Association of Telomere Length of Peripheral Blood Leukocytes With Hematopoietic Relapse, Malignant Transformation, and Survival in Severe Aplastic Anemia

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Severe aplastic anemia is characterized by life-threatening cytopenias and a profound diminution of bone marrow progenitor cells. Clinical and laboratory evidence implicate it as an immune mediated disorder in which oligoclonal cytotoxic T cells target and destroy hematopoietic progenitor cells resulting in profound marrow failure.1 Severe aplastic anemia can be cured by hematopoietic stem cell transplantation, but in older patients and when a histocompatible sibling donor is unavailable, immunosuppressive therapy with antithymocyte globulin (ATG) plus cyclosporine is effective.1 The majority of patients, 60% to 70%, respond with hematologic improvement to immunosuppression. However, relapses occur in about one-third of responders and clonal evolution is observed in 10% to 15% of cases, which manifests late as myelodysplasia.1

Although immune destruction of hematopoietic cells is the proximate cause of severe aplastic anemia, recently target cell abnormalities have been identified as risk factors in bone marrow failure. Mutations in telomerase complex genes resulting in extremely short telomeres have been described in some patients with apparently acquired severe aplastic anemia.2-5 Telomeres are nucleotide repeats at the ends of the chromosomes that function as protective caps to prevent erosion of genomic DNA

Context Critically short telomeres produce apoptosis, cell senescence, and chromosomal instability in tissue culture and animal models. Variations in telomere length have been reported in severe aplastic anemia but their clinical significance is unknown.

Objective To investigate the relationship between telomere length and clinical outcomes in severe aplastic anemia.

Design, Setting, and Patients Single institution analysis of 183 patients with severe aplastic anemia who were treated in sequential prospective protocols at the National Institutes of Health from 2000 to 2008. The pretreatment leukocyte age-adjusted telomere length of patients with severe aplastic anemia consecutively enrolled in immunosuppression protocols with antithymocyte globulin plus cyclosporine for correlation with clinical outcomes were analyzed.

Main Outcome Measures Hematologic response, relapse, clonal evolution, and survival.

Results There was no relationship between hematologic response and telomere length with response rates of 56.5% of 46 patients in the first, 54.3% of 46 in the second, 60% of 45 in the third, and 56.5% of 46 in the fourth quartiles. Multivariate analysis demonstrated that telomere length was associated with relapse, clonal evolution, and mortality. Evaluated as a continuous variable, telomere length inversely correlated with the probability of hematologic relapse (hazard ratio [HR], 0.16; 95% confidence interval [CI], 0.03-0.69; P = .01). The probability of clonal evolution was higher in patients in the first quartile (24.5%; 95% CI, 8.7%-37.5%) than in quartiles 2 through 4 (8.4%; 95% CI, 3.2%-13.3%; P = .009), and evolution to monosomy 7 or complex cytogenetics was more common in the first quartile (18.8%; 95% CI, 3.5%-31.6%) than in quartiles 2 through 4 (4.5%; 95% CI, 0.5%-8.2%; P = .002). Survival between these 2 groups differed, with 66% (95% CI, 52.9%-82.5%) surviving 6 years in the first quartile compared with 83.8% (95% CI, 77.3%-90.9%) in quartiles 2 through 4 (P = .008).

Conclusion In a cohort of patients with severe aplastic anemia receiving immunosuppressive therapy, telomere length was unrelated to response but was associated with risk of relapse, clonal evolution, and overall survival.
TELOMERE LENGTH AND OUTCOME IN SEVERE APLASTIC ANEMIA

METHODS

Patient and Treatment Details

Patients were enrolled into 3 sequential treatment-naive severe aplastic anemia protocols from November 2000 to May 2008 at the National Institutes of Health in Bethesda, Maryland. Race/ethnicity was self-reported and collected as part of the prospective clinical research protocols in which the patients were enrolled. All consecutive patients treated with ATG plus cyclosporine for whom sufficient samples were available for testing were included. A total of 248 patients enrolled into treatment protocols during the analysis period: 19 were not treatment-naive, 16 received alternative immunosuppression other than ATG plus cyclosporine, and for 30 a sufficient sample was not available for analysis. A total of 183 patients were included in the analysis. Patients (or legal guardians) signed informed consent according to approved protocols by the institutional review board of the National Heart, Lung, and Blood Institute. For protocol entry purposes, severe aplastic anemia was defined as bone marrow cellularity of less than 30% and severe pancytopenia with at least 2 of the following peripheral blood count criteria: (1) absolute neutrophil count less than 500/µL; (2) absolute reticulocyte count less than 60,000/µL; (3) platelet count less than 20,000/µL. Chromosomes were assayed after in vitro exposure of lymphocytes to diepoxybutane and in some cases also to mitomycin C to exclude Fanconi anemia. Patients with inherited severe aplastic anemia or evidence of a clonal hematologic disorder as inferred from bone marrow cytogenetics were excluded from enrolling into these treatment protocols.

Hematologic response, defined as no longer meeting criteria for severe aplastic anemia, was determined at 6 months following ATG and, for the current analysis, adopted as the criterion for hematologic recovery. Patients who relapsed by definition required reinstitution of immunosuppression. Clonal evolution was defined as the appearance of a new cytogenetic abnormality on bone marrow cytogenetics after immunosuppressive therapy. All patients were tested for mutations in the telomerase complex genes TERT and TERC, as previously described. Patients underwent 1 of 4 regimens: 3 were based on horse ATG plus cyclosporine and 1 rabbit ATG plus cyclosporine regimen. The 3 horse-ATG regimens were standard horse ATG plus cyclosporine, horse ATG/cyclosporine/mycophenolate mofetil, and horse ATG/cyclosporine/sirolimus and have been described previously in detail. There was no difference in clinical outcome among the 3 horse-ATG regimens, which were combined for this analysis. Cyclosporine was discontinued after 6 months in all but 47 responders to horse-ATG plus cyclosporine who had their cyclosporine dose tapered after 6 months.

Telomere Length Measurement

Telomere length of pretreatment peripheral blood leukocytes was assessed by quantitative polymerase chain reaction (PCR) as previously described. Total leukocytes were separated by ammonium-based lysis of red blood cells and DNA extracted using the DNeasy Blood kit (Qiagen, Valencia, California). Polymerase chain reactions assays were performed in a 7500 Real Time PCR System (Applied Biosystems, Foster City, California). Each sample's telomere length (x) was based on the telomere to single copy gene ratio (T:S ratio) and based on the calculation of the ΔCt, \[ C_{\text{T(teromes)}/C_{\text{single gene}}} \]. Telomere length was expressed as the relative T:S ratio, which was normalized to the average T:S ratio of reference sample \[ 2^{(\Delta \text{Ct} - \Delta \text{Ct0})/2\Delta \text{Ct}} \], used for the standard curve, as reference sample, and as validation sample. To make comparable the results from different plate runs, the results of each plate were approved only if the relative T:S ratio of the validation reference sample fell within a 3% variation. Laboratory personnel conducting the telomere length assay were blinded to patients' clinical outcomes prior to statistical analysis.

Statistical Methods

Age-adjusted telomere length for each patient was computed by subtracting his/her linear predicted telomere length from the observed telomere length. Nonparametric Cox regression based on splines with continuous age-adjusted telomere length was used as an exploratory analysis for the probability distributions of time to relapse, time to evolution, and overall survival. We then evaluated the effects of age-adjusted telomere length quartiles on the event probabilities of these clinical outcomes using the Cox proportional hazard model. Based on the survival curves per quartile for each of the clinical events, quartiles with similar event probabilities were grouped in further analysis. Consequently, for analysis of time to clonal evolution and survival, patients in the first quartile formed a distinct group, and patients in the second, third, and fourth quartiles were combined due to their similar clinical outcomes. As the relationship between telomere length and relapse was more linear, there was no apparent threshold that discriminated those at higher risk of this outcome. Summary statistics (means, proportions, and stan-
standard deviations) stratified by age-adjusted telomere quartiles were used to describe patients’ age, sex, and other baseline characteristics. P values based on multisample tests for proportions and the analysis of variance tests were used to compare patients’ baseline characteristics. P values from the log-rank tests were used to evaluate the overall covariate effects in the univariate and multivariate Cox proportional hazard models. Numerical results were computed using the S-PLUS software package (TIBCO Software Inc, Palo Alto, California). Two-sided P values were used throughout and considered statistically significant if <.05.

RESULTS

Telomere length was measured in pretreatment leukocytes in a total of 183 patients who received initial therapy at our institution and categorized in quartiles after age adjustment (Table 1). Age distribution among all 4 quartiles was similar (Table 1 and eFigure 1 available at http://www.jama.com). Median follow-up in all patients was 55 months (range, 0.1-116 months) and for surviving patients 64 months (range, 6-116 months).

Multivariate Analysis of Telomere Length on the Rate of Response, Relapse, Clonal Evolution, and Survival

In multivariate logistic regression, telomere length was not associated with response at 6 months. Reported covariates predictive of response included the reticulocyte and lymphocyte counts. In a multivariate Cox proportional hazard model, shorter telomeres were associated with relapse, clonal evolution, and mortality (Table 2). Evaluated as a continuous variable, telomere length was inversely associated with relapse, for clonal evolution and mortality, those in quartiles 2 through 4 had a rate about one-third compared with the first quartile (Table 2).

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Factor</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>P Value</th>
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<tr>
<td>Patients, No. (%)</td>
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<td>46 (25)</td>
<td>45 (25)</td>
<td>46 (25)</td>
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<td>Sex, No. (%) [SD]</td>
<td>Male</td>
<td>105 (67)</td>
<td>29 (63)</td>
<td>26 (67)</td>
<td>25 (56)</td>
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<tr>
<td>Female</td>
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<td>78 (43)</td>
<td>17 (37)</td>
<td>20 (44)</td>
<td>20 (44)</td>
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<td>Race/ethnicity, No. (%) [SD]</td>
<td>White</td>
<td>91 (60)</td>
<td>23 (50)</td>
<td>24 (52)</td>
<td>25 (56)</td>
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<td>Black</td>
<td>45 (25)</td>
<td>9 (20)</td>
<td>12 (26)</td>
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<td>17 (37)</td>
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<td>Hispanic</td>
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<td>11 (24)</td>
<td>7 (15)</td>
<td>8 (18)</td>
<td>9 (20)</td>
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<td>Asian</td>
<td>12 (7)</td>
<td>3 (7)</td>
<td>3 (7)</td>
<td>5 (11)</td>
<td>3 (7)</td>
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<td>Age, mean (SD), y</td>
<td>35 (1.5)</td>
<td>37 (3.3)</td>
<td>34 (3)</td>
<td>34 (2.9)</td>
<td>34 (3.1)</td>
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<td>Immunosuppression, No. (%) [SD]</td>
<td>Horse ATG/CsA</td>
<td>70 (38)</td>
<td>16 (35)</td>
<td>15 (33)</td>
<td>23 (51)</td>
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<td>Horse ATG/CsA/MMF</td>
<td>48 (26)</td>
<td>17 (37)</td>
<td>9 (20)</td>
<td>11 (24)</td>
<td>11 (24)</td>
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<tr>
<td>Horse ATG/CsA/sirolimus</td>
<td>35 (19)</td>
<td>5 (11)</td>
<td>11 (24)</td>
<td>8 (18)</td>
<td>11 (24)</td>
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<tr>
<td>Rabbit ATG/CsA</td>
<td>30 (16)</td>
<td>8 (17)</td>
<td>11 (24)</td>
<td>3 (7)</td>
<td>8 (17)</td>
</tr>
<tr>
<td>Blood counts, mean (SD), µL</td>
<td>ARC</td>
<td>20 464 (1371)</td>
<td>24 352 (2820)</td>
<td>22 181 (3265)</td>
<td>20 158 (2474)</td>
</tr>
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<td>ALC</td>
<td>1312 (48)</td>
<td>1328 (121)</td>
<td>1285 (92)</td>
<td>1322 (71)</td>
<td>1313 (96)</td>
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<tr>
<td>ANC</td>
<td>363 (22)</td>
<td>456 (46)</td>
<td>382 (48)</td>
<td>346 (35)</td>
<td>266 (39)</td>
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<tr>
<td>ANC&lt;200 No. (%) [SD]</td>
<td>67 (37)</td>
<td>11 (24)</td>
<td>17 (37)</td>
<td>16 (36)</td>
<td>23 (50)</td>
</tr>
<tr>
<td>Platelet</td>
<td>10 852 (1423)</td>
<td>9304 (923)</td>
<td>9848 (888)</td>
<td>14 911 (5519)</td>
<td>9435 (1157)</td>
</tr>
</tbody>
</table>

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; ATG, antithymocyte globulin; CsA, cyclosporine; MMF, mycophenolate mofetil.

Response and Relapse According to Telomere Length

One hundred and four patients (57%) responded to immunosuppressive therapy. There was no correlation between telomere length at first presentation and the probability of response. The response rate for patients in the first quartile was 56.3% (95% confidence interval [CI], 41.6%-71.4%); in the second quartile, 54.3% (95% CI, 39.4%-69.3%); in the third quartile, 60% (95% CI, 43.1%-74.9%); and in the fourth quartile, 56.5% (95% CI, 41.6%-71.4%). Of the 59 unresponsive patients with telomere length in quartiles 2 through 4, 37 underwent a second course of immunosuppression and 13 responded; of the 20 patients in the first quartile, 10 underwent a second course of immunosuppression and 2 responded.

Twenty-six of the 104 patients who responded to a first course of immunosuppressive therapy later relapsed with a 6-year relapse rate of 26.3% (95% confidence interval [CI], 18.6%-34.1%).
Clonal Evolution
Survival

Blood counts per µLc

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relapse HR (95% CI)</th>
<th>P Value</th>
<th>Clonal Evolution HR (95% CI)</th>
<th>P Value</th>
<th>Survival HR (95% CI)</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td>Telomere length</td>
<td>0.16 (0.03-0.69)a</td>
<td>.01</td>
<td>0.29 (0.11-0.76)b</td>
<td>.01</td>
<td>0.35 (0.17-0.73)c</td>
<td>.005</td>
</tr>
<tr>
<td>Age per year</td>
<td>1.03 (1.01-1.05)</td>
<td>.005</td>
<td>1.03 (1.00-1.05)</td>
<td>.01</td>
<td>1.03 (1.02-1.05)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Blood counts per µLc</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ARC</td>
<td>0.99 (0.63-1.55)</td>
<td>.96</td>
<td>1.10 (0.55-2.19)</td>
<td>.79</td>
<td>0.63 (0.41-0.97)</td>
<td>.03</td>
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<tr>
<td>ALC</td>
<td>1.31 (0.55-3.10)</td>
<td>.54</td>
<td>1.16 (0.51-2.66)</td>
<td>.72</td>
<td>0.89 (0.48-1.66)</td>
<td>.72</td>
</tr>
<tr>
<td>ANC</td>
<td>1.20 (0.67-2.16)</td>
<td>.54</td>
<td>0.70 (0.38-1.27)</td>
<td>.24</td>
<td>0.95 (0.65-1.38)</td>
<td>.78</td>
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<tr>
<td>Platelet</td>
<td>0.80 (0.46-1.38)</td>
<td>.42</td>
<td>0.76 (0.45-1.28)</td>
<td>.30</td>
<td>1.11 (0.73-1.67)</td>
<td>.63</td>
</tr>
</tbody>
</table>

Abbreviations: ALC, absolute lymphocyte count; ANC, absolute neutrophil count; ARC, absolute reticulocyte count; CI, confidence interval; HR, hazard ratio.

a Continuous telomere length was used for relapse due to the linear relationship between relapse and telomere length.

b For clonal evolution and survival, short telomere was defined as an age-adjusted telomere length < first quartile and long telomere as an age-adjusted telomere length > first quartile.
c Natural log-transformed ARC, ALC, ANC, and platelet count were used to reduce the skewness of these variables.

Clonal Evolution and Survival

According to Pretreatment Telomere Length

Clonal evolution and survival initially were analyzed per quartile, a higher rate for these outcomes was observed for those with shorter telomeres (overall log-rank P=.02 and P=.06, respectively, for differences among all quartiles; data not shown). In univariate analysis, those in quartiles 2 through 4 clustered more favorably than those with a telomere length in the first quartile who had a higher risk of clonal evolution and mortality (Figure 2A and Figure 2A). When the relationship between telomere length and clonal evolution and survival was analyzed using the estimated log-hazard curve, differences in outcome were observed for those in first quartile (data not shown). When the quartiles according to the estimated event probability curves were grouped, the cumulative incidence of clonal evolution was 8.4% (95% CI, 3.2%-13.3%) for those in quartiles 2 through 4 compared with 24.5% (95% CI, 8.7%-37.5%) among those with shortest telomeres in the first quartile (P=.009, log-rank; Figure 2A). Of the 10 patients who evolved in the longer telomere group, 4 had monosomy 7 (1 patient developed t(12;13) prior to evolving to monosomy 7), 1 deletion 13q, 1 loss of chromosome Y, 1 loss of chromosome 18, 1 t(6;14), 1 deletion of chromosome 3, and 1 had complex cytogenetic abnormalities. Of the 9 patients in the shortest telomere group (first quartile) who evolved, 6 had monosomy 7 (2 patients developed deletion 13q prior to evolving to monosomy 7), 1 had deletion 13q, 1 had t(9;19), and 1 evolved to complex cytogenetics (which followed a loss of chromosome Y abnormality). More evolution to monosomy 7 or complex cytogenetics was observed in the shortest (first quartile) telomere group (18.8%; 95% CI, 3.5%-31.6%) than those with longer telomeres (4.5%; 95% CI, 0.3%-8.2%; P=.002, log-rank; Figure 2B).

Worse survival was observed in patients with the shortest telomere length (first quartile) while the curves for the remaining quartiles clustered to yield a similar survival rate. The survival proportions between the 2 groups differed significantly, with 66% (95% CI, 52.9%-82.5%) in the shortest telomere (first quartile) group surviving 6 years compared with 83.8% (95% CI, 77.3%-90.9%) in those with longer telomeres in quartiles 2 through 4 (P=.008, log-rank; Figure 3A). In the longer telo-
meme group, 12 died from complications of pancytopenia, 3 died after hematopoietic stem cell transplantation, 2 from progression to myelodysplasia, 1 from heart failure, and 1 in a traffic accident. In the shortest telomere group, 3 died from complications of pancytopenia, 5 died after stem cell transplants, 3 died after clonal evolution, 1 from failure to thrive, 1 from a traffic collision, and 1 from unknown causes. Among the 19 deaths in the longer telomere group, 3 occurred after relapse or clonal evolution, and among the 14 deaths in the first quartile, 5 occurred after relapse or clonal evolution.

**COMMENT**

When first observed, short telomeres of leukocytes in acquired severe aplastic anemia was presumed to be secondary to hematopoietic stress. The discovery of loss-of-function mutations in genes of the telomerase complex (TERC and TERT) established a genetic etiology for telomere attrition in marrow failure. Telomerase mutations are etiologic in the constitutional marrow failure syndrome dyskeratosis congenita and are also found in a minority of patients with acquired aplastic anemia. However, in the present series, only 1 patient later tested positive for a TERT mutation (codon A202T; his leukocytes' telomere length was less than the 10th percentile). Therefore, the current study describes a relationship between variations of telomere length within the normal range and severe aplastic anemia clinical outcomes in patients who (with a single exception) lacked known genetic explanations for shorter telomeres.

Telomere length was not associated with response to immunosuppression. Why some patients do not respond is unknown; insufficient number of hematopoietic stem cells, inadequate immunosuppression, and a nonimmune etiology for marrow failure each have been suggested. That shorter telomeres were not associated with unresponsiveness to immunosuppression indicates that this parameter does not distinguish a nonimmune etiology group. In our cohort, stem cell reserves appeared sufficient for recovery after therapy.

Clonal evolution to myelodysplasia is a major adverse event in severe aplastic anemia; it cannot be routinely predicted and usually signals a poor prognosis. In particular, the finding of monosomy 7 on bone marrow cytogenetics is associated with persistent pancytopenia unresponsive to immunosuppression and progression to myelodysplasia. The current work shows that telomere length relates to the development of abnormal marrow clones, in particular monosomy 7, with serious clinical consequences.

Prediction of important disease-related complications is critical to risk stratification and patient management. The major problems of immunosup-
pressive therapy in severe aplastic anemia are unresponsiveness, relapse, and clonal evolution. Recently, we reported that pretreatment reticulocyte count was predictive of response to immunosuppression; however, there are no recognized predictors for relapse and clonal evolution. In the current study, we showed that pretreatment telomere length was associated with relapse and clonal evolution, 2 serious late events in severe aplastic anemia. When absolute reticulocyte count and telomere length were combined in our cohort, 3 groups were observed: (1) a favorable group with high reticulocyte count and longer telomeres; (2) an intermediate group with high reticulocyte count and shorter telomeres or a low reticulocyte count and longer telomere length; and (3) a poor-risk group with low reticulocyte count and shorter telomere length (Figure 3B). Antithymocyte globulin plus cyclosporine may be adequate for the most favorable group; in contrast, better regimens are needed for the poor-risk group. For example, androgen treatment offers a potential for in vivo modulation of telomere length and for those at greater risk for late complications after immunosuppression, higher-risk protocols such as stem cell transplants in older patients and alternative sources of stem cells might be considered earlier in younger patients.

Telomere attrition is not simply a biomarker; rather, a plausible mechanism for destabilization of the genome has been inferred from basic telomere biology. Ample in vitro and animal experimentation indicate that critical shortening of telomeres causes chromosome instability, tumor formation, and cancer progression. Normally, senescence would preclude cells with critically short telomeres from tumorigenesis, but cells with malignant potential may escape by failure of mechanisms such as p53 signaling of DNA damage or activation of alternative modes of telomere maintenance. In telomerase “knockout” mice (Terc−/−), aneuploidy and end-to-end fusions were observed in fibroblasts after 4 generations, suggesting an inability to protect chromosome ends when telomerase was deficient. Furthermore, on breeding of telomerase-deficient knockout mice with short telomeres with wild-type mice with long telomeres, chromosome fusions and signal-free ends occurred preferentially on chromosomes with critically short telomeres.

Clinically, telomere length has been associated with human cancer. In dyskeratosis congenita, the incidence of cancers was 11-fold higher than that of the general population, with high rates of tongue cancer and leukemia. In leukemia patients with no underlying telomerase disorder, hypomorphic mutations in TERT were 3-fold more frequent in patients than in controls. Telomere attrition has been implicated in a variety of solid organ malignancies. In Barrett esophagus, there was an increased risk of developing esophageal adenocarcinoma in individuals who had shorter leukocyte telomere length at first clinical presentation. Furthermore, as in our study, telomere length was an independent predictor for progression to esophageal cancer after correction for other covariates. Premature shortening of telomeres of leukocytes and in colonic epithelia in a few patients has been correlated with cancer progression in ulcerative colitis. Our current data are consistent with findings in other inflammatory diseases that predispose to cancer, in that the risk of clonal evolution was increased in those with pretreatment, age-adjusted telomere length in the lower quartile. However, in contrast to many investigations of predisposition to gastrointestinal tract tumors, we determined telomere length months to years before malignant transformation and in those hematopoietic cells directly subject to dysplasia and leukemia. Indeed, bone marrow cells from patients with short telomeres in vitro showed increased number of telomere-free chromosomes, aneuploidy, and chromosomal translocations not seen in cells of patients with longer telomeres (our unpublished data).

Our study has strengths and limitations. The strengths include the homogeneous cohort enrolled into our research protocols with specified diagnostic criteria, immunosuppressive drug administration, supportive care, and prospectively determined and defined clinical outcomes. We monitor our patients indefinitely at specified intervals, and our protocols require periodic assessment for adverse events like relapse and clonal evolution. Our study is limited due to the retrospective nature of the analysis and the relatively small number of patients, which did not allow for validation testing in a separate cohort or for reliable subgroup analysis. As a research facility and quaternary referral center, our patient population also may not be representative and our results not necessarily generalizable. Therefore, our results need to be replicated to validate the observed associations and to determine reliable telomere length thresholds that could be incorporated in treatment algorithms.

In conclusion, our data show that in a cohort of patients with severe aplastic anemia receiving immunosuppressive therapy, telomere length was not associated with response but was associated with risk of relapse, clonal evolution, and overall survival.

Author Contributions: Dr Scheinberg had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Scheinberg and Cooper contributed equally to this work.

Study concept and design: Scheinberg, Cooper, Wu, Calado, Young.

Acquisition of data: Scheinberg, Cooper, Sloand, Calado, Young.

Analysis and interpretation of data: Scheinberg, Cooper, Wu, Calado, Young.

Drafting of the manuscript: Scheinberg, Cooper, Calado, Young.

Critical revision of the manuscript for important intellectual content: Scheinberg, Cooper, Sloand, Wu, Calado, Young.

Statistical analysis: Wu, Calado.

Administrative, technical, or material support: Scheinberg, Sloand, Young.

Study supervision: Calado, Young.

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REFERENCES


