The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

Telomere Length Testing of Lymphocytes Alone By Flow-FISH Is a Highly Sensitive and Specific Test to Screen for a Telomere Biology Disorder in a Cohort of Children and Very Young Adults with Bone Marrow Failure

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Background Uncovering an underlying telomere biology disorder (TBD) in patients with bone marrow failure (BMF) has crucial implications for treatment and outcomes, including hematopoietic cell transplantation and malignancy risk. Telomere length (TL) testing of peripheral blood leukocytes by clinical Flow-FISH has become an important test in diagnosing TBDs. In seminal studies, Alter et al. compared age-adjusted TL lengths (< or $\geq 1^{st}$ percentile) in lymphocytes (lymphs), granulocytes (grans), and four lymph subsets in individuals with a known TBD and their unaffected relatives. They found that very low (VL) TL (<1st percentile) in lymphs had a sensitivity (Sens) of 97%, specificity (Spec) of 91%, and positive predictive value (PPV) of 85% for a TBD diagnosis. VL TL in 3 or 4 lymph subsets had slightly better test performance with a Sens of 98%, Spec of 94%, and PPV of 89%. We hypothesized that test performance characteristics of flow FISH would be different if applied to a 'real-world' cohort, which could inform the use of TL in lymphs alone versus in the four lymph subsets for different clinical indications.

Methods We performed a search of the electronic medical record to identify all individuals who had Flow-FISH performed at Texas Children's Hospital from 2007 - 2022 (Figure 1). We excluded those who had the 2-panel test (lymphs and grans) or family TL cascade testing. We collected the indication for and results of Flow-FISH, demographic and clinical characteristics, and genetic testing information via chart review. A composite score (Comp Score) was created to define TBD (Comp Score of 1 or 2) vs. Not TBD (Comp Score <1). Performance of lymph TL alone and TL in 3 or 4 lymph subsets in predicting TBD was evaluated using Sens, Spec, PPV, NPV, area under ROC curve (AUC), and Cohen's Kappa statistic (k).

Results Our final cohort included 433 individuals who received the 6-panel test (lymphs, grans, and four lymph subsets) with a median age of 9.7 years, range 0.06-22.6. Top indications for Flow-FISH were severe aplastic anemia (SAA) (n=106), mild aplastic anemia (AA) (i.e., not fulfilling criteria for MAA or SAA) (103), cytopenias without known or documented bone marrow hypoplasia or dysplasia (70), moderate aplastic anemia (MAA) (37), non-therapy-related myelodysplastic syndrome (26), and an incidental TBD gene variant identified through comprehensive sequencing (17). Twenty-two subjects (5.1%) had a TBD, 17 (3.9%) of which had a pathogenic/likely pathogenic variant (P/LPV) in a known TBD gene (Figure 1). Seven of those with SAA (6.6%) had VL TL in lymphs, 5 (4.7%) of which also had VL TL in 3 or 4 of the lymph subsets, and only 1 (0.9%) had a TBD based on Comp Score. Importantly, none had VL TL in 3 or 4 of the lymph subsets without VL TL in lymphs. Five of those with MAA (13.5%) had VL TL in lymphs, all of which had VL TL in 3 or 4 lymph subsets. Three had a TBD based on Comp Score, and each had a PV in a TBD gene; a TBD variant was not identified in two despite exome sequencing and chromosomal microarray testing. Eighteen of those with mild AA (17.5%) had VL TL in lymphs, 15 (14.6%) also had VL TL in 3 or 4 lymph subsets, 9 (8.7%) had a TBD based on Comp Score, and 7 (6.8%) had a P/LPV in a TBD gene.

The Sens of TL in lymphs < vs. $\geq 1^{st}$ percentile and TL < 1st percentile in 3 or 4 vs. < 3 lymph subsets against TBD based on Comp Score in those with AA regardless of severity was 100%, and the Spec ranged from 90.4% (lymph TL testing alone in mild AA) to 96.2% (lymph subsets testing in SAA) (Table 1). The PPV for both test approaches was low ($\leq$20%) in SAA due to the low prevalence of TBD in this population. PPV was between 50-60% in the other AA groups. The AUC for detecting a TBD in the various AA groups was comparably high for both test approaches (0.95-0.98). The two tests had a substantial to almost perfect agreement (k=0.82, p<0.001).

Conclusions This single institutional cohort demonstrates that the prevalence of TBD in young patients presenting with SAA is remarkably low, and the Sens and Spec of TL testing in lymphs alone are sufficiently high to detect the rare TBD patient and prompt additional testing (e.g., genetics) in few patients due to false positive testing. The prevalence of TBD is higher in those with MAA or mild AA, yet both test approaches detect all TBD patients. Spec level in mild AA is marginally higher with

lymph subset TL testing but, at ˜94%, underscores the need for other clinical parameters (e.g., genetics) to establish a TBD diagnosis in a small percentage of patients.

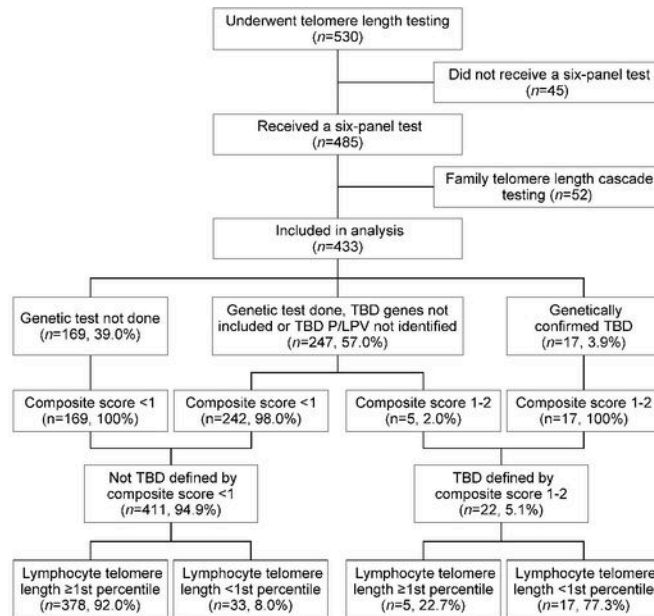**Disclosures** No relevant conflicts of interest to declare.

Figure 1: Flowchart of study population.

		Not TBD (composite score <1)	**TBD** (composite score 1-2)	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**AUC**
1A. Lymphocyte telomere length <1st percentile vs. ≥1st percentile								
All patients (N=433)								
≥1st percentile		378	5	77.3%	92.0%	34.0%	98.7%	0.85
<1st percentile		33	17					
SAA (n=106)								
≥1st percentile		99	0	100.0%	94.3%	14.3%	100.0%	0.97
<1st percentile		6	1					
MAA (n=37)								
≥1st percentile		32	0	100.0%	94.1%	60.0%	100.0%	0.97
<1st percentile		2	3					
Mild AA (n=103)								
≥1st percentile		85	0	100.0%	90.4%	50.0%	100.0%	0.95
<1st percentile		9	9					
MAA or Mild AA (n=140)								
≥1st percentile		117	0	100.0%	91.4%	52.2%	100.0%	0.96
<1st percentile		11	12					
1B. Cases with 3 or 4 vs. <3 lymphocyte subsets <1st percentile								
All patients (N=433)								
<3 subsets		390	5	77.3%	94.9%	44.7%	98.7%	0.86
3 or 4 subsets		21	17					
SAA (n=106)								
<3 subsets		101	0	100.0%	96.2%	20.0%	100.0%	0.98
3 or 4 subsets		4	1					
MAA (n=37)								
<3 subsets		32	0	100.0%	94.1%	60.0%	100.0%	0.97
3 or 4 subsets		2	3					
Mild AA (n=103)								
<3 subsets		88	0	100.0%	93.6%	60.0%	100.0%	0.97
3 or 4 subsets		6	9					
MAA or Mild AA (n=140)								
<3 subsets		120	0	100.0%	93.8%	60.0%	100.0%	0.97
3 or 4 subsets		8	12					
PPV, positive predictive value; NPV, negative predictive value								
AUC, Area under the Receiver operating characteristic (ROC) curve								
TBD: telomere biology disorder; MAA: moderate aplastic anemia; SAA: severe aplastic anemia; AA: aplastic anemia								

Table 1: The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) for the lymphocyte telomere length tests against the TBD determined by the composite score

Figure 1

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