in *PIGA*, *BCOR*, and *DNMT3A* at VAFs ranging from 0.2% to 15% (Figure 1). CH in lymphoid-related genes was only found in a single patient with a *STAT3* p.S614R mutation at VAF of 3% exclusive to the CD8<sup>+</sup> fraction.

**Conclusion:** HAAA has a distinct immunologic and molecular profile than non-HAAA patients. PNH clones were less frequent in HAAA and patterns of cytokines were significantly different than the non-HAAA cohort. CH in *PIGA* and *BCOR*, highly associated with AA, were not present in our small HAAA cohort. Our results indicate a distinct underlying pathophysiology that initiates the immune destruction resulting in marrow failure in HAAA patients. Confirming these findings in a larger cohort and correlating with clinical outcomes is ongoing.

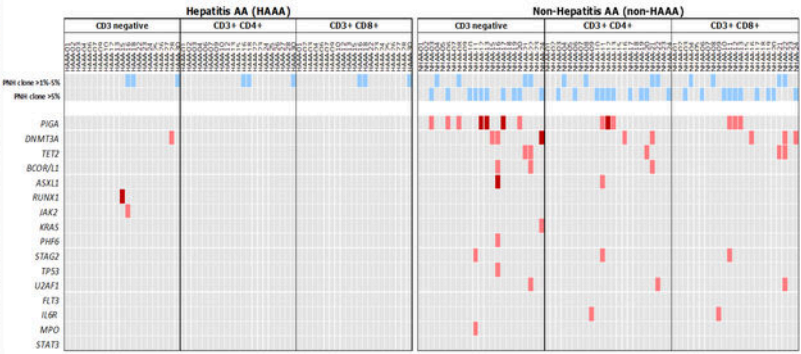
**Disclosures** No relevant conflicts of interest to declare.

**Table 1: Demographics and Clinical Characteristics of Hepatitis-associated aplastic anemia (HAAA) and non-hepatitis-associated aplastic anemia (non-HAAA) cohorts.**

Number of patients (n, %)	HAAA (N=36)	Non-HAAA (N=24)	Healthy Control (N=23)	P value
<b>Gender</b>				0.7
Male	20 (57%)	16 (67%)	13 (57%)	
Female	16 (43%)	8 (33%)	10 (43%)	
<b>Age, years (median, range)</b>				0.92**
<18	11 (33%)	10 (42%)	0	
18-59	16 (50%)	11 (46%)	13 (57%)	
>60	9 (25%)	3 (12%)	10 (43%)	
<b>Race</b>				0.39
White	20 (57%)	12 (50%)	11 (48%)	
Black or African American	5 (17%)	8 (33%)	8 (35%)	
Asian	1 (3%)	1 (4%)	3 (13%)	
Multiple Race/Unknown	4 (13%)	3 (13%)	1 (4%)	
<b>Severity of Disease</b>				
SAA	13 (43%)	13 (54%)	-	
VSAA	16 (50%)	11 (46%)	-	
MMA	1 (3%)	0	-	
<b>GPI negative neutrophil by flow (&gt;1%)</b>	3/27 (15%)	14/24 (58%)	-	<b>0.0008</b>
<b>GPI negative neutrophil clone size (VAF, %)</b>	1.4%	7.4%	-	<b>0.53</b>
<b>Pre-treatment blood counts (median, range)</b>				
ANC (KUL)	0.19 (0.0-1.2)	0.26 (0.0-0.7)	-	0.82
PLT (KUL)	10.5 (1.4-7)	8.1 (2-22)	-	0.11
ARC (KUL)	14.6 (2.4-104)	17.3 (1.6-60.4)	-	0.47
<b>Somatic Mutations*</b>				
CD3+ (n of mutations)	4	13	3	<b>0.006</b>
CD4+ (n of mutations)	0	7	2	<b>0.95</b>
CD8+ (n of mutations)	0	8	4	<b>0.84</b>

\*Twenty-one HAAA patients for somatic mutation analysis. \*\*Comparison of HAAA and non-HAAA only. Abbreviations: SAA, severe aplastic anemia; VSAA, very severe aplastic anemia; MMA, moderate aplastic anemia; GPI, glycosylphosphatidylinositol; VAF, variant allele frequency; ANC, absolute neutrophil count; PLT, platelet count; ARC, absolute reticulocyte count; IST, immunosuppressive therapy.

**Figure 1: Clonal landscape of HAAA versus non-hepatitis AA. Oncoprint of 45 patients with available ECS. Abbreviations: HAAA, hepatitis-associated aplastic anemia; ECS, error-corrected sequencing.**



**Figure 1**

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