RESEARCH ARTICLE

High pre-transplant TREC levels indicate good prognosis after hematopoietic stem cell transplantation [version 1; peer review: 1 approved, 1 approved with reservations]

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Abstract

Background

Thymus-dependent T-cell reconstitution plays a role in immune recovery after stem cell transplantation (HSCT). High pre-HSCT thymic function has been associated with higher survival, lower incidence of acute and chronic graft versus host disease (GVHD) and lower incidence of infections. The aim of this study was to analyze the relationship between pre-HSCT peripheral blood levels of T-cell receptor excision circles (TREC) and post-HSCT clinical events in recipients of HLA-identical hematopoietic stem cell transplants.

Method

Delta deletion signal joint TRECs (sjTRECs) formed by the dREC-yja rearrangement were quantified by real time PCR in peripheral blood lymphocytes of 62 HSCT recipients.

Results

Univariate analysis revealed an association between low TREC levels and a higher incidence of grade II-IV acute GVHD ($p=0.026$), bacterial infection ($p=0.005$) and cytomegalovirus infection ($p=0.033$), whereas high TREC levels were associated with higher overall survival ($p=0.028$). In the multivariate analysis, low pre-HSCT TREC levels remained independently associated with lower survival ($p=0.032$; RR 2.6), occurrence of grade II-IV acute GVHD ($p=0.031$; RR: 2.5), bacterial infection ($p=0.006$, RR: 6.6) and cytomegalovirus infection ($p=0.039$; RR:2.8).

Conclusion

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	1	2
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Any reports and responses or comments on the article can be found at the end of the article.

Our results corroborate the concept that pre-HSCT recipient's thymic function is an important predictor of risk for acute grade II-IV GVHD and infection.

Keywords

T cell receptor excision circle, thymic function, stem cell transplantation

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Introduction

Although a hematopoietic stem cell transplant (HSCT) is an effective treatment for several types of malignant blood diseases, it can cause a number of different side effects, mainly related to opportunistic infections and graft versus host disease (GVHD). The immediate post-transplant period is followed by severe immunodeficiency resulting in persistent susceptibility to infection^{1–5}. Even more prolonged immunodeficiency arises from inadequacy in effective CD4+ T-cell and B cell reconstitution^{6–9}. Regeneration of adequate T-cell numbers and repertoire are key elements in the recovery of immunity, required for protection against infection¹⁰.

Post-transplant T-cells are derived from mature T-cells present in the donor graft, and T-cells developing *de novo* in the recipient from donor stem cells. It is likely that the latter pathway of differentiation leads to long-term immune reconstitution^{11,12}. It has been suggested that the reconstitution and maintenance of effective T-cell immunity after HSCT is dependent on education about T-cell precursors in the thymus, as shown by the correlation between increased thymic output and an increment in naïve T-cells, and broader TCR repertoires after transplantation^{13–15}. Factors that can delay thymic function include age, cell source, histocompatibility leukocyte antigen mismatches, GVHD and direct thymic damage from chemoradiotherapy^{11,16–19}.

The production of a functional T-cell receptor requires the deletion of the TCR δ gene, which is positioned within the TCR α - locus²⁰. The deleted TCR δ -genes remain present as extrachromosomal circular products called signal joints T-cell receptor excision circles (sjTRECs). These products are stable²¹, are not duplicated during mitosis, and are therefore diluted with each cellular division²². Thus, sjTRECs are markers of developmental proximity to the thymus, and their concentrations in peripheral blood can be used to estimate thymic output and evaluate thymic function in patients after stem cell transplantation²³.

It has been reported that TREC levels correlate to post-transplant outcomes^{18,24,25}. Several factors affecting thymic output have been suggested, but there is still some controversy^{11,26–28}. Herein we tested whether pre-transplant thymic function, assessed by the quantification of TRECs in the peripheral blood, may predict a prognosis in the setting of HLA-identical HSCT with a large prevalence of high risk disease. Furthermore, we also assessed TREC levels before and during acute GVHD.

Materials and methods

Patients, donors and transplantation characteristics

Over a consecutive 54-month period, 62 allogeneic stem cell transplant recipients from the UNIFESP (Bone Marrow Transplant Unit of Sao Paulo Hospital and Pediatric Bone Marrow Transplant Unit of the Pediatric Institute of Oncology) and Santa Marcelina Hospital, Brazil, between 2003 and 2007, were included. The group was comprised of children and adults who received a myeloablative preparative regimen and unmanipulated bone marrow or peripheral blood stem cell graft from an HLA-matched related donor. Patients submitted a stem cell transplant with a T-cell depleted graft and children with severe combined immunodeficiency were excluded. The risk of disease was classified based on the

International Bone Marrow Transplant Register criteria (www.cibmtr.org). Patients' characteristics, treatment and complications after transplantation are summarized in Table 1. This study was approved by the Institutional Medical Ethics Committee (number CEP 0532/02) and all patients or guardians gave informed consent before their enrollment.

Table 1. Patients characteristics.

Characteristics	N=62
Recipient age, median (range)	26(2–58)
Age over 25 y	31
Diagnosis, n	
Acute Lymphoblastic Leukemia	12
Acute Myeloid Leukemia	16
Myelodysplastic Syndrome	7
Aplastic Anemia	6
Hodgkin disease	2
Chronic Myeloid Leukemia	9
Non Hodgkin Lymphoma	10
Risk Disease*, n	
Nonmalignant	6
Malignant low risk	25
Malignant intermediate risk	14
Malignant high risk	17
GVHD Prophylaxis, n	
CSA + MTX	42
CSA	13
Others	7
Conditioning Regimen, n	
Bussulphan based	39
TBI based	13
Others	10
Engraftment in days, median (range)	14(10–25)
Median cells infused (range)	
TNC/Kg $\times 10^8$	7.0(2.3–11)
CD34/Kg $\times 10^6$	4.9(2.1–12)
Cells Source	
Bone Marrow	30
Peripheral blood stem cell	32
Major ABO incompatibility	13
Acute GVHD	
Absent	31
Grade I	8
Grade II-IV	23
Chronic GVHD	
Absent	18
Localized	11
Extensive	23
Cytomegalovirus reactivation	22
Severe Bacterial Infection	14
Fungal infection	3
Relapse	6
Follow up in months, median (range)	18(0.5–60)

CSA – cyclosporine, MTX – methotrexate, TBI – total body irradiation, TNC – total nucleated cell, GVHD – graft versus host disease.

GVHD prophylaxis, supportive care and end points

GVHD prophylaxis consisted predominantly of cyclosporine or cyclosporine and short course methotrexate. Cyclosporine levels were monitored weekly and kept at the therapeutic range of 150–300 ng/dl during the first 60 days, and then the drug dose was continuously tapered through the next 180 days, until a complete suspension, depending on disease status at the time of transplantation and the presence or absence of GVHD. Antibacterial, antifungal and antiviral prophylaxis consisted of 160mg of trimethoprim-sulfamethoxazol 3 times/week on alternative days for *Pneumocystis jiroveci*, fluconazole 150mg once/day for fungal prophylaxis and acyclovir 400mg twice/day or valacyclovir 500mg twice/day for *Herpes simplex* Surveillance cytomegalovirus (CMV) antigenemia testing was performed for all patients. Diagnosis and clinical grading of acute and chronic GVHD were performed according to established criteria^{29–31}. Bacterial, cytomegalovirus and fungal infection, sepsis and septic shock were defined according to previously established criteria^{32–34}.

Sample collection

Buffy coat samples were obtained and frozen before the conditioning regimen for the 62 patients. Thirty-one patients developed acute GVHD, and we were able to collect samples within 2 weeks before acute GVHD, and in ongoing GVHD from 16 of these patients. A control group was composed of 27 healthy donors from whom peripheral blood or bone marrow was collected before G-CSF stimulation.

Clinical data

Sixty-two patients received a myeloablative conditioning regimen followed by allogeneic HCT (Table 1). During the follow-up period of 18 months, among all patients, there were 14 severe bacterial infections, 22 CMV infections, six relapses and three fungal infections. Acute GVHD (aGVHD) occurred in 31 patients, and the incidence of grade I, II and III-IV was 13%, 18% and 19%, respectively. Among the 52(83.8%) who survived for at least 100 days after transplantation, 34(65%) developed chronic GVHD (cGVHD). As the number of relapses and fungal infection were low in our cohort, we focused the analysis on overall survival and occurrence of GVHD and severe bacterial infections.

Relative quantification of sjTREC by real-time –PCR

To perform the TREC assay, total DNA was extracted from buffy-coat by the DTAB/CTAB method (dodecyl trimethyl-ammonium bromide/cetyl trimethyl-ammonium bromide)³⁵, and DNA concentrations were adjusted to 100ng/ul. One μ l of DNA was used in all reactions. Delta deletion sjTRECs formed by δ REC- ψ J α rearrangement were amplified and quantified using SYBR-Green PCR Master Mix and an ABI 7000 instrument (Applied Biosystems, Foster City, CA). The standard curve was generated as previously described³⁶. Primers for sjTRECs were: forward primer (5'CATC-CCTTTCAACCATGC'3) and reverse primer (5'CGTGCCTAAC-CCTGCAGC'3), which produced an approximately 102 base-pair product. To improve TREC detection, two steps of amplification were performed. The first PCR conditions were: 50°C for 2 minutes and 95°C for 10 minutes, followed by 10 cycles of 95°C for 15 seconds and 63°C for 1 minute. Afterwards, 1ul was utilized in second-step amplification. The second PCR conditions were: 95°C

for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 63°C for 1 minute. TREC values obtained by RT-PCR were normalized by the proportion of lymphocytes in the blood cell count. This count was performed in the same peripheral blood sample that was utilized for DNA isolation. All DNA samples were amplified in triplicate and universal PCR precautions were taken to avoid amplification contaminations along with negative control reactions.

Statistical analysis

A receiver-operating-characteristic (ROC) curve was used to obtain the most discriminating overall survival cut-off for TREC values. Correlation between Gaussian distribution variables was calculated with Spearman correlation. Survival analysis was calculated using Kaplan-Meier plot and log rank test. Cumulative incidence analysis was calculated using death as a competing event for infections, acute or chronic GVHD. Cox proportional hazard model analysis was used to identify independent risk factors for death, infection, and acute or chronic GVHD, reviewed in the context of hematopoietic cell transplantation³⁷. The variables included in the model were those that were marginally significant, p -value ≤ 0.10 , in the univariate analyses. Non-parametric (Wilcoxon and Mann-Whitney) tests were utilized to compare TREC levels between patients with high and low disease risk, and before and during ongoing acute GVHD. All p values were two tailed and a value <0.05 was considered to indicate statistical significance. The analysis was done using the SPSS v10.0 Statistical Software (Chicago, Illinois, 1992).

Results

Correlation between TREC levels and age

Because we used a modified assay for TREC detection, we first wanted to confirm that it gives similar results to previous studies^{38,39}. To this end, we analyzed the relationship between TREC levels and age and, as expected, observed a highly significant negative correlation in healthy control individuals as well as in patients before transplantation ($r=-0.46$ and $r=-0.70$, respectively; $p\leq 0.0001$).

TREC levels and high/low risk disease

Although the pre-transplant TREC levels have been previously associated with post-transplant prognosis²⁴, we sought to repeat this analysis in our population of patients because it had a much bigger proportion of malignant high-risk diseases (27% vs 1% in 24). Indeed, we observed higher levels of TRECs in patients with no high-risk disease (nonmalignant, low and intermediate risk) compared to high-risk disease patients in both adult and children populations ($p<0.001$, Figure 1A and Figure 1B), thus making critical the inclusion of this factor in further analysis.

TREC levels and survival

Using the receiver-operating-characteristic (ROC) curve, we found a cut-off value of 134 TREC/ μ g of lymphocyte DNA that discriminated between patients that survived or not for at least 6 months after transplantation (65% of sensitivity and 60% of specificity). Based on this cut-off, patients were divided into “high” and “low” TREC groups. We observed higher survival in “high” TREC group ($p=0.028$) (Figure 2) with almost twice the survivors in the “high” TREC group vs the “low” TREC group at 18 months after transplantation (65% vs. 37%, respectively). Applying a multivariate analysis including TREC level, cell source (peripheral blood), recipient

Figure 1. **A)** Comparison of TRECs levels between high-risk disease (n=7) and others (nonmalignant, low and intermediated risk disease; n=24) in children before stem cell transplantation ($p<.001$). **B)** Comparison of TRECs levels between high risk disease (n=10) and others (nonmalignant, low and intermediated risk disease; n=21) and in adults before stem cell transplantation ($p<.001$).

Overall Survival

Figure 2. Overall survival after stem cell transplantation stratified by TREC levels. Patients with high TREC (n=35) are shown as a solid line, while the patients with low TREC (n=27) are plotted as a dashed line. Log rank $p=.028$.

age (>25 years), ABO incompatibility, sex mismatch, and high risk disease, we found that worse survival was independently associated ($p<0.05$) to low TREC values before transplantation (RR: 2.6) and high risk disease (RR: 2.2) (Table 2).

We also observed a higher incidence of grades II-IV aGVHD (Figure 3) among low TREC patients ($p=0.02$), with the incidence of aGVHD grade II-IV in high and low TREC groups of 25.7% and 57.8%, respectively. Multivariate analysis showed that low TREC levels (RR 2.5, $p<0.03$) and the antecedent of high risk disease (RR: 5.2 $p<0.025$) were independently associated with an increased incidence of aGVHD grades II-IV (Table 2).

Although we did not find an association between TREC levels and cGVHD, the expected associations of cGVHD with age⁴⁰⁻⁴² and source of cells for transplant⁴³⁻⁴⁵ were detected (Table 2).

TREC levels and infections

Next we performed the same analysis for bacterial and cytomegalovirus infection. There was a higher incidence of severe bacterial infection in the low TREC (42%) compared to the high TREC (8.5%) group ($p=0.005$) (Figure 4). Considering other variables, only low TREC level and peripheral blood as a source of transplant independently associated to bacterial infections (RRs: 6.6 and 4.2, respectively) (Table 2). CMV infection was also more

Table 2. Cox Model Analysis.

	Univariate	Multivariate		
	p	RR	CI	p
Overall Survival				
Low TREC	0.028	2.6	1.1-6.3	0.032
Age more than 25y	0.47	----	----	0.39
F→M vs others	0.64	----	----	----
Peripheral blood vs bone marrow	0.13	----	----	----
High risk disease vs others	0.05	2.2	1.1-4.7	0.044
ABO Incompatibility	0.26	----	----	----
Grade II-IV Acute GVHD				
Low TREC	0.026	2.5	1.3-5.9	0.031
Age more than 25y	0.036	----	----	0.15
F→M vs others	0.035	----	----	0.94
Peripheral blood vs bone marrow	0.40	----	----	----
High risk disease vs others	0.02	5.2	1.2-5.9	0.025
ABO Incompatibility	0.26	----	----	----
Extensive Chronic GVHD				
Low TREC	0.48	----	----	0.54
Age more than 25y	0.008	----	----	0.11
F→M vs others	0.42	----	----	----
Peripheral blood vs bone marrow	0.005	----	----	0.14
High risk disease vs others	0.51	----	----	----
ABO Incompatibility	0.19	----	----	----
Bacterial Infection				
Low TREC	0.005	6.6	1.7-25	0.006
Age more than 25y	0.090	----	----	0.95
F→M vs others	0.69	----	----	----
Peripheral blood vs bone marrow	0.037	4.2	1.2-15.6	0.028
High risk disease vs others	0.36	----	----	----
ABO Incompatibility	0.43	----	----	----
Cytomegalovirus Infection				
Low TREC	0.033	2.8	1.4-5.7	0.039
Age more than 25y	0.15	----	----	0.59
F→M vs others	0.65	----	----	----
Peripheral blood vs bone marrow	0.93	----	----	----
High risk disease vs others	0.16	----	----	----
ABO Incompatibility	0.23	----	----	----

(F→M) denotes female donor into male recipient, RR indicates relative ratio
95% CI, 95% confidence interval

frequently observed in the low TREC patients (63% vs. 22%, $p<0.03$) (Figure 5). Furthermore, low TREC values before transplantation and high risk disease had independent impact on the incidence of CMV infection (Table 2).

Post-transplant TREC levels in relation to acute GVHD

With the result of pre-transplant thymus function associated with aGVHD, we wondered if thymus output after transplantation (i.e. TREC levels) would also be related to aGVHD. For this purpose we assessed TRECs levels in blood samples collected before (pre-aGVHD) and during aGVHD from 16 patients. The time period between pre-aGVHD and aGVHD samples was 11.5 ± 8 days. Comparing TREC levels between pre-aGVHD and aGVHD samples, we observed increased levels during aGVHD (147 vs. 480 TRECs/ μ g of lymphocyte DNA, $p=0.015$). One could suggest that this difference may be explained by the gradual increase in TREC levels after transplantation. In which case, the more time between pre-aGVHD and GVHD samples, the larger the increment in TREC levels. The analysis, however, showed no correlation between distance in time and TREC levels (Dataset 1). In addition, there was no correlation between the number of days after transplantation (17 to 49 days) and TREC levels in this time period. This indicates that we have observed an increase in TREC levels associated with aGVHD independently on the overall increase in TREC levels after transplantation reported previously^{25,46}.

Grade II-IV Acute GVHD

Figure 3. Cumulative incidence of acute GVHD (%) in the low-level TREC (n=27) group compared with the high-level group (n=35). Patients with high TREC are shown as a solid line, while the patients with low TREC are plotted as a dashed line. Log rank $p=.026$.

Grade II-IV Acute GVHD

Figure 4. Cumulative incidence of bacterial infection in the low level TREC (N=27) group compared with the high level group (N=35). Patients with high TREC are shown as a solid line, while the patients with low TREC are plotted as a dashed line. Log rank $p=.005$.

Cytomegalovirus Infection

Figure 5. Cumulative incidence of cytomegalovirus infection in the low-level TREC (n=27) group compared with the high-level group (n=35). Patients with high TREC are shown as a solid line, while the patients with low TREC are plotted as a dashed line. Log rank $p=.027$.

Dataset 1. sjTREC before transplant - patient dataset

<http://dx.doi.org/10.5256/f1000research.7330.d108555>

1. **Patient:** patient's number after anonymization
2. **Age:** patient's age in years
3. **sjTREC copies:** TRECs copynumber before transplantation
4. **Diagnosis:** disease that motivated the transplant indication
5. **Donor gender:** gender of donor
6. **Receptor gender:** gender of receptor
7. **ABO donor:** ABO blood group in donor
8. **ABO receptor:** ABO blood group in receptor before transplantation
9. **Cell source:** cell source from donor (peripheral blood or bone marrow)
10. **Conditioning regimen:** chemotherapy and/or radiotherapy utilized before stem cell infusion (Flu- fludarabine; Cy – cyclophosphamide; Mel – melphalan; TBI – total body irradiation; VP – etoposide)
11. **GVHD prophylaxis:** drugs utilized to prevent acute graft versus host disease (CSA – cyclosporine; MTX – methotrexate and Methylprednisolone)
12. **Acute GVHD grading:** Glucksberg criteria for acute GVHD classification
13. **Diagnosis Acute GVHD II-IV -days after transplant:** when the diagnosis of acute GVHD was performed after transplantation (in days).
14. **CMV reactivation:** occurrence or not of cytomegalovirus reaction after stem cell transplantation
15. **CMV reactivation after transplant, days:** when the diagnosis of cytomegalovirus reactivation was performed after transplantation (in days)
16. **Bacterial infection:** occurrence or not of bacterial infection after stem cell transplantation
17. **Bacterial infections after transplant, days:** when the diagnosis of bacterial infection was performed after transplantation (in days)
18. **Extensive chronic GVHD:** occurrence or not of extensive chronic graft versus host disease after stem cell transplantation
19. **Extensive Chronic GVHD diagnosis, days after transplant:** when the diagnosis of extensive chronic graft versus host disease was performed after transplantation (in days)
20. **Follow up, in months:** follow up after stem cell transplantation (in months)
21. **Status:** patient status after transplantation (alive or death)

sjTREC pre and ongoing aGVHD before spreadsheet

1. **Patient:** patient' number after anonymization
2. **Acute GVHD diagnosis, days:** when the diagnosis of acute GVHD was performed after transplantation (in days).
3. **Sample collected before aGVHD, days:** number of the days before emergence of acute GVHD when the sample was harvested
4. **Sample collected - Ongoing GVHD, days:** the day after stem cell transplant when the sample was harvested. All patients was under acute GVHD activity
5. **Steroids use, days:** number of the days under steroids use when the sample was harvested.
6. **Grade acute GVHD:** Glucksberg criteria for acute GVHD classification
7. **Pre acute GVHD:** sjTREC copies number before acute GVHD patient
8. **Ongoing acute GVHD:** sjTREC copies number in ongoing acute GVHD patient
9. **Corticoids pre acute GVHD:** if patient had taken steroids before acute GVHD diagnosis
10. **Corticoids ongoing GVHD:** if patient was taking steroids when the acute GVHD diagnosis was performed.

Discussion

It has been postulated that a good immune reconstitution after HSCT means a better prognosis for the patients under stem cell transplantation^{47,48}. The evaluation of immune reconstitution assessed by TREC quantification has been a matter of research. Here we analyzed the impact of pre-transplant thymic function in patients undergoing an HSCT. Although it has been reported that TREC levels correlate to post-transplant outcome^{5,18,24,25,49} it was not clear whether these observations are results of association of TREC levels with the severity of disease risk, especially considering a strong relation between disease risk and TREC levels (Figure 1A and 1B). In contrast to previously published work²⁴, we analyzed a cohort of patients with a much higher proportion of malignant high-risk diseases, which allowed us to address the above mentioned concern. Thus, our results suggest that TREC levels is an independent marker for complications after HSCT.

Interestingly, we found that low TREC levels were associated with a higher probability of bacterial infection and aGVHD. This suggests that TREC levels are not just associated with high or low T-cell response after transplantation, but rather reflect an overall response to damage as well as potential capacity to establish immune homeostasis after transplantation. Indeed, in the setting of high-risk disease, patients were treated prior to transplantation with multiples

cycles of chemotherapy and/or radiotherapy, potential contributors to thymic injury. Cortical and medullary thymic epithelial cells are dramatically altered after radiotherapy and chemotherapy^{14,50} as damage of thymic stroma precludes normal thymocyte development⁵¹ and probably the generation of TRECs.

Diminished levels of TRECs have been reported during chronic GVHD, or with a previous history of acute or chronic GVHD^{10,11}. However, others studies have failed to demonstrate a direct association between TRECs levels and acute or chronic GVHD^{46,52}. In a mouse model of aGVHD, TRECs levels were analyzed in the spleen and thymus, but not in blood, making difficult the direct comparison of these experiments in mice with our results⁵³. TREC levels during acute GVHD have not been reported in patients. We, however, observed an increase of TREC levels during ongoing acute GVHD in comparing to the TREC levels two weeks before aGVHD. Interestingly, herein observed increases in TREC levels during acute GVHD are very similar to our previous observation of increased TREC levels during acute cardiac rejection³⁸ suggesting that similar events may be occurring in these two pathological processes in the blood. The explanation for these results, on one hand, could be an increased output of TREC positive naïve T-cells into circulation, stimulated by the ongoing immune response²⁵. On the other hand, this result might indicate the exit of activated/effector cells from the circulation into target tissues and a consequent increase in a naïve T-cell pool in the blood. Keeping in mind the limitations of our analysis (a small number of patients and an indirect comparison), additional research is necessary in order to draw definitive conclusions about changes in post-transplant TREC levels in relation to aGVHD.

In this work we demonstrated a relatively easy way of normalizing TREC values determined by RT-PCR, without the concomitant need for flow cytometry to estimate the numbers of CD3+ cells. Notably, a cut-off established by ROC-curve analysis, which could discriminate between a good or bad prognosis, was similar to the value found at the 60th percentile, after TREC values distribution in percentiles from published works^{24,25}. Using our cut-off, we

observed very similar results when compared to published results in relation to survival, acute GVHD, bacterial and CMV infections²⁴. Finally, we have shown disease risk as one the most important sources of TREC level variability in patients before HSCT. Further studies should consider this factor in order to establish how TREC levels can be used in diagnostics.

In conclusion, our results further support the idea that the thymic competence before transplantation is a critical factor for a good prognosis after hematopoietic stem cell transplantation.

Data availability

F1000Research: Dataset 1. sjTREC before transplant - patient dataset, [10.5256/f1000research.7330.d108555](https://doi.org/10.5256/f1000research.7330.d108555)

Author contributions

AMA- designed the study, participated in patients enrolment, performed experiments, analyzed results, wrote manuscript; KK- performed experiments, analyzed results; AS- participated in patients enrolment and study design; JSRO- designed the study, participated in patients enrolment, contributed to analyses of results; MGL- designed the study, contributed to analyses of results, contributed to guidance of the laboratory part of the project; NS and AM- designed the study, analyzed results, wrote manuscript, provided overall leadership of the project. All authors have seen and agreed to the final content of the manuscript.

Competing interests

The authors declared no competing interests.

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References

- Marks DI, Cullis JO, Ward KN, *et al.*: **Allogeneic bone marrow transplantation for chronic myeloid leukemia using sibling and volunteer unrelated donors. A comparison of complications in the first 2 years.** *Ann Intern Med.* 1993; **119**(3): 207–214.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hansen JA, Gooley TA, Martin PJ, *et al.*: **Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia.** *N Engl J Med.* 1998; **338**(14): 962–968.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Hongeng S, Krance RA, Bowman LC, *et al.*: **Outcomes of transplantation with matched-sibling and unrelated-donor bone marrow in children with leukaemia.** *Lancet.* 1997; **350**(9080): 767–771.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Kernan NA, Bartsch G, Ash RC, *et al.*: **Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program.** *N Engl J Med.* 1993; **328**(9): 593–602.
[PubMed Abstract](#) | [Publisher Full Text](#)
- Wils EJ, van der Holt B, Broers AE, *et al.*: **Insufficient recovery of thymopoiesis predicts for opportunistic infections in allogeneic hematopoietic stem cell transplant recipients.** *Haematologica.* 2011; **96**(12): 1846–1854.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Small TN, Papadopoulos EB, Boulad F, *et al.*: **Comparison of immune reconstitution after unrelated and related T-cell-depleted bone marrow transplantation: effect of patient age and donor leukocyte infusions.** *Blood.* 1999; **93**(2): 467–480.
[PubMed Abstract](#)
- Small TN, Avigan D, Dupont B, *et al.*: **Immune reconstitution following T-cell depleted bone marrow transplantation: effect of age and posttransplant graft**

- rejection prophylaxis. *Biol Blood Marrow Transplant*. 1997; 3(2): 65–75.
[PubMed Abstract](#)
8. Storek J, Gooley T, Witherspoon RP, *et al.*: Infectious morbidity in long-term survivors of allogeneic marrow transplantation is associated with low CD4 T cell counts. *Am J Hematol*. 1997; 54(2): 131–138.
[PubMed Abstract](#) | [Publisher Full Text](#)
 9. Storek J, Espino G, Dawson MA, *et al.*: Low B-cell and monocyte counts on day 80 are associated with high infection rates between days 100 and 365 after allogeneic marrow transplantation. *Blood*. 2000; 96(9): 3290–3293.
[PubMed Abstract](#)
 10. Talvensaari K, Clave E, Douay C, *et al.*: A broad T-cell repertoire diversity and an efficient thymic function indicate a favorable long-term immune reconstitution after cord blood stem cell transplantation. *Blood*. 2002; 99(4): 1458–1464.
[PubMed Abstract](#) | [Publisher Full Text](#)
 11. Weinberg K, Blazar BR, Wagner JE, *et al.*: Factors affecting thymic function after allogeneic hematopoietic stem cell transplantation. *Blood*. 2001; 97(5): 1458–1466.
[PubMed Abstract](#) | [Publisher Full Text](#)
 12. Douek DC, Vescio RA, Betts MR, *et al.*: Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. *Lancet*. 2000; 355(9218): 1875–1881.
[PubMed Abstract](#) | [Publisher Full Text](#)
 13. Dumont-Girard F, Roux E, van Lier RA, *et al.*: Reconstitution of the T-cell compartment after bone marrow transplantation: restoration of the repertoire by thymic emigrants. *Blood*. 1998; 92(11): 4464–4471.
[PubMed Abstract](#)
 14. Mackall CL, Fleisher TA, Brown MR, *et al.*: Age, thymopoiesis, and CD4+ T-lymphocyte regeneration after intensive chemotherapy. *N Engl J Med*. 1995; 332(3): 143–149.
[PubMed Abstract](#) | [Publisher Full Text](#)
 15. Roux E, Dumont-Girard F, Starobinski M, *et al.*: Recovery of immune reactivity after T-cell-depleted bone marrow transplantation depends on thymic activity. *Blood*. 2000; 96(6): 2299–2303.
[PubMed Abstract](#)
 16. Douek DC, McFarland RD, Keiser PH, *et al.*: Changes in thymic function with age and during the treatment of HIV infection. *Nature*. 1998; 396(6712): 690–695.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Fallen PR, McGreavey L, Madrigal JA, *et al.*: Factors affecting reconstitution of the T cell compartment in allogeneic haematopoietic cell transplant recipients. *Bone Marrow Transplant*. 2003; 32(10): 1001–1014.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Sairafi D, Mattsson J, Uhlin M, *et al.*: Thymic function after allogeneic stem cell transplantation is dependent on graft source and predictive of long term survival. *Clin Immunol*. 2012; 142(3): 343–350.
[PubMed Abstract](#) | [Publisher Full Text](#)
 19. Politikos I, Boussiotis VA: The role of the thymus in T-cell immune reconstitution after umbilical cord blood transplantation. *Blood*. 2014; 124(22): 3201–3211.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 20. Livak F, Schatz DG: Identification of V(D)J recombination coding end intermediates in normal thymocytes. *J Mol Biol*. 1997; 267(1): 1–9.
[PubMed Abstract](#) | [Publisher Full Text](#)
 21. Livak F, Schatz DG: T-cell receptor alpha locus V(D)J recombination by-products are abundant in thymocytes and mature T cells. *Mol Cell Biol*. 1996; 16(2): 609–618.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Takeshita S, Toda M, Yamagishi H: Excision products of the T cell receptor gene support a progressive rearrangement model of the alpha/delta locus. *EMBO J*. 1989; 8(11): 3261–3270.
[PubMed Abstract](#) | [Free Full Text](#)
 23. Wu X, Zhu K, Du X, *et al.*: Frequency analysis of TRBV subfamily sJTRECcs to characterize T-cell reconstitution in acute leukemia patients after allogeneic hematopoietic stem cell transplantation. *J Hematol Oncol*. 2011; 4: 19.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 24. Clave E, Rocha V, Talvensaari K, *et al.*: Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. *Blood*. 2005; 105(6): 2608–2613.
[PubMed Abstract](#) | [Publisher Full Text](#)
 25. Chen X, Barfield R, Benaim E, *et al.*: Prediction of T-cell reconstitution by assessment of T-cell receptor excision circle before allogeneic hematopoietic stem cell transplantation in pediatric patients. *Blood*. 2005; 105(2): 886–893.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Przybylski GK, Kreuzer KA, Siegert W, *et al.*: No recovery of T-cell receptor excision circles (TRECs) after non-myeloablative allogeneic hematopoietic stem cell transplantation is correlated with the onset of GVHD. *J Appl Genet*. 2007; 48(4): 397–404.
[PubMed Abstract](#) | [Publisher Full Text](#)
 27. Clave E, Bussan M, Douay C, *et al.*: Acute graft-versus-host disease transiently impairs thymic output in young patients after allogeneic hematopoietic stem cell transplantation. *Blood*. 2009; 113(25): 6477–6484.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Castermans E, Hannon M, Dutrieux J, *et al.*: Thymic recovery after allogeneic hematopoietic cell transplantation with non-myeloablative conditioning is limited to patients younger than 60 years of age. *Haematologica*. 2011; 96(2): 298–306.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. Glucksberg H, Storb R, Fefer A, *et al.*: Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation*. 1974; 18(4): 295–304.
[PubMed Abstract](#) | [Publisher Full Text](#)
 30. Sullivan KM, Agura E, Anasetti C, *et al.*: Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol*. 1991; 28(3): 250–259.
[PubMed Abstract](#)
 31. Przepiorka D, Weisdorf D, Martin P, *et al.*: 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant*. 1995; 15(6): 825–828.
[PubMed Abstract](#)
 32. Ljungman P, De Bock R, Cordonnier C, *et al.*: Practices for cytomegalovirus diagnosis, prophylaxis and treatment in allogeneic bone marrow transplant recipients: a report from the Working Party for Infectious Diseases of the EBMT. *Bone Marrow Transplant*. 1993; 12(4): 399–403.
[PubMed Abstract](#)
 33. American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. *Crit Care Med*. 1992; 20(6): 864–874.
[PubMed Abstract](#)
 34. Lossos IS, Breuer R, Or R, *et al.*: Bacterial pneumonia in recipients of bone marrow transplantation. A five-year prospective study. *Transplantation*. 1995; 60(7): 672–678.
[PubMed Abstract](#)
 35. Gustinich S, Manfioletti G, Del Sal G, *et al.*: A fast method for high-quality genomic DNA extraction from whole human blood. *Biotechniques*. 1991; 11(3): 298–300, 302.
[PubMed Abstract](#)
 36. Shulzhenko N, Morgun A, Rampim GF, *et al.*: Monitoring of intragraft and peripheral blood TIRC7 expression as a diagnostic tool for acute cardiac rejection in humans. *Hum Immunol*. 2001; 62(4): 342–347.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Klein JP, Rizzo JD, Zhang MJ, *et al.*: Statistical methods for the analysis and presentation of the results of bone marrow transplants. Part I: unadjusted analysis. *Bone Marrow Transplant*. 2001; 28(10): 909–915.
[PubMed Abstract](#) | [Publisher Full Text](#)
 38. Morgun A, Shulzhenko N, Socorro-Silva A, *et al.*: T cell receptor excision circles (TRECs) in relation to acute cardiac allograft rejection. *J Clin Immunol*. 2004; 24(6): 612–616.
[PubMed Abstract](#) | [Publisher Full Text](#)
 39. Svaldi M, Lanthaler AJ, Dugas M, *et al.*: T-cell receptor excision circles: a novel prognostic parameter for the outcome of transplantation in multiple myeloma patients. *Br J Haematol*. 2003; 122(5): 795–801.
[PubMed Abstract](#) | [Publisher Full Text](#)
 40. Ringden O, Paulin T, Lönnqvist B, *et al.*: An analysis of factors predisposing to chronic graft-versus-host disease. *Exp Hematol*. 1985; 13(10): 1062–1067.
[PubMed Abstract](#)
 41. Atkinson K, Horowitz MM, Gale RP, *et al.*: Risk factors for chronic graft-versus-host disease after HLA-identical sibling bone marrow transplantation. *Blood*. 1990; 75(12): 2459–2464.
[PubMed Abstract](#)
 42. Kondo M, Kojima S, Horibe K, *et al.*: Risk factors for chronic graft-versus-host disease after allogeneic stem cell transplantation in children. *Bone Marrow Transplant*. 2001; 27(7): 727–730.
[PubMed Abstract](#) | [Publisher Full Text](#)
 43. Mohty M, Kuentz M, Michallet M, *et al.*: Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood*. 2002; 100(9): 3128–3134.
[PubMed Abstract](#) | [Publisher Full Text](#)
 44. Anderson D, DeFor T, Burns L, *et al.*: A comparison of related donor peripheral blood and bone marrow transplants: importance of late-onset chronic graft-versus-host disease and infections. *Biol Blood Marrow Transplant*. 2003; 9(1): 52–59.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Cutler C, Giri S, Jeyapalan S, *et al.*: Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J Clin Oncol*. 2001; 19(16): 3685–3691.
[PubMed Abstract](#)
 46. Hochberg EP, Chillemi AC, Wu CJ, *et al.*: Quantitation of T-cell neogenesis in vivo after allogeneic bone marrow transplantation in adults. *Blood*. 2001; 98(4): 1116–1121.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Parkman R, Cohen G, Carter SL, *et al.*: Successful immune reconstitution decreases leukemic relapse and improves survival in recipients of unrelated cord blood transplantation. *Biol Blood Marrow Transplant*. 2006; 12(9): 919–927.
[PubMed Abstract](#) | [Publisher Full Text](#)
 48. Kim DH, Sohn SK, Won DI, *et al.*: Rapid helper T-cell recovery above 200 × 10⁶/l

- at 3 months correlates to successful transplant outcomes after allogeneic stem cell transplantation. *Bone Marrow Transplant*. 2006; **37**(12): 1119–1128.
[PubMed Abstract](#) | [Publisher Full Text](#)
49. Wils EJ, Rombouts EJ, van Mourik I, *et al.*: **Stem cell factor consistently improves thymopoiesis after experimental transplantation of murine or human hematopoietic stem cells in immunodeficient mice.** *J Immunol*. 2011; **187**(6): 2974–2981.
[PubMed Abstract](#) | [Publisher Full Text](#)
 50. Adkins B, Gandour D, Strober S, *et al.*: **Total lymphoid irradiation leads to transient depletion of the mouse thymic medulla and persistent abnormalities among medullary stromal cells.** *J Immunol*. 1988; **140**(10): 3373–3379.
[PubMed Abstract](#)
 51. Hollander GA, Wang B, Nichogiannopoulou A, *et al.*: **Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes.** *Nature*. 1995; **373**(6512): 350–353.
[PubMed Abstract](#) | [Publisher Full Text](#)
 52. Storek J, Joseph A, Espino G, *et al.*: **Immunity of patients surviving 20 to 30 years after allogeneic or syngeneic bone marrow transplantation.** *Blood*. 2001; **98**(13): 3505–3512.
[PubMed Abstract](#) | [Publisher Full Text](#)
 53. Krenger W, Schmidlin H, Cavadini G, *et al.*: **On the relevance of TCR rearrangement circles as molecular markers for thymic output during experimental graft-versus-host disease.** *J Immunol*. 2004; **172**(12): 7359–7367.
[PubMed Abstract](#) | [Publisher Full Text](#)
 54. de Moraes Arantes A, Seber A, Kawemura K, *et al.*: **Dataset 1 in: High pre-transplant TREC levels indicate good prognosis after hematopoietic stem cell transplantation.** *F1000Research*. 2015.
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The paper presented by de Moraes Arantes *et al.* corroborates those of others that suggest the potential significance of pretransplant TREC levels on, infection, GVHD and survival outcomes after allogeneic transplant. While very interesting, it is difficult to make firm conclusions considering the fact that there are numerous potential interactions that could influence outcome. For example, age, use of TBI and cell source have themselves been associated with risks of acute and/or chronic GVHD. An older patient might be expected to have lower pretransplant TRECs because of age. A younger with a non malignant patient might be expected to have higher TREC's because of age and lack of prior treatment. Although not shown, use of bone marrow is more likely in children than in adults. As many of these issues are not random, it is possible that these intriguing findings are not related to pretransplant TRECs but to some other factor or group of factors. Still, the data argues for larger studies as the relevant technologies are more readily available today.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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The authors examine the effect of pre transplant TREC levels and outcome of hematopoietic transplantation. TRECs are new thymic emigrants and are related to thymic function.

Critique

The authors confirm the findings reported by Clave et al ten years ago (their reference 24), that higher pre transplant TREC levels are associated with less GVHD, fewer infections and better survival.

Clave E, Rocha V, Talvensaari K, *et al.*: Prognostic value of pretransplantation host thymic function in HLA-identical sibling hematopoietic stem cell transplantation. *Blood*. 2005; 105(6): 2608–2613.

These findings are not surprising in that improved thymic function would enhance immune reconstitution, which would be expected to be protective against infection. Thymic dysregulation occurs with GVHD.

Competing Interests: No competing interests were disclosed.

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