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.2-5 Telomeres are nucleotide repeats at the ends of the chromosomes that function as protective caps to prevent erosion of genomic DNA. Consequently, 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.

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during cell division. Telomeric DNA can be elongated by the telomerase complex, which is composed of a reverse transcriptase catalytic subunit (encoded by telomerase reverse transcriptase [TERT]), an RNA template (encoded by telomerase RNA component [TERC]), and associated proteins. To determine the effect of telomere attrition in acquired severe aplastic anemia, we measured telomere length pretreatment in consecutive patients at our institution who had received ATG plus cyclosporine since 2000 and analyzed its relationship with hematologic recovery, relapse, clonal evolution, and survival.

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: \[ C_t(telomeres)/C_t(single\ gene) \]. Telomere length was expressed as the relative T:S ratio, which was normalized to the average T:S ratio of reference sample \[ 2^{-ΔC(telomeres−gene)} \] 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 char-
teristics. 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 com-
puted using the S-PLUS software pack-
age (TIBCO Software Inc, Palo Alto,
California). Two-sided P values were
used throughout and considered sta-
tistically significant if <.05.

RESULTS

Telomere length was measured in pre-
treatment leukocytes in a total of 183
patients who received initial therapy at
our institution and categorized in quar-
tiles after age adjustment (Table 1). Age
distribution among all 4 quartiles was
similar (Table 1 and eFigure 1 avail-
able at http://www.jama.com). Me-
dian 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 lympho-
cyte counts.12 In a multivariate Cox
proportional hazard model, shorter
telomeres were associated with relapse, clonal evolution, and mortal-
ity (Table 2). Evaluated as a continu-
ous 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).

Response and Relapse According
to Telomere Length

One hundred and four patients (57%)
responded to immunosuppressive
therapy. There was no correlation be-
tween telomere length at first presen-
tation and the probability of response.
The response rate for patients in the first
quartile was 56.3% (95% confidence in-
terval [CI], 41.6%-71.4%); in the sec-
ond 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 pa-
tients with telomere length in quar-
tiles 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 immu-
nosuppressive therapy later relapsed
with a 6-year relapse rate of 26.3% (95%-

### Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>Factor</th>
<th>All Patients</th>
<th>1 (0.53-1.93)</th>
<th>2 (0.78-1.41)</th>
<th>3 (1.04-1.67)</th>
<th>4 (1.26-1.93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, No. (%)</td>
<td>183</td>
<td>46 (25)</td>
<td>46 (25)</td>
<td>45 (25)</td>
<td>46 (25)</td>
</tr>
<tr>
<td>Sex, No. (%) [SD]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Male                        | 105 (67) [3.7]| 29 (63) [7.2]| 26 (67) [7.4]| 25 (56) [7.5]| 25 (54) [7.4]| .84
| Female                      | 78 (43) [3.7]| 17 (37) [7.2]| 20 (44) [7.4]| 20 (44) [7.5]| 21 (47) [7.4]|
| Race/ethnicity, No. (%) [SD]|              |              |              |              |              |
| White                       | 91 (50) [3.7]| 23 (50) [7.5]| 24 (62) [7.4]| 25 (56) [7.5]| 19 (41) [7.3]| .57
| Black                       | 45 (25) [3.2]| 9 (20) [5.9] | 12 (26) [6.5]| 7 (16) [5.5] | 17 (37) [7.2]| .97
| Hispanic                    | 35 (19) [2.9]| 11 (24) [6.4]| 7 (15) [5.4] | 8 (18) [5.8] | 9 (20) [5.9] | .34
| Age, mean (SD), y           | 35 (1.5)     | 37 (3.3)     | 34 (3)       | 34 (2.9)     | 34 (3.1)     | .89
| Immunosuppression, No. (%) [SD]|              |              |              |              |              |
| Horse ATG/CsA               | 70 (38) [3.6]| 16 (35) [7.1]| 15 (33) [7.0]| 23 (51) [7.5]| 16 (35) [7.1]| .24
| Horse ATG/CsA/MMF           | 48 (26) [3.3]| 17 (37) [7.2]| 9 (20) [5.9]| 11 (24) [6.5]| 11 (24) [6.4]| .26
| Blood counts, mean (SD), µL |              |              |              |              |              |
| ARC                         | 20 464 (1371)| 24 352 (2820)| 22 181 (3265)| 20 158 (2474)| 15 159 (2177)| .12
| ALC                         | 1312 (48)    | 1328 (121)   | 1285 (92)    | 1322 (71)    | 1313 (96)    | .89
| ANC                         | 363 (22)     | 456 (46)     | 382 (48)     | 346 (35)     | 266 (39)     | .02
| ANC<200 No. (%) [SD]        | 67 (37) [3.6]| 11 (24) [6.4]| 17 (37) [7.2]| 16 (36) [7.2]| 23 (50) [7.5]| .08
| Platelet                    | 10 852 (1423)| 9304 (923)   | 9848 (888)   | 14 911 (5519)| 9435 (1157)  | .67

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

aP values were based on the F statistics for comparing all 4 quartiles.
bLog-transformed ARC, ALC, ANC, and platelet count were used for the analysis of variance models.
CI, 17%-34.5%). In 3 additional patients, a cytogenetic abnormality was identified at the time of their relapse and considered to have evolved for the purpose of analysis. Among responders, the rate of relapse inversely correlated with telomere length; risk for this outcome increased with shorter telomere length (Figure 1). Overall log-rank P value for difference in relapse among quartiles was not significant (P=.08; Figure 1, only responding patients were at risk for relapse, reducing statistical power). However, when telomere length was evaluated as a continuous variable, the rate of relapse increased as telomere length shortened (HR, 0.16; P=.01; Table 2). Among the 9 relapsed patients in quartiles 3 through 4, 8 were treated with immunosuppression, and of the 7 patients who are evaluable to date, 6 have responded; and among the 17 in quartiles 1 and 2, 16 were treated with immunosuppression, and of the 15 evaluable patients to date, 12 have responded. Thus, shorter telomeres were associated with greater risk of relapse but did not preclude hematologic recovery with further immunosuppression.

**Clonal Evolution and Survival According to Telomere Length**

When the incidence for 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 3A). When the relationship between telomere length and clonal evolution and survival were 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 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-

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**Table 2. Multivariate Cox Proportion Hazard Model for Relapse, Clonal Evolution, and Survival**

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relapse HR (95% CI)</th>
<th>P</th>
<th>Clonal Evolution HR (95% CI)</th>
<th>P</th>
<th>Survival HR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere length</td>
<td>0.16 (0.03-0.69)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>.01</td>
<td>0.29 (0.11-0.76)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>.01</td>
<td>0.35 (0.17-0.73)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;.001</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 µL&lt;sup&gt;c&lt;/sup&gt;</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>
</tr>
<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>
</tr>
<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.

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

<sup>b</sup>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.

<sup>c</sup>Natural log-transformed ARC, ALC, ANC, and platelet count were used to reduce the skewness of these variables.
Reticulocyte Count (ARC) was defined as 25,000/µL or higher and a low ARC of less than 25,000/µL. A high ARC was associated with a longer survival and longer telomere length, whereas a low ARC was associated with shorter survival and shorter telomere length. A group with intermediate survival was defined by either a low ARC and longer telomere length (quartiles 2 through 4) or a high ARC and with the shortest telomere length (first quartile). A high ARC was associated with a longer survival, whereas a low ARC was associated with a shorter survival. This suggests that telomere length and ARC are independent predictors of survival in patients with severe aplastic anemia. Overall survival according to pretreatment telomere length and ARC is shown in Figure 2. The survival probability by telomere length quartile is shown in Figure 3A. The survival probability by telomere length and ARC is shown in Figure 3B.

**COMMENT**

When first observed, short telomeres of leukocytes in acquired severe aplastic anemia were 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.
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.\(^{15-22}\) Normally, senescence would preclude cells with critically short telomeres from tumorigenesis,\(^ {23}\) but cells with malignant potential may escape by failure of mechanisms such as p53 signaling of DNA damage\(^ {24}\) 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.\(^ {25}\) 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.\(^ {26}\)

Clinically, telomere length has been associated with human cancer.\(^ {27}\) 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.\(^ {28}\) In leukemia patients with no underlying telomerase disorder, hypomorphic mutations in TERT were 3-fold more frequent in patients than in controls.\(^ {29}\) 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.\(^ {30}\) Furthermore, as in our study, telomere length was an independent predictor for progression to esophageal cancer after correction for other covariates.\(^ {30}\) Premature shortening of telomeres of leukocytes and in colonic epithelia in a few patients has been correlated with cancer progression in ulcerative colitis.\(^ {21,22}\) 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 chromosomal ends, 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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