RESEARCH ARTICLE

Disrupted lymphocyte homeostasis in hepatitis-associated acquired aplastic anemia is associated with short telomeres

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Hepatitis-associated aplastic anemia (HAA) is a variant of acquired aplastic anemia (AA) in which immune-mediated bone marrow failure (BMF) develops following an acute episode of seronegative hepatitis. Dyskeratosis congenita (DC) is an inherited BMF syndrome characterized by the presence of short telomeres, mucocutaneous abnormalities, and cancer predisposition. While both conditions may cause BMF and hepatic impairment, therapeutic approaches are distinct, making it imperative to establish the correct diagnosis. In clinical practice, lymphocyte telomere lengths (TL) are used as a first-line screen to rule out inherited telomeropathies before initiating treatment for AA. To evaluate the reliability of TL in the HAA population, we performed a retrospective analysis of TL in 10 consecutively enrolled HAA patients compared to 19 patients with idiopathic AA (IAA). HAA patients had significantly shorter telomeres than IAA patients ($P = 0.009$), including four patients with TL at or below the 1st percentile for age-matched controls. HAA patients had no clinical features of DC and did not carry disease-causing mutations in known genes associated with inherited telomere disorders. Instead, short TLs were significantly correlated with severe lymphopenia and skewed lymphocyte subsets, features characteristic of HAA. Our results indicate the importance of caution in the interpretation of TL measurements in HAA, because, in this patient population, short telomeres have limited specificity.

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Introduction

Acquired aplastic anemia (AA) affects both children and adults, and is characterized by low blood counts and severely hypocellular bone marrow. The underlying pathophysiology of AA is thought to be T-cell-mediated destruction of early hematopoietic cells [1]. The diagnosis of AA is made in part by excluding other disorders that can present with bone marrow failure (BMF) [2].

Dyskeratosis congenita (DC) is a multisystem BMF syndrome, caused by mutations of genes involved in telomere maintenance [3]. Although DC classically presents in childhood with stereotypical mucocutaneous changes of skin, nails, and mucosa, milder forms of telomere dysfunction, such as those associated with mutations in *TERT* and *TERC* genes, can present with bone marrow failure indistinguishable from AA both in children and adults [4]. Furthermore, patients with telomere disorders, particularly those caused by *TERT* and *TERC* mutations, can present with a variety of liver abnormalities including hepatic inflammation, fibrosis, and cirrhotic liver failure [5,6]. In clinical practice, lymphocyte telomere length (TL) measurements are used as a first-line screen to rule out inherited telomeropathies before initiating treatment for AA [7,8].

During routine clinical screening, we observed that several patients with classic features of hepatitis-associated AA (HAA), a subset of AA that occurs concurrent to or following an acute episode of seronegative hepatitis [9], had lymphocyte TL at or below the first percentile of age-matched controls, in the range similar to that of inherited telomere disorders [10,11]. To confirm our initial observation, we retrospectively analyzed TL measurements obtained at the time of diagnosis in 10 consecutively enrolled HAA patients, and compared them to TL in patients with non-hepatitis associated AA. Our results show that a subgroup of patients with HAA present with significantly shorter TL at diagnosis, leading to a diagnostic challenge in differentiating between HAA and inherited telomeropathies based on TL measurement.

Additional Supporting Information may be found in the online version of this article.

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Methods

Patients and study oversight. The Children's Hospital of Philadelphia (CHOP) Bone Marrow Failure Syndrome (BMFS) cohort is an open prospective/retrospective cohort for the study of molecular mechanisms of BMFS, approved by the CHOP Institutional Review Board. Written informed consent from all study participants or their legal guardians was obtained prior to study participation in accordance with the Declaration of Helsinki. All consecutively enrolled patients with pediatric-onset AA, referred to the CHOP Comprehensive BMFS Center between 2009 and 2015, who had clinical telomere length (TL) measurement within 6 months of diagnosis and prior to therapy initiation, were eligible for this analysis. The diagnosis of AA and disease severity classification were made according to previously published criteria, and required exclusion of inherited BMFS [12,13]. HAA was defined according to established criteria [9,14] as severe bone marrow aplasia within 6 months of a documented seronegative (non-A, non-B, non-C) hepatitis, where hepatitis status was characterized as an increase in serum transaminases to at least three times the upper limit of normal.

Telomere length measurement. Telomere length (TL) measurements of peripheral blood lymphocytes were performed by fluorescence *in situ* hybridization coupled with flow cytometry (flow-FISH) [15] as a part of the clinical diagnostic evaluation by a CLIA-certified TL testing center (Repeat Diagnostics, North Vancouver, Canada), that provides a reference to respective TL of age-matched healthy controls.

Lymphocyte subset analysis. Lymphocyte subset analysis of patients' peripheral blood was performed by flow cytometry as a part of standard clinical evaluation by the CLIA-certified CHOP Clinical Immunology Laboratory. Median absolute lymphocyte counts for age-matched normal controls were obtained from the established reference of immunophenotyping of lymphocyte subpopulations in childhood [16].

Sanger sequencing. Sanger sequencing for inherited mutations in the *TERT* and *TERC* genes was performed by polymerase chain reaction (PCR) amplification of the 16 exons of the *TERT* gene and of the single exon of the *TERC* gene, followed by bi-directional Sanger sequencing, using standard techniques [17]. Oligonucleotide sequences were used as previously published [11], with minor modification. Genotyping for the two polymorphic variants in *TERT* and *RTEL1* genes was performed similarly; all oligonucleotide sequences are listed in Supporting Information.

Whole exome sequencing (WES) and bioinformatic analysis. WES was performed on DNA extracted from the patients' bone marrow aspirate and paired skin fibroblast DNA as described previously [18], using Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) at the BGI@CHOP High Throughput Sequencing Center. Exome libraries were constructed with Agilent SureSelect All Exon V4 + UTRs kit (Agilent Technologies, Santa Clara, CA). Paired-end WES to 150X average depth was performed using the Illumina HiSeq 2500 platform, according to the manufacturer's recommendations. Constitutional calling on bone marrow–skin biopsy pairs was performed with VarScan2 [19], using parameters *–min-coverage 4,–min-var-freq 0.08,–P-value 0.05,–strand-filter 1–min-avg-qual 20,* with downstream filtering and annotation in SNP & Variation Suite v8.0 (Golden Helix, Bozeman, MT) to identify constitutional variants in the known genes associated with DC: *ACD, CTC1, DKC1, NHP2, NOP10, PARN, RTEL1, TERC, TERT, TINF2, USB1, and WRAP53.* All nonsynonymous coding or splice-site variants were manually curated in Integrative Genomics Viewer [20]. To determine minor allele frequencies in the population, variants were annotated against databases of genomic variation (dbSNP, 1000Genomes Project, NHLBI GO Exome Sequencing Project, Exome Aggregation Consortium, and the Supercentenarian 17 [21–25]). To estimate potential impact on protein function, variants were further subjected to *in silico* functional annotation using a panel of five functional prediction algorithms—SIFT [26], PolyPhen-2 [27], MutationTaster [28], FATHMM [29], and MutationAssessor [30].

Statistics. Fisher's exact test was used to compare gender, TL above and below the 1st percentile for age, and disease severity between the two groups (HAA vs. IAA). Wilcoxon test was used to compare age, total lymphocytes counts, $CD3^+$ T lymphocytes, $CD19^+$ B lymphocytes, $CD3^+CD4^+$ T cells, $CD3^+CD8^+$ T cells, and CD4/CD8 ratios between the two groups. Linear regression was used to compare telomere lengths between the two groups adjusting for patients' age. The telomere lengths in IAA and HAA groups were correlated with total lymphocyte and lymphocyte subset ($CD3^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$ and $CD19^+$) counts via the Pearson correlation coefficient. All statistical comparisons were two-sided and a P value <0.05 was considered statistically significant.

Results

Clinical presentation

Twenty nine consecutively enrolled patients with pediatric-onset AA were included in this study: 10 had HAA and 19 had idiopathic AA not related to hepatitis (IAA) (Table I). The median age at diagnosis was 8.0 years (range 1–19 years). AA disease severity was very severe in 6 patients, severe in 21 patients, and moderate in 2 patients.

TABLE I. Patient Characteristics of Hepatitis-associated and Idiopathic Aplastic Anemia Patients

Patient characteristic	Overall ($n = 29$)	IAA ($n = 19$)	HAA ($n = 10$)	P value
Age at diagnosis, y, median (range)	8 (1-19)	9 (1-19)	8 (3-17)	0.982
Gender, *n* (%)				0.450
Female	12 (41)	9 (47)	3 (30)	
Male	17 (59)	10 (53)	7 (70)	
Disease severity, *n* (%)				0.552
Severe	21 (73)	13 (68)	7 (70)	
Very severe	6 (21)	3 (16)	3 (30)	
Moderate	2 (7)	2 (11)	0 (0)	
Median lymphocyte telomere length				**0.009**
≤1st percentile of age-matched controls	4	0	4	
>1st percentile of age-matched controls	25	19	6	

IAA, idiopathic acquired aplastic anemia, not associated with hepatitis; HAA, hepatitis-associated acquired aplastic anemia. Y, years. kb, kilobases.

There was no significant difference in age ($P = 0.982$), gender ($P = 0.450$) or AA disease severity ($P = 0.552$) between the HAA and IAA patient groups. In agreement with prior reports [9], the median time from the diagnosis of seronegative hepatitis to the development of cytopenias was 3 weeks, ranging from under 1 week to 4 months (Table II). Transaminases peaked at a median of 2,589 U L^{-1} for alanine aminotransferase (range 865–3,461 U L^{-1}) and 2,161 U L^{-1} for aspartate aminotransferase (range 391–6,707 U L^{-1}), with total bilirubin peaking at a median of 12.45 milligrams/dL (range 0.9–52 mg dL^{-1}). Seven of the ten patients had a diagnostic liver biopsy; one patient underwent an orthotopic liver transplant for acute liver failure, whereas in other patients hepatitis resolved following corticosteroid therapy or was self-limited.

Patients with hepatitis-associated aplastic anemia have significantly shorter lymphocyte telomere lengths than patients with idiopathic AA

Strikingly, the lymphocyte telomere lengths (TL) were significantly shorter in HAA patients, compared to patients with IAA; on average, TL in HAA patients were 0.9713 kilobases shorter after accounting for the patients' age ($P = 0.009$). Notably, 4 of 10 HAA patients had telomeres at or below the 1st percentile of age-matched normal controls, within the diagnostic range for telomeropathies [10,31]; in contrast, none of the nineteen IAA patients in our cohort had TL below the 1st percentile ($P = 0.009$) (Table I, Fig. 1A). None of the HAA patients with very low TL had clinical features of classic DC.

To ensure that the significantly lower telomere lengths in the HAA patients were not caused by an occult telomere disorder, we used whole exome sequencing to screen three of the four patients with TL at or below the 1st percentile for mutations in known genes associated with DC (Supporting Information Table I) [32,33]. Although we identified several polymorphic variants (Supporting Information Table II), our analysis revealed no disease-causing mutations. Two of the three patients carried rare single nucleotide polymorphisms (SNPs) with a minor allele frequency below 1% in the general population [21,22,24]; these were examined more closely. Patient 484.1 was found to carry two rare variants: heterozygous polymorphism (dbSNP rs35719940) in *TERT* (c.2995G>A, p.Ala999Thr), with an allele frequency of 0.9% overall and 2% in the European population, as well as a homozygous polymorphism (dbSNP rs190887884) in *RTEL1* (c.2546G>A, p.Gly849Asp), with an allele frequency of 0.6% overall and 0.99% in the European population; the latter was previously described as a nonsegregating, benign variant in a study of familial

TABLE II. Clinical Characteristics of Ten Patients With Hepatitis-associated Aplastic Anemia

Patient ID	Age (years)	Median TL (kilobases)	Peak ALT (U L^{-1})	Peak AST (U L^{-1})	Total bilirubin (mg dL^{-1})	Liver biopsy performed	Hepatitis to AA interval (months)	AA severity	Cytogenetics	PNH flow cytometry*	AA therapy
484.01	8	5.9	3,461	4,551	25.6	Yes	1	SAA	Normal	Negative	IST
466.01	12	6.7	2,586	1,639	9.3	Yes	4	VSAA	Normal	Minor (<1%)	IST
274.01	5	6.9	1,607	3,434	11.8	Yes	3	SAA	Normal	n/a	Allo-BMT
487.01	8	6.9	1,711	2,296	21.6	Yes	1	SAA	Normal	Minor (<1%)	IST
492.01	10	7.6	2,592	1,775	13.1	No	0.5	SAA	Normal	Negative	Allo-BMT
412.01	17	8.3	3,431	6,707	52	Yes	Diagnosed concurrently	SAA	Normal	1.40%	Died prior to therapy
409.01	7	9.1	2,814	3,471	19	Yes	2	SAA	Normal	Negative	Allo-BMT
369.01	7	9.2	3,286	2,025	4.7	Yes	Diagnosed concurrently	VSAA	Normal	Negative	Allo-BMT
275.01	11	9.4	865	391	1.6	No	Diagnosed concurrently	SAA	Normal	Minor (<1%)	Allo-BMT
450.01	3	9.8	1,030	599	0.9	No	Diagnosed concurrently	VSAA	Normal	Negative	IST

TL, telomere lengths; ALT, alanine aminotransferase; AST, aspartate aminotransferase; SAA, severe AA; VSAA, very severe AA; PNH, paroxysmal nocturnal hemoglobinuria; Allo-BMT, allogeneic bone marrow transplant; IST, immunosuppression therapy. *, PNH clone size as measured by the % of CD55- and CD59-negative granulocytes.

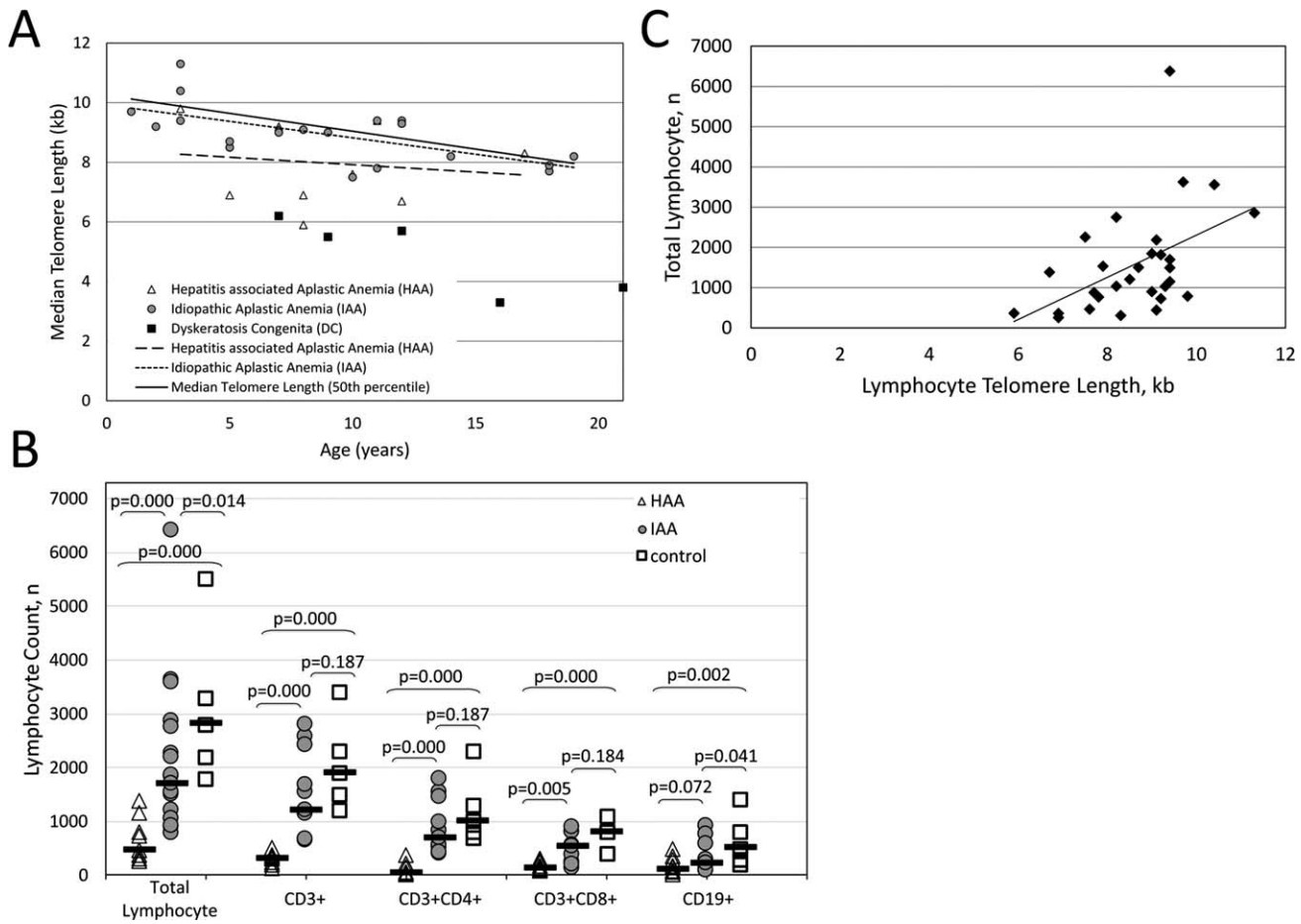

Figure 1. Disrupted lymphocyte homeostasis in hepatitis-associated acquired aplastic anemia is associated with short telomeres. A. A scatter plot depicting median telomere length in kilobases (kb) on the *Y* axis, plotted against the patients' age in years on the *X* axis. Values for patients with hepatitis-associated aplastic anemia (HAA) are shown with white triangles, and those for idiopathic aplastic anemia (IAA) are shown with gray circles. For comparison, values for patients with genetically confirmed dyskeratosis congenita (DC) are shown with black squares. The solid line in the figure indicates the 50th percentile of normal median telomere length for age-matched controls. The regression lines for HAA and AA patients are shown as a large dashed and small dashed lines, respectively. B. Shown are the lymphocyte counts within the specified lymphocyte subsets in patients with HAA (white triangles), IAA (gray circles), and age-matched normal controls (white rectangles), including: absolute total lymphocyte count; $CD3^+$ T cells; $CD3^+CD4^+$ T cells; $CD3^+CD8^+$ T cells; and $CD19^+$ B cells. The horizontal black bars represent the median for each lymphocyte population. For total lymphocytes as well as for all T lymphocyte subsets, HAA patients showed significant differences compared to IAA patients; for each intergroup comparison, the corresponding p-value is listed above the bracket. C. A scatter plot depicting median telomere length in kilobases (kb) on the *X* axis plotted against the patients' absolute lymphocyte count on the *Y* axis; regression line is shown as a black line. All AA patients are included.

interstitial pneumonia [34]. This patient's parents, both in good health, were genotyped and confirmed to be heterozygous carriers of the rs190887884 in *RTEL1*; one of the parents was also confirmed to be a heterozygous carrier of rs35719940 in *TERT* (Supporting Information Fig. 1). Lymphocyte TL measurements of both parents were normal, at 50th percentile of age-matched normal controls, suggesting that neither variant is disease-causing. Similarly, patient 487.1 was found to be heterozygous for two rare variants: a polymorphism (dbSNP rs754768798) in *RTEL1* (c.3103C>T, p.Pro1035Ser) with a reported frequency of 4 in 116296 alleles in the Exome Aggregation Consortium [25], as well as a novel variant in *WRAP53* (c.1597G>A, p.Gly533Ser). *In silico* analysis of functional impact using five independent functional prediction algorithms suggested that both variants are functionally benign (Supporting Information Table II); in agreement, the patient's TL repeated at 1 year after diagnosis showed a median lymphocyte TL increased to between the 1st and 10th percentile.

The fourth patient with TL below the 1st percentile underwent an upfront matched related donor bone marrow transplant and maintained follow-up in our center for only 1 year after transplant. Although we were unable to perform WES due to the lack of sufficient available germline DNA material, we were able to test for germline mutations in *TERT* and *TERC* by Sanger sequencing, with no pathogenic mutations identified. Additionally, the patient's pretransplant conditioning contained a high dose alkylating agent, cyclophosphamide at 200 mg kg^{-1}, which was tolerated without organ toxicity as evidenced by normal pulmonary and liver function tests at the patient's last follow-up at our center 1 year after transplant. While an uncomplicated transplant course does not completely exclude an occult telomeropathy, the patient's complete lack of transplant-related complications strongly argues against a severe form of an inherited telomere disorder.

For three of the four patients with TL at or below the 1st percentile at diagnosis, lymphocyte TL was repeated 1 year after therapy. The fourth patient was unavailable for lymphocyte TL analysis for reason of having received a bone marrow transplant. Of the three patients who were able to be tested, two patients demonstrated an increase in median lymphocyte TL: median lymphocyte TL increased to between the 1st and 10th percentile in one patient and to ~50th percentile in the second patient. The third patient remained lymphopenic with TL below the 1st percentile.

Disrupted lymphocyte homeostasis in hepatitis-associated aplastic anemia is associated with short telomeres

Because specific subsets and activation states of lymphocytes are associated with distinct telomerase activity [35–37], we hypothesized that differences in lymphocyte populations associated with the unique inflammatory state of HAA [9,38] could partly account for the significantly shorter TL in this population, as well as for the subsequent increase in median telomere length in a subset of patients at 1-year post-therapy. While both HAA and IAA patients were found to have lower total and B lymphocyte counts than age-matched normal controls, compared to IAA patients, HAA patients exhibited significantly lower absolute lymphocytes ($P = 0.0002$), $CD3^+$ T lymphocytes ($P = 0.0001$), CD4+ and CD8+ T lymphocyte subsets ($P = 0.0001$ and $P = 0.0048$, respectively) (Fig. 1B), and, in agreement with prior studies [9,38], a significantly decreased CD4/CD8 ratio ($P = 0.0174$) (data not shown). Importantly, the median TL showed a significant correlation with lymphocyte counts (Pearson correlation coefficient 0.47, $P = 0.0095$) (Fig. 1C). Of note, all four patients with the sub-1st percentile telomere lengths appeared to have more severe hepatic dysfunction as evidenced by significant hyperbilirubinemia and marked transaminitis; in contrast, patients with milder hepatic dysfunction, as evidenced by milder hyperbilirubinemia and the clinical decision to forgo a liver biopsy, had higher TL (Table II).

Discussion

Using a combination of lymphocyte TL measurements, WES, and lymphocyte subset analysis, we have shown that patients with HAA have significantly lower lymphocyte TL at diagnosis than patients with idiopathic AA, including 40% of HAA patients with TL below the 1st percentile of age-matched controls. Our results underline the lack of specificity of lymphocyte TL as a screening test for inherited telomere disorders in HAA, and provide evidence that disrupted lymphocyte homeostasis associated with inflammatory disorders such as HAA can affect lymphocyte TL, limiting its clinical utility in this patient population.

The association of AA with seronegative autoimmune hepatitis is well recognized, with a history of non-A, B, or C hepatitis present in 5–10% of AA patients [39]. Although the clinical syndrome of HAA is generally fairly distinct, characterized by marked transaminitis preceding or concurrent with the onset of cytopenias in a previously healthy pediatric or young adult patient [9], it can occasionally mimic an occult telomere disorder. Patients with inherited mutations in *TERT* and *TERC* can present with cytopenias in conjunction with severe seronegative hepatitis and hepatic necrosis, which may require liver transplantation [6]. Similarly, a case of an apparent familial bone marrow failure syndrome presenting as classical HAA has been reported, with the patient's course notable for failure to respond to immunosuppressive therapy, progression to myelodysplasia, and subsequent death 44 days post-transplant due to multiple transplant-related complications [40], suggestive of an undiagnosed telomeropathy. The recognized potential for devastating consequences of a missed occult telomere syndrome [41] highlights the importance of considering inherited syndromes in the differential diagnosis of HAA. Importantly, our results show that the diagnostic evaluation should thoughtfully incorporate other data beyond the lymphocyte TL measurements. Similar to other inflammatory conditions associated with antigen-driven replicative exhaustion and telomere shortening [42–44], HAA has been linked to a variety of lymphocyte abnormalities [9,14], and our study demonstrates that lymphopenia and skewed lymphocyte subsets correlate with shorter telomere lengths in HAA.

Limitations of our study include a relatively small sample size. However, AA is a rare disease with an incidence of one to two cases per million, of which HAA comprises a much smaller fraction of 5–10%. Recognizing the rarity of HAA, our study represents one of the largest single-institution cohorts of HAA. Future systematic studies performed through multi-institutional collaborations that incorporate a larger patient population will be needed to better define the kinetics of telomere shortening and recovery in HAA, and to delineate other clinicopathologic characteristics that can better define this patient population.

In sum, our finding that abnormally diminished and skewed T cell populations in HAA are associated with very short lymphocyte TL suggests that the disrupted lymphocyte homeostasis intrinsic to the pathogenesis of pediatric HAA [9,14] may limit the specificity of TL as a screening tool to exclude DC in patients with HAA. Although further studies are needed to elucidate the etiology of shortened lymphocyte telomeres, our results indicate the critical importance of caution in the use of TL measurements to diagnose telomere disorders in patients presenting with hepatitis and bone marrow aplasia.

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References

1. Young NS. Current concepts in the pathophysiology and treatment of aplastic anemia. Hematol Am Soc Hematol Educ Prog 2013;2013:76–81.
2. Scheinberg P, Young NS. How I treat acquired aplastic anemia. Blood 2012;120:1185–1196.
3. Dokal I. Dyskeratosis congenita. Hematol Am Soc Hematol Educ Prog 2011;2011:480–486.
4. Townsley DM, Dumitriu B, Young NS. Bone marrow failure and the telomeropathies. Blood 2014;124:2775–2783.
5. Hartmann D, Srivastava U, Thaler M, et al. Telomerase gene mutations are associated with cirrhosis formation. Hepatology 2011;53:1608–1617.
6. Calado RT, Regal JA, Kleiner DE, et al. A spectrum of severe familial liver disorders associate with telomerase mutations. PLoS One 2009;4:e7926.
7. Williams DA, Bennett C, Bertuch A, et al. Diagnosis and treatment of pediatric acquired aplastic anemia (AAA): An initial survey of the North American Pediatric Aplastic Anemia Consortium (NAPAAC). Pediatr Blood Cancer 2014;61:869–874.
8. Hartung HD, Olson TS, Bessler M. Acquired aplastic anemia in children. Pediatr Clin North Am 2013;60:1311–1336.
9. Brown KE, Tisdale J, Barrett AJ, et al. Hepatitis-associated aplastic anemia. N Engl J Med 1997;336:1059–1064.
10. Alter BP, Baerlocher GM, Savage SA, et al. Very short telomere length by flow fluorescence in situ hybridization identifies patients with dyskeratosis congenita. Blood 2007;110:1439–1447.
11. Du HY, Pumbo E, Ivanovich J, et al. TERC and TERT gene mutations in patients with bone marrow failure and the significance of telomere length measurements. Blood 2009;113:309–316.
12. International Agranulocytosis and Aplastic Anemia Study. Incidence of aplastic anemia: The relevance of diagnostic criteria. Blood 1987;70:1718–1721.
13. Davies JK, Guinan EC. An update on the management of severe idiopathic aplastic anaemia in children. Br J Haematol 2007;136:549–564.
14. Lu J, Basu A, Melenhorst JJ, et al. Analysis of T-cell repertoire in hepatitis-associated aplastic anemia. Blood 2004;103:4588–4593.
15. Baerlocher GM, Vulto I, de Jong G, et al. Flow cytometry and FISH to measure the average length of telomeres (flow FISH). Nat Protoc 2006;1:2365–2376.
16. Comans-Bitter WM, de Groot R, van den Beemd R, et al. Immunophenotyping of blood lymphocytes in childhood. Reference values for lymphocyte subpopulations. J Pediatr 1997;130:388–393.
17. Green MR, Sambrook J. Molecular Cloning: A Laboratory Manual, 4th ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press; 2012.
18. Babushok DV, Perdigones N, Perin JC, et al. Emergence of clonal hematopoiesis in the majority of patients with acquired aplastic anemia. Cancer Genet 2015;208:115–128.
19. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: Somatic mutation and copy number alteration discovery in cancer by exome sequencing. Genome Res 2012;22:568–576.
20. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative genomics viewer (IGV): High-performance genomics data visualization and exploration. Brief Bioinform 2013;14:178–192.
21. Sherry ST, Ward MH, Kholodov M, et al. dbSNP: The NCBI database of genetic variation. Nucleic Acids Res 2001;29:308–311.
22. Abecasis GR, Auton A, Brooks LD, et al. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491:56–65.
23. Gierman HJ, Fortney K, Roach JC, et al. Whole-genome sequencing of the world's oldest people. PLoS One 2014;9:e112430.
24. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012;337:64–69.
25. Exome Aggregation Consortium (ExAC). Cambridge, MA. Available at: http://exac.broadinstitute.org. Accessed on November 7, 2015.
26. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4:1073–1081.
27. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet 2013. Chapter 7:Unit7.20.
28. Schwarz JM, Cooper DN, Schuelke M, et al. MutationTaster2: Mutation prediction for the deep-sequencing age. Nat Methods 2014;11:361–362.
29. Shihab HA, Gough J, Cooper DN, et al. Predicting the functional, molecular, and phenotypic consequences of amino acid substitutions using hidden Markov models. Hum Mutat 2013;34:57–65.
30. Reva B, Antipin Y, Sander C. Predicting the functional impact of protein mutations: Application to cancer genomics. Nucleic Acids Res 2011;39:e118.
31. Wilson DB, Link DC, Mason PJ, et al. Inherited bone marrow failure syndromes in adolescents and young adults. Ann Med 2014;46:353–363.
32. Bertuch AA. The molecular genetics of the telomere biology disorders. RNA Biol 2015 Sep 23 [Epub ahead of print].
33. Dokal I, Vulliamy T, Mason P, et al. Clinical utility gene card for: Dyskeratosis congenita—Update 2015. Eur J Hum Genet 2015;23. doi:10.1038/ejhg.2014.170 [Epub ahead of print].
34. Cogan JD, Kropski JA, Zhao M, et al. Rare variants in RTEL1 are associated with familial interstitial pneumonia. Am J Respir Crit Care Med 2015;191:646–655.
35. Son NH, Murray S, Yanovski J, et al. Lineage-specific telomere shortening and unaltered capacity for telomerase expression in human T and B lymphocytes with age. J Immunol 2000;165:1191–1196.
36. Lin J, Epel E, Cheon J, et al. Analyses and comparisons of telomerase activity and telomere length in human T and B cells: Insights for epidemiology of telomere maintenance. J Immunol Methods 2010;352:71–80.
37. Weng NP, Levine BL, June CH, et al. Human naive and memory T lymphocytes differ in telomeric length and replicative potential. Proc Natl Acad Sci USA 1995;92:11091–11094.
38. Ikeda T, Morimoto A, Nakamura S, et al. A marked decrease in CD4-positive lymphocytes at the onset of hepatitis in a patient with hepatitis-associated aplastic anemia. J Pediatr Hematol Oncol 2012;34:375–377.
39. Locasciulli A, Bacigalupo A, Bruno B, et al. Hepatitis-associated aplastic anaemia: Epidemiology and treatment results obtained in Europe. A report of the EBMT aplastic anaemia working party. Br J Haematol 2010;149:890–895.
40. Breakey VR, Meyn S, Ng V, et al. Hepatitis-associated aplastic anemia presenting as a familial bone marrow failure syndrome. J Pediatr Hematol Oncol 2009;31:884–887.
41. Fogarty PF, Yamaguchi H, Wiestner A, et al. Late presentation of dyskeratosis congenita as apparently acquired aplastic anaemia due to mutations in telomerase RNA. Lancet 2003;362:1628–1630.
42. van Baarle D, Nanlohy NM, Otto S, et al. Progressive telomere shortening of Epstein-Barr virus-specific memory T cells during HIV infection: Contributor to exhaustion? J Infect Dis 2008;198:1353–1357.
43. Effros RB, Allsopp R, Chiu CP, et al. Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. Aids 1996;10:F17–22.
44. Gadalla SM, Wang T, Haagenson M, et al. Association between donor leukocyte telomere length and survival after unrelated allogeneic hematopoietic cell transplantation for severe aplastic anemia. JAMA 2015;313:594–602.