Telomere Length in Children with Acquired Severe Aplastic Anemia

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Abstract

Objective: This study aimed to assess telomere length in children with severe aplastic anemia and to correlate this parameter with the response to immunosuppressive therapy

Method: The study group consisted of 18 children aged with severe aplastic anemia (SAA) treated with rabbit antithymocyte globulin and cyclosporine from one of hematology center. Telomere length was analyzed in all patients and in 20 parents. Control group was composed of 12 healthy children and 12 healthy adults. Telomere length in peripheral blood was assessed using polymerase chain reaction (PCR).

Result: There was statistical significance between telomere length in patients with SAA and healthy children (p=0.03852) as well as in parents of SAA children and adults from control group (p=0.01086). There was no difference concerning response to IST in relation to telomere length (p=0.7859).

Conclusion: Our studies confirmed that pediatric patients with SAA and their parents have shorter telomeres compared to healthy population. The assessment of telomere length in diseases of short telomeres, including aplastic anemia, should be a diagnostic standard. Future studies are necessary to confirm the role of telomere length as an independent prognostic risk factor and a good predictor of prognosis in SAA.

Keywords: Aplastic anemia; Children; Telomere length; Immunosuppressive treatment

Introduction

Augured severe aplastic anemia (SAA) is rare disorder of bone marrow failure which if not appropriately treated is highly fatal. SAA is characterized by morphologic marrow features, namely hypocellularity, and resultant peripheral cytopenias [1-7]. Now most of the patients survive because they can be successfully treated with either hematopoietic stem cell transplantation (HSCT) or immunosuppressive therapy (IST) [8-12]. The choice of the treatment is dependent on a patients’ age and availability of HLA-matched family donor. Hematopoietic stem cell transplantation is considered as the treatment of choice in children with HLA-matched family donor and in young adults before 40 years of age. In other cases immunosuppressive therapy is carried out [4,8-17]. The long-term results of those two methods are comparable. Long-lasting remission in 60-75% of patients above 5 years of age, treated with HSCT as well as IST, was proven by numerous studies [8-13,17-25]. However, serious adverse effects of above-mentioned therapies still exist. Graft-versus-Host Disease (GvHD) is one of the major issues after HSCT, on the other hand after IST non-response occurs. Additionally IST is connected with higher relapse rate and higher probability of transformation in to myelodysplastic syndrome (MDS) or malignant disease, including acute myeloblastic leukemia (AML) [17-21]. Despite multiple attempts, a factor enabling to qualify children to proper therapeutic arm in terms of treatment outcome and the occurrence of adverse effects has not been established yet [22-25]. The prognostic value of initial neutrophil, reticulocyte or platelet count, presence of paroxysmal nocturnal hemoglobinuris clone is questionable [14,16,17,22,23,25]. Only the patient’s age at the onset of the disease seems to influence the clinical outcome. Younger patients respond better to IST in comparison with older patients. The search for a reliable prognostic factor is still being conducted [14,16,17,22-25]. Telomere shortening is observed in about 1/3 of patients with SAA, and mutations in genes of telomerase complex (TERC, TERT) were identified in about 5-10% of SAA patients [26-34]. Initially telomere shortening was considered to be a consequence of oxidative stress in hematopoietic system [35,36]. Moreover, the mutations in TERC and TERT were identified as etiological background of telomere shortening. Patients with those mutations are found to possess significantly shorter telomeres than age-matched healthy individuals [34-36]. As SAA belongs to the short telomeres diseases, an attention has been paid to telomere length as potential predictor of long-term treatment outcome [37-41].

This study was aimed to assess telomere length in children with severe aplastic anemia (SAA) and to determine whether this parameter can be used to predict response to immunosuppressive therapy.

Material and Methods

Eighteen patients with severe aplastic anemia on immunosuppressive treatment with no HLA-matched family donor were enrolled in one polish pediatric hematology and oncology center in years 2009-2013. The mean age of enrolled subjects was 14.35 years (range: 7-19), study group included 9 boys and 9 girls. All patients fulfilled criteria of severe aplastic anemia: bone marrow cellularity...
<25% of reference value for the age or 25-50% with <30% of hematopoietic reservoir and additionally neutrophil count (ANC) <0.5 × 10^9/L and/or platelet count <20 × 10^9/L [38]. All patients had congenital aplastic anemia. In all cases paroxysmal nocturnal hemoglobinuria was excluded. Diagnostic tests for PNH were performed with the use of flow cytometry with CD55 and CD59 antigen expression analysis on neutrophils and red blood cells. The chromosomal fragility test was performed as Fanconi anemia screening. In all patients had bone marrow cyrogenetic analysis was performed to diagnose chromosomal defects.

**Protocol therapy**

Patients received rabbit antithymocyte globulin (r-ATG, Lymphoglobulin, Genzyme) at 3.75 mg/kg/mc intravenously at 1-5 days, cyclosporine (CSA, Sandimmun Neoral, Novartis Pharma) at 5 mg/kg orally at days 1-180. CSA dose was modified to maintain serum drug level of 100-200 ng/ml. Granulocyte colony stimulating factor (G-CSF, Filgrastim– Neupogen, Hoffmann-La Roche) was administered subcutaneously or intravenously only to patients with severe infection non-responding to antibiotics and antifungal medications. The criteria of remission were evaluated at days 112, 180 and 360 after treatment initialization. Complete remission (CR) was defined as ANC >1.5 × 10^9/L, PLT >100 × 10^9/L and hemoglobin level >11.0g/L. Criteria for partial remission (PR) were defined as follows: ANC >0.5 × 10^9/L, PLT >20 × 10^9/L and hemoglobin level >8.0 g/L. Children were divided into three groups: non responders (NR) – 6 children with refractory disease, early remissions (E-CR) – 6 children with early response to treatment defined as complete or partial remission at day 112, and late remissions (L-R) – 6 children who achieved CR or PR at days 180-360. Telomere length was analyzed in all patients and in 20 parents of 11 children with SAA (11 mothers and 9 fathers aged 39-55 years). Control group was composed of 12 healthy children (3 girls and 9 boys, aged 3-17) and 12 healthy adults aged 31-57 years. Each participant/parents signed informed consent and agreed for blood sampling.

**Statistical analysis**

Telomere/single copy gene (T/S) ratio for samples was calculated from human reference DNA (Applied Biosystems), standard curve and T/S ratios values were expressed in relation to telomere length in controls. Control quartiles leukocyte telomere length (LTL) were categorized into quartiles based on the weighted sample distribution of LTL. The R Project for Statistical Computing was used for statistical analysis, p value <0.05 was considered statistically significant [41]. Mann–Whitney U test or Kruskal-Wallis test was employed as appropriate. Linear regression analysis was carried out with the use of Excel (Microsoft) and Statistica software.

Table 1: Telomere length values in severe aplastic anemia (SAA) and healthy children.
When analyzing telomere length between healthy adults and parents of children with SAA, it was found that the parents of our patients have shorter telomeres and these differences are statistically significant (0.01086).

In the whole group of 18 SAA patients treated with IST, 12 (66.6%) of them responded to the therapy. Seven patients out of 9 achieved remission in the group of children with short telomeres, 4 out of 7 in the group of children with medium telomeres and 1 out of 2 in children with long telomeres. No significance between telomere length and response to treatment was found (p=0.7859). There was a significant difference (p=0.013) between telomere length in healthy children and children with SAA who responded to treatment after day 180 (Figure 2). The difference was not detected between non-responders and late responders (L-CR) (p=0.065).

In healthy children, telomere length correlated with platelet (p=0.008, R²=0.5166), white blood cell (p=0.007, R²=0.5223)
neutrophil ($p=0.025, R^2=0.41$) and monocyte ($p=0.02, R^2=0.3986$) count and all the correlations were statistically significant (Figure 4).

![Figure 4: Linear regression analysis of correlation between T/S ratio and peripheral blood counts (A) Hgb, (B) WBC, (C) Monocytes, (D) Lymphocytes, (E) Granulocytes (F) Platelets in healthy children.](image)

In the course of 5-year follow-up, one relapse 3 years after remission was noted. No clonal disease or transformation into malignant disease was observed. No chromosomal abnormalities or PNH clones were found. All patients live in remission: 17 after IST and one after IST and BMT from unrelated donor.

**Discussion**

Congenital aplastic anemia's such as Dyskeratosis congenital (DS), Fanconi anemia (FA), Diamond-Blackfan anemia (DBA) belong to a group of short telomeres diseases [26-31]. Short telomeres are detected in around one third of patients with acquired severe aplastic anemia [25-30,35,37]. Our study confirms significantly shorter telomeres in patients with SAA in comparison to healthy controls ($p=0.001136$) and indicates that parents of SAA children have shorter telomeres than adults from control group. Other studies also highlighted this phenomenon in adults with SAA [25-30], Tutelman et al. confirmed telomere shortening in neutrophils, T- and B-lymphocytes as well as NK cells in the group of 28 patients with SAA [37]. Pavesi et al. assessed telomere length in patients with bone marrow failure syndromes (BMFS) such as congenital bone marrow failure (DS, DBA, FA) acquired aplastic anemia and concluded that telomere length was a handy clue in a diagnostic process of children with no phenotypic features [40]. Young observed correlation between length of telomeres in SAA patients and blood cell count in pancytopenic patients. Clonal transformation to myelodysplasia and acute leukemia and long-term survival were also dependent to the telomeres length [32]. In the observed group there was only one relapse and no clonal transformations, thus such observation could not be confirmed. Surprisingly in the control group (but not in patients) a significant correlation between leukocyte, platelet, neutrophil, monocyte count and telomere length was observed. In a search for prognostic factors for IST response, we have shown that there was no relationship between telomere length and treatment response ($p=0.7859$). Sakaguchi et al. study demonstrated that the measurement of telomere length of lymphocytes is useful for predicting the response to IST in patients with AA [35]. Multivariate analysis showed that telomere length shorter than -1.0 SD (hazard ratio (HR): 22.0; 95%CI: 4.19-115; $p<0.001$), platelet count at diagnosis less than $25 \times 10^9/L$ (HR: 13.9; 95%CI: 2.00-96.1; $p=0.008$), and interval from diagnosis to immunosuppressive therapy longer than 25 days (HR: 4.81; 95%CI: 1.15-20.1; $p=0.031$) were the significant variables for poor response to immunosuppressive therapy. But in our study we not found correlation between this parameters and response to IST. To begin with, there are several differences between the current study and Sakaguchi et al. study, including the methods of telomere length measurement and patients' characteristics [35]. In our study, the telomere length of pretreatment total leukocytes was assessed by quantitative polymerase chain reaction (PCR) but in Chinese study measured the telomere length of lymphocytes using flow-FISH. Another difference between the two studies was the distribution of patients’ diagnosis. Patients in our study was restricted to children with severe AA, and very severe AA, patients with moderate AA were not included. In contrast, 20 of 64 AA patients in Sakaguchi et al. study had moderate disease [35].

A large retrospective analysis of 183 SAA patients on ATG-based IST assessing association of treatment results with initial telomere length in peripheral blood leukocytes was conducted by National Institutes of Health (NIH) [36]. A special attention was drawn to relation between telomere length and hematological response 6 months after treatment initialization, SAA relapse, chromosomal disorders and subsequent after-effects (clonal and malignant diseases). There was no association between initial telomere length and hematological response 6 months after treatment initiation, however relapse rate was significantly higher in patients with shorter telomeres. Higher rate of clonal cytogenetic abnormalities was observed in patients with shorter telomeres in comparison to patients with longer telomeres. Moreover, the risk of chromosomal abnormalities such as monosomy 7 was also noted in group of patients with shorter telomeres. Overall survival differed six years after IST initiation and was connected with initial telomere length. TERC/TERT mutation was found only in one person among 183 SAA patients [35].

Tutelman et al. showed that short telomeres and PNH clone in children with SAA is a predictive factor of good IST response [37]. In addition, an increased frequency of chromosomal breaks and chromosomal aberrations, particularly monosomy 7, is commonly found in AA patients. In our group no chromosomal abnormalities or PNH clones were found.

During the 5-years follow-up we observed only one patient with relapse. None of the children developed MDS or AML. All patients in our study group live in remission.

**Conclusion**

Our studies confirmed that patients with SAA and their parents have shorter telomeres compared to healthy population. The assessment of telomere length in diseases of short telomeres, including aplastic anemia, should be a diagnostic standard. Future studies are necessary to confirm the role of telomere length as an independent prognostic risk factor and a good predictor of prognosis in SAA.

**References**

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