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# RECENT THYMIC EMIGRANTS AND PROGNOSIS IN T- AND B-CELL CHILDHOOD HEMATOPOIETIC MALIGNANCIES

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**The concentration of T-cell receptor rearrangement excision DNA circles (TRECs) in peripheral blood mononuclear cells (PBMCs) is currently known to be a marker of recent thymic emigrants. We evaluated the hypothesis that TREC values would be lower in childhood T-cell hematopoietic malignancies than in childhood B-cell acute lymphoblastic leukemia (ALL) or healthy controls because the former category may reflect compromised thymic function. From the Greek national childhood leukemia/lymphoma database we obtained all 30 available T-cell leukemia/non-Hodgkin's lymphoma cases, 30 age- and sex-matched childhood B-cell origin cases of ALL and 60 healthy hospital controls. We compared TREC levels in PBMCs using a real-time PCR assay. There was highly significant reduction of TREC values in children with T-cell malignancies (median 3,100 TRECs/$10^6$ PBMCs), whereas children with B-cell origin ALL had slightly but nonsignificantly lower TREC values compared to healthy children (medians 19,300 and 22,500 TRECs/$10^6$ PBMCs, respectively). During a median follow-up period of about 19 months, only 4 children died. All of them had a T-cell hematopoietic malignancy and relatively low TREC values. The number of TRECs was higher among healthy girls than among healthy boys, and a similar pattern was evident in T-cell malignancies. It appears that there is a pattern of concordance of high TREC values with better disease prognosis in hematologic childhood malignancies. This applies to specific disease entities with better prognosis (B-cell origin ALL having higher TREC values than T-cell leukemia/lymphoma) and to gender, another important predictor of prognosis conditional on disease entity (girls having higher TREC values than boys); however, it may also be true for the survival of individual patients. These preliminary findings can be used as hypothesis-generating indications that should be confirmed in larger data sets.**


**Key words:** *thymic emigrant; leukemia; lymphoma; T-cell receptor rearrangement excision circle; acute lymphoblastic leukemia*

T cells recognize infected or antigen-presenting cells through their T-cell receptors (TCRs). Thymic output of T lymphocytes can be measured by quantifying a unique feature of the TCR rearrangement process.[1–3] Episomal DNA bioproducts of the TCR rearrangement, TCR rearrangement excision circles (TRECs), can be detected in recent thymic emigrants (RTEs) in the peripheral blood.[3–5] The frequency of TRECs is considered to be the most accurate marker of T-cell neogenesis. Accordingly, this relatively newly developed technique for the assessment of thymic output (and possibly the only available alternative to $CDR45RA^+CD62$ lymphocyte measurement) may be used as a correlate of not only recently migrated T cells but also memory cells reverted in phenotype.[6,7]

T- and B-cell malignancies represent clonal expansions of T and B cells, respectively, at different stages of development as a result of rearrangements of either TCR genes or immunoglobulin genes.[8] In particular, acute lymphoblastic leukemia (ALL) represents either clonal expansion of maturationally arrested cells at specific stages of thymic ontogeny (T-cell ALL) or B-cell clonal expansion at an extrathymic site.[9] The thymus provides the inductive microenvironment for TCR rearrangement, which is a prerequisite for T-cell leukemia. Thus, we have hypothesized that the thymic function may be defective in children with T-cell ALL. In contrast, it would remain essentially unaffected in children with B-cell ALL because the abnormal differentiation during B-cell development occurs extrathymically. We have also speculated that children with T-cell leukemia and low TREC values may have worse prognosis compared to those with high TREC values. To evaluate these hypotheses, we undertook an epidemiologic study in Athens, Greece, using recently developed molecular techniques.

## MATERIAL AND METHODS

During the period 1 January 1996 to 31 October 1999, a total of 207 cases of childhood leukemia and 90 cases of childhood lymphoma, for which adequate biologic samples were available, were diagnosed in Greece by a network of childhood hematologists/oncologists. All cases were diagnosed by bone marrow or lymph node immunophenotyping. To substitute for the lack of a national cancer registry in Greece, this network, comprising all pediatric hematologists/oncologists, was developed with the aims of nationwide, continuous registration and subsequent epidemiologic study of children diagnosed with leukemia (from 1980 onward) or with lymphoma (from 1994 onward). The database essentially reached complete coverage for both malignancies by 1995, and the respective data have been used in a series of national and international studies investigating risk factors for childhood hematopoietic malignancies among Greek children.[10–14] From the leukemia series, exclusions were made for the following reasons: 43 for being of nonlymphoblastic type, 7 because they concerned infants $<12$

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months old and 8 for inadequate blood sampling or initiation of chemotherapy before blood sampling. Of the remaining 149 cases of acute ALL in children older than 1 year, 19 were of T type and the remaining were of B-cell origin. Among the 90 cases of childhood lymphoma, 12 were diagnosed with non-Hodgkin's type of T-cell origin but 1 was excluded because the patient, an 11-year-old boy, had received chemotherapy before blood sampling.

The 19 cases of T-cell leukemia and the 11 cases of T-cell non-Hodgkin's lymphoma (NHL) were grouped together because of their presumed common etiology,[15] to ensure an adequate number of childhood T-cell hematologic malignancies (leukemias/lymphomas). Each case of the T-cell leukemia/lymphoma series was matched for gender and age (±6 months) to a case of B-cell leukemia from the same database, using random selection whenever more than 1 eligible B-cell leukemia case was available. Thus, a total of 30 cases of childhood T-cell leukemia/lymphoma and 30 cases of childhood B-cell leukemia were enrolled.

The control group was comprised of essentially healthy children hospitalized for minor surgery or traumas during the same period and in the same institution as the corresponding index child. This pool of controls was used for the selection of 60 comparison patients matched for gender and age (±6 months) to each of the childhood leukemia/lymphoma cases. Again, a random selection process was used when more than 1 comparison child was eligible.

The Review Board of the University of Athens Medical School approved the study protocol, and the parents of all children were informed and agreed to participate. Parents or guardians of eligible cases provided information through an interviewer-administered, precoded questionnaire. Blood samples were obtained by venipuncture; thereafter, peripheral blood mononuclear cells (PBMCs) were isolated from specimens and cryopreserved within 6 hr from the time of collection, according to the protocol followed by the National Retroviruses Reference Center of Greece. Genomic DNA was extracted from all samples at the National Retroviruses Reference Center, which is based at the Department of Hygiene and Epidemiology, Athens Medical School.

Genomic DNA samples were shipped blinded to the Aaron Diamond AIDS Research Center (New York, NY), where TREC values were measured using a molecular beacon–based real-time multiplex PCR assay.[5] A CCR5 sequence was also used to measure cell equivalents in the input DNA. In each genomic DNA sample, PBMCs were quantified as 1 cell per 2 CCR5 copies and TREC values were calculated as the number of TRECs per $10^6$ PBMCs, following the protocol previously described.[5]

*Statistical analysis*

Because the distribution of TREC values per study participant was positively skewed in every category studied, the analysis was based on log-transformed values (to the base of 10); in this instance, the mean of the log-transformed values is the geometric mean and closely approximates the median value. Routine statistical procedures using the nonparametric Mann-Whitney test and multiple regression were performed as appropriate. To compare TREC values between girls and boys, a standard analysis of covariance was used. $p < 0.05$ (2-sided) was considered significant.

## RESULTS

Among control children with B-cell origin ALL, 21 were boys and 9 were girls, with mean age at diagnosis of 7.86 years. None had died by the end of the observation period. Among T-cell malignancy cases, 21 were boys and 9 were girls, with mean age at diagnosis of 7.97 years; 3 boys and 1 girl died after a median survival of about 15 months. Among the healthy comparison children, 42 were boys and 18 were girls, with a mean age of 7.95 years.

Table I shows the median, quartiles, geometric mean and 95% confidence intervals of the geometric mean of TREC values (in thousands) per $10^6$ PBMCs. There was a substantial and highly significant ($p < 0.001$) reduction of TREC values in children with T-cell malignancies, whereas children with B-cell ALL had slightly and nonsignificantly lower TREC values compared to healthy children.

**TABLE I** – DESCRIPTIVE CHARACTERISTICS OF TRECS/$10^6$ PBMCS VALUES (IN THOUSANDS) AMONG CASES WITH B-CELL ALL, T-CELL ALL/NHL AND HEALTHY CONTROLS

| | Number | Median | Quartiles[1] | | Geometric mean | 95% Confidence interval |
|---|---|---|---|---|---|---|
| | | | 1st | 3rd | | |
| B-cell ALL | 30 | 19.3 | 5.2 | 51.4 | 16.0 | 9.5–26.3 |
| T-cell ALL/NHL | 30 | 3.1 | 0.8 | 14.5 | 2.2 | 1.0–5.1 |
| Healthy controls | 60 | 22.5 | 10.7 | 39.5 | 21.3 | 20.8–21.9 |

[1]Upper cut-off points.

Table II shows the median and quartiles of the number of TRECs per $10^6$ PBMCs among children with B-cell ALL or T-cell malignancies and among healthy children by gender. The number of TRECs was significantly higher among healthy girls than among healthy boys, and a similar pattern was evident with respect to T-cell hematopoietic malignancies, whereas a nonsignificantly higher number was observed in B-cell subjects. Adjustment for age through standard analysis of covariance (boys were about 2 years older than girls) reduced, but did not eliminate, the difference, which remained statistically significant among controls ($p < 0.018$) and borderline significant among children with T-cell malignancies ($p < 0.058$). There were no substantial differences between children with T-cell ALL and those with T-cell NHL (data not shown). During a median follow-up period of 17.8 months, none of the children with B-cell origin leukemia died. In contrast, during a median follow-up period of 19.9 months, 4 children with T-cell malignancies died. All 4 deceased children, presented in detail in Table III, had TREC values equal to or lower than the corresponding category-specific median.

## DISCUSSION

Childhood hematopoietic malignancies may arise from thymic or extrathymic clonal expansions of T (T-cell malignancies) or B (B-cell malignancies) cells. Therefore, thymic function, as assessed by the number of TRECs, may be defective among children with T-cell ALL or lymphomas, whereas it would remain essentially unaffected in children with B-cell origin ALL.

Our findings appear to provide evidence that childhood T-cell malignancies are characterized by substantially reduced values of TRECs/$10^6$ PMBCs compared to B-cell childhood leukemia and controls, whereas a small and nonsignificant difference exists between children belonging to the latter 2 groups. Moreover, because in T-cell leukemia, cells with TRECs (*i.e.,* T cells) represent a higher proportion of PBMCs than in B-cell ALL, it can be inferred that the mean number of TRECs per T cell is overestimated in the former instance (measured TREC are contributed to by a larger than assumed number of T cells) and underestimated in the latter. Thus, the contrast between B- and T-cell leukemia with respect to TREC values is even larger than that shown in Table I. Moreover, because there are 2 important sources of variation of TREC values in B-cell malignancies (TRECs/T cell and proportion of T cells among all mononuclear cells) and only 1 in normal children (TRECs/T cell), the overall variation appears to be higher among B-cell ALL cases than among normal children, notwithstanding the similarity of the corresponding median values.

TREC values are positively associated with adequate function of the thymus[1–3,16–18] and, consequently, with cellular immunity. Therefore, it is tempting to speculate that the reduced number of TRECs in childhood T-cell malignancies could be related to worse prognosis compared to childhood B-cell malignancies. Support for this notion is provided by the relatively low number of TRECs among the 4 deceased children, all of whom belonged to the group

**TABLE II** – TREC VALUES (MEDIAN AND QUARTILES) PER $10^6$ PBMCS (IN THOUSANDS) AND LOG 10-TRANSFORMED TREC MEAN VALUES AMONG B-CELL ALL, T-CELL ALL/NHL AND HEALTHY CONTROLS BY GENDER

| Diagnosis group | 1st quartile[1] | Median | 3rd quartile[1] | *p* for comparison by gender[2] | *p* for comparison by gender[3] |
|---|---|---|---|---|---|
| B-cell ALL | | | | | |
| Male | 4.4 | 13.6 | 52.2 | NS | NS |
| Female | 13.5 | 22.6 | 62.1 | | |
| T-cell ALL/NHL | | | | | |
| Male | 0.2 | 1.7 | 9.6 | 0.09 | 0.058 |
| Female | 2.4 | 5.5 | 36.7 | | |
| Healthy controls | | | | | |
| Male | 7.8 | 16.1 | 33.0 | 0.005 | 0.018 |
| Female | 22.8 | 27.1 | 61.9 | | |

[1]Upper cut-off points.–[2]*p* value derived from Mann-Whitney test.–[3]*p* value derived from multiple regression following log 10 transformation and adjustment for age.

**TABLE III** – AGE AT DIAGNOSIS, GENDER, SURVIVAL TIME (MONTHS), TRECS/$10^6$ PBMCS (IN THOUSANDS) AND LOG-TRANSFORMED TREC VALUES OF CHILDREN WHO DIED DURING FOLLOW-UP

| Diagnosis | Age at diagnosis (years) | Gender | Survival time (months) | TRECs |
|---|---|---|---|---|
| T-cell ALL | 10.2 | Male | 24.2 | 1.4 |
| T-cell ALL | 3.0 | Male | 10.3 | 1.2 |
| T-cell NHL | 5.9 | Female | 15.7 | 5.4 |
| T-cell ALL | 7.9 | Male | 9.3 | 0.08 |

with T-cell malignancies. The data in this respect, however, are too sparse to allow firm statistical substantiation or strong conclusions.

Cellular immunity is important for the control of carcinogenic processes, and this forms the theoretical basis for the use of DNA vaccines against cancer development.[19–23] Moreover, in certain forms of cancer, effective treatment is associated with thymic hyperplasia.[24–27] Childhood T-cell malignancies may have a worse prognosis compared to B-cell ALL because T-cell malignancies are inherently associated with compromised cellular immunity on account of the thymic involvement. The limited data in our study on the poor survival of children with small TREC values provide some support to this hypothesis, as does the well-known predominance of girls over boys in the survival from childhood hematopoietic malignancies. Both the deceased children with T-cell malignancies in our study (Table III) and children of male gender (Table II) are characterized by lower TREC values. Indeed, the lower TREC values among boys compared to girls (aged in 87% of instances between 4 and 14 years), a finding that has not been previously reported, may contribute to the worse prognosis of boys *vs.* girls in several forms of childhood malignancy and perhaps other conditions.

Among the advantages of the present study is that TRECs were measured using a reliable and well-calibrated method. A potential pitfall in the measurement of TRECs as surrogate markers of thymic dysfunction, however, may appear in cases of increased cell division, as has been shown in HIV-1 infection.[28] The study samples were selected from a well-defined basis and carefully matched for gender and age. The study size was sufficient for the documentation of the striking difference in numbers of TRECs between childhood T-cell malignancies and childhood B ALL, but it was not large enough to evaluate the association of TRECs with survival among children with T-cell malignancies given the therapeutic advances that have been made with regard to treatment of childhood leukemia and the relatively short follow-up period. Lastly, although the study was designed as a case-control investigation, it did not have the shortcomings of this type of design because its explicit objective was to ascertain cross-sectional associations rather than to search for etiologic factors.

In conclusion, our study provides preliminary evidence of a pattern of concordance of high TREC values with better survival in hematopoietic childhood malignancies. This applies to disease entities (B-cell ALL *vs.* T-cell leukemia/lymphoma) and gender, an important predictor of prognosis conditional on disease entity (girls *vs.* boys); but it may also be true for the survival of individual patients. Given the small study size, however, these findings should be interpreted with caution and further confirmed in other population groups.

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