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Malnutrition and Immune Cell Subsets in Children Undergoing Kidney Transplantation

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Abstract

Background: Malnutrition, including obesity and undernutrition, among children is increasing in prevalence and is common among children on renal replacement therapy. The effect of malnutrition on the pre-transplant immune system and how the pediatric immune system responds to the insult of both immunosuppression and allotransplantation is unknown. We examined the relationship of nutritional status with post-transplant outcomes and characterized the peripheral immune cell phenotypes of children from the Immune Development of Pediatric Transplant (IMPACT) study.

Methods: Ninety-eight patients from the IMPACT study were classified as having obesity, undernutrition, or normal nutrition-based pre-transplant measurements. Incidence of infectious and

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Author Contributions

BIS conceived of the design and analysis and wrote the paper. HJL performed the analysis and wrote the paper. RE, PG, EFR, MS, BW, OMM, NJM, and ADK wrote the paper. LS generated the data. CZ performed the analysis. ETC conceived and designed the analysis and wrote the paper.

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The authors of this manuscript have no conflicts of interest to disclose.

alloimmune outcomes at 1-year post-transplantation were compared between nutritional groups using Gray's test and Fine-Gray subdistribution hazards model. Event-free survival was estimated by Kaplan-Meier method and compared between groups. Differences in immune cell subsets between nutritional groups over time were determined using generalized estimating equations accounting for the correlation between repeated measurements.

Results: We did not observe that nutritional status was associated with infectious or alloimmune events or event-free survival post-transplant. We demonstrated that children with obesity had distinct T and B cell signatures relative to those with undernutrition and normal nutrition, even when controlling for immunosuppression. Children with obesity had a lower frequency of CD8 Tnaive cells 9-month post-transplant ($p<0.001$), a higher frequency of CD4 CD57+PD1- T cells, and lower frequencies of CD57-PD1+ CD8 and CD57-PD1- CD8 T cells at 12-month transplant ($p<0.05$ for all).

Conclusions: Children with obesity have distinct immunophenotypes that may influence the tailoring of immunosuppression.

1. Introduction

Pediatric kidney transplantation is the preferred method of renal replacement in children and clinical outcomes have progressively improved over the past 25 years[1]. Unfortunately, the prevalence of malnutrition encompassing both obesity and undernutrition is rising among both adults[2] and children[3]. More specifically, obesity among pediatric patients on renal replacement therapy (RRT) and transplant recipients is high, with up to 12% of patients on RRT with obesity in one European study[4] and 18% in a US study[5]. Between 8 and 13% of patients undergoing transplants in the US have obesity, with an increased prevalence in more contemporary cohorts[6 7]. Furthermore, in a study that examined the body mass index (BMI) of different organ transplant recipients, kidney transplant recipients were most likely to be overweight or have obesity (19% vs. 11% in liver and 6% in heart transplant recipients)[8].

Obesity is known to be proinflammatory[12] and when present at the time of transplant has been associated with multiple adverse events in pediatric kidney transplantation. First, obesity has been shown to correlate with metabolic syndrome among transplant recipients with manifestations including hypertension, dyslipidemia, and glucose intolerance[13, 14]. Additionally, patients undergoing kidney transplants who were overweight at baseline, or who were exposed to steroids were most likely to develop obesity in a study of pediatric solid organ recipients[8]. Finally, obesity has also been associated with worse post-transplant outcomes including increases in rates of delayed graft function (DGF), acute rejection, and increased hospitalizations as well as decreased graft, and patient survival[7, 15–17].

With regards to undernutrition, chronically ill children in general are at higher risk for undernutrition with one study finding that greater than 30% of chronically ill children attending outpatient visits are malnourished[9]. Furthermore, end-stage renal disease is associated with undernutrition with approximately 20–45% of children classified with this malnourished state[10]. Importantly, we have also previously shown that undernutrition may impair transplant recipients' ability to mount protective immune responses to common viral pathogens[11].

Increasingly, it is understood that nutritional status and the immune system reciprocally influence each other. Whereas obesity may enrich for certain T cell subsets, certain T cell subsets may influence glycemic homeostasis[18–20]. We have previously shown that pediatric patients on RRT or undergoing kidney transplants possess distinct T cell signatures characterized by an increase in senescent cells markedly by a greater frequency of CD57+ CD8 T cells than age-matched controls[21]. Others have shown that multiple early childhood events can influence the pediatric immune system[22].

Given the association of malnutrition with poor clinical outcomes after pediatric kidney transplantation and the potential for nutritional status to modulate immunologic function, we sought to understand the effects of pre-transplantation (baseline) nutritional status on relevant immune cell populations in a well-characterized cohort of pediatric kidney transplant recipients. We utilized the Immune Development in Pediatric Transplant (IMPACT) cohort to determine how baseline nutritional status (obesity, undernourished, or normal nutrition) was related to T and B cell phenotypes and clinical outcomes over time in the first year post-transplant.

2. Methods

2.1 Patients and Study Design

We performed a secondary analysis of the IMPACT study[11] examining the relationship of nutritional status with immunologic characteristics. Briefly, the IMPACT study was a multi-center prospective observational trial (NCT00951353) that ran from July 2009-March 2012. Pediatric kidney transplant recipients aged 1–20 years, were monitored for the first year after their transplant. The Institutional Review Board of each site reviewed and approved the protocol. Following enrollment, patient anthropometric measures (including height weight and body mass index [BMI]) were collected as well as general baseline characteristics. Patients were clinically managed according to each site's preference without specific protocol. Regimens including induction with anti-thymocyte globulin (ATG) and/or IL-2 receptor blockade (Daclizumab or Basiliximab) and maintenance immunosuppression with or without steroids were used. Patients also were managed with calcineurin inhibitors, mTOR inhibitors, and antimetabolites. One hundred six (106) of the 125 patients enrolled underwent kidney transplantation. Ninety-eight (98) patients with complete height and weight data at baseline were included in the present study. Patients were stratified by nutritional status at baseline as obesity (BMI z-score greater than +2 standard deviations [SD]), undernutrition (BMI, or weight-for-age, or height-for-age z-scores lower than −2 SDs), or normal nutrition based upon World Health Organization classification[23]. Patients who had BMI z-scores > 2 SD but height-for-age Z-scores < −2 SD were classified in the obesity but not undernutrition group.

2.2 Flow Cytometry

Peripheral blood was obtained at multiple time points including baseline (pre-transplant), 1,3,6,9, and 12 months for flow cytometry. Memory subsets of CD4+ and CD8+ T cells were analyzed using the following categorization: naïve (Tnaïve, CD45RA+CCR7+), central memory (Tcm, CD45RA-CCR7+), effector memory (Tem, CD45RA-CCR7-), and effector

memory RA (Temra, CD45RA+CCR7-). Samples were also examined for CD57 and PD1, considered markers of senescence/exhaustion. T regulatory (Treg) cells were defined as CD4+CD25+CD127- expression.

We also assessed the B cell subset frequency which included: naïve B cells (CD27-IgD+), transitional B cells (CD27-CD38bright), and plasma cells (CD27+CD38bright). Gating strategies are as previously described[24].

2.3 Statistical Analysis

Missing data was present in the flow cytometry data with a maximum missing percentage of 29% (for Treg at 9-month) per cell line per time point. Given that missing data was only present in the flow outcome data, a complete case analysis was conducted[25]. A pre-selected list of confounders, including age, sex, race, ATG use, CMV/EBV serostatus, etc., was examined between nutritional groups using Kruskal-Wallis tests for continuous variables and chi-square tests or Fisher's exact tests for categorical variables. ATG use was the only variable that was not balanced between nutritional groups and was included as a covariate in all regression models.

The primary clinical outcome was the first clinical event after kidney transplantation, defined as experiencing either the alloimmune or infectious outcomes, as previously described[11, 24]. Patients were followed from the time of transplantation until the alloimmune or infectious outcomes, or 1-year post-transplantation, whichever occurred first. The alloimmune and infectious events were considered competing risks because the occurrence of one event altered the risk of having another event. The cumulative incidences of the alloimmune and infectious outcomes were compared between nutritional groups at 1-year post-transplantation using Gray's test and Fine-Gray subdistribution hazards models. Event-free survival was estimated by Kaplan-Meier method and compared between groups using the log-rank test and Cox proportional hazards model.

To investigate the effects of nutritional status on both the absolute count and frequency of T cell subsets over time, linear regression models using generalized estimating equations to account for within-subject correlation were fit. The models included nutritional status, time (baseline, 1, 3, 6, 9, 12 months), interaction between time and nutritional status, and ATG usage. Interaction terms were further removed from the model if not significant. To protect against multiple comparisons, pairwise comparisons between time points were made only if the overall omnibus test was statistically significant.

All statistical tests used a two-sided significance level of 0.05 without adjusting for multiplicity. All analyses were performed using R 3.5.1 (R Core Team, Vienna, Austria) and SAS 9.4 (SAS Institute, Cary, NC).

3. Results

Cohort Characteristics

There was a total of 98 children included in the study. Fifty-eight (58) had normal nutrition, 11 had obesity, and 29 had undernutrition at baseline. There were no significant differences

between the groups in terms of demographics or immunosuppressive treatment, except that patients with obesity were more likely to undergo thymoglobulin induction (p=0.019, Table 1).

Nutritional status is not associated with clinical outcomes

In this secondary analysis, we examined the effect of nutritional status on either alloimmune or infectious complications after transplantation (regression results in Supplemental Table 1). We did not see any association with either overall event-free survival (log-rank test p=0.5), cumulative incidence of alloimmune event (Gray's test p=0.69), or cumulative incidence of infection event (Gray's test p=0.92), however, this study was not powered to examine this association. Of note, 19 patients (21%) changed nutritional category at 12 months compared with baseline with the majority of these changes occurring as a transition to normal weight from either obesity (n=4) or undernourished (n=9).

Nutritional status is associated with memory T cell subsets

We next examined the association of nutritional status with CD4 and CD8 memory markers. Although there was no difference in CD4 memory T cell frequency over time (data not shown), the frequency of CD8 Tnaive cells was decreased and that of CD8 Temra cells was increased in the obesity group at later time points compared to the normal nutrition and undernutrition groups (Figure 1 A & C). When examining absolute counts, there was a greater number of CD8 Temra cells in the obesity group compared to the undernourished and normal growth groups at later time points in a model which included an interaction with time (Supplemental Figure 1C). Of note, there were no differences in the frequency or absolute count of CD4 Treg between groups (data not shown).

Nutritional status is associated with markers of terminal differentiation

Among CD4 T cells, there was an increased frequency of CD57+PD1- CD4 T cells at 12 months post-transplant among patients with obesity (+1.82%, 95% CI: 0.46% to 3.17%, p=0.0086, Figure 2C).

There were differences in frequency of all four CD8 T cell CD57/PD1 subsets. There were decreased frequencies of CD8+CD57-PD1- T cells at 9- and 12-months post-transplant in the group with obesity and a decreased frequency of CD8+CD57-PD1+ T cells at 12 months compared to patients with normal nutrition(Figure 3 A & D). Overall, the mean frequency of CD8+CD57+PD1+ and CD8+CD57+PD1- in the children with obesity were higher compared to the normal nutrition and undernutrition groups (Figure 3 B & C). Similar trends among absolute counts of CD8 T cells for CD57/PD1 subsets were also seen (Supplemental Figure 2).

Nutritional status is associated with Naïve B cell frequencies but differences do not persist

Compared to normal nutrition patients, patients with obesity had a higher frequency of naïve B cells at baseline through 3 months post-transplant, though this difference did not persist over time (Figure 4). There were no differences in the absolute count of B cells over time.

4. Discussion

In the present study, we do not observe that children with malnutrition either obesity or undernutrition undergoing renal transplantation had significantly different alloimmune and infectious outcomes compared to children with normal nutrition. However, we report a distinct T and B cell signature among children with obesity compared to those with normal nutrition or undernutrition. Children with obesity had large differences in their T cell phenotypes with more memory polarized and senescent CD8 T cell phenotypes that, though present at baseline, are also accentuated over time. Additionally, children with obesity had a higher frequency of naïve B cells at baseline, which persisted until 3 months post-transplantation. These findings give potential insight into the immunological consequences of obesity, the choice of immunosuppression, and its impact on pediatric kidney transplantation.

Previously, we have shown that ATG induction is associated with broad changes in the pediatric immune system regardless of nutritional status[24]. Some of these changes, including an increase in certain memory and/or senescent T cell types in both the CD4 and CD8 compartment, were observed to a greater extent in patients with obesity compared to those with normal nutrition and undernutrition. These findings point to the potential for an interaction between obesity and induction that may be of clinical significance, especially in the context of a pediatric immune system that is undergoing large developmental changes. Specifically, an increase in mature T cell phenotypes over time may lead to increased T cell help and therefore a change in the relative frequency of naïve B cells, especially in the context of many new alloantigens. This may be why we see a convergence in the percentage of naïve B cells over time between children with and without obesity.

Previous research on the role of obesity in transplantation has shown that obesity is generally associated with worsened graft outcomes in both pediatric and adult populations[26–28]. In children, obesity and overweight status have been associated with both decreased death-censored graft survival and hazard of death in more contemporary studies[5, 7, 16, 29]. The immunological changes seen in our cohort with obesity are potentially concordant with this finding. As T cells become senescent they become increasingly dysregulated and are more prone to aberrant activation[30] which may lead to activation, inflammation, and ultimately graft damage even in the face of immunosuppressive medications.

Additionally, it is well known that obesity modulates the immune system[18–20]. One interesting finding is that obesity leads to a dysregulated response to infection in a mouse model with over-exuberant memory T cell activation[18]. Our findings are consistent with this literature, showing that indeed obesity may act as a chronic immune stimulus and is associated with polarization towards a memory T cell phenotype over time, especially in the CD8 compartment. Another direct effect of obesity is an accelerated involution of the thymus[31]. This could have two potential effects. First, this may lead to a greater amount of repopulation occurring via peripheral homeostatic proliferation which is known to skew towards memory phenotypes both generally[32] and in response to polyclonal antibody depletion specifically[33], which is consistent with our data. Secondly, it may affect the

efficacy of calcineurin inhibitors as these drugs have a very narrow therapeutic window and are exceedingly lipophilic[34]. This may cause them to be at least partially sequestered in a fatty thymus and have important consequences in children with obesity, including altering their efficacy.

Another clinically relevant finding in the present study is that there was an increase in the frequency of CD4+CD57+PD1- T cells among patients with obesity. We have previously shown that this subset of T cells is one of perhaps multiple antigen experienced, memory-type T cells that are resistant to costimulation blockade based immunosuppression and may mediate costimulation blockade resistant rejection[35–37]. Overall, our data suggest the use of costimulation blockade (i.e. belatacept) based regimens may be problematic in obese patients, as these regimens are less effective when more memory-type T cells are present. More recently, we have performed a series of *in vitro* studies that also show that these memory-type T cells may be relatively sensitive to rapamycin[38]. Finally, rapamycin has been shown to have multiple saltatory effects in mouse models of obesity, suggesting it may be a useful adjunct in this population regardless of its specific immune impact[39–43].

Although our study highlights the T and B cell phenotypes associated with malnutrition, it has several limitations. First, it is retrospective in nature, and therefore the assessment of any endpoint with obesity is potentially confounded. Next, there was a disproportionate number of patients who received thymoglobulin, however, we controlled for this in our regression analysis. Finally, there were a relatively small number of patients with obesity in the study limiting clinical comparisons, including with regards to differential usage of prednisone with these patients. However, this represents one of the few efforts to longitudinally characterize the immune phenotype of pediatric transplant recipients across all aspects of malnutrition, including those with obesity, a population of patients that is likely to increase in prevalence over time.

5. Conclusion

In conclusion, we observed differences in the T and B cell phenotypes among children with obesity relative to children with normal nutrition or undernutrition. While we did not find an association of nutritional status with either infectious or alloimmune outcomes, our study was not powered to do so. These findings may have implications for the appropriate immunosuppression of children with obesity receiving a kidney transplant.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Abbreviations:

ATG	Anti-Thymocyte Globulin
BMI	Body Mass Index
DGF	Delayed Graft Function
IMPACT	Immune Development of Pediatric Transplant
RRT	Renal Replacement Therapy

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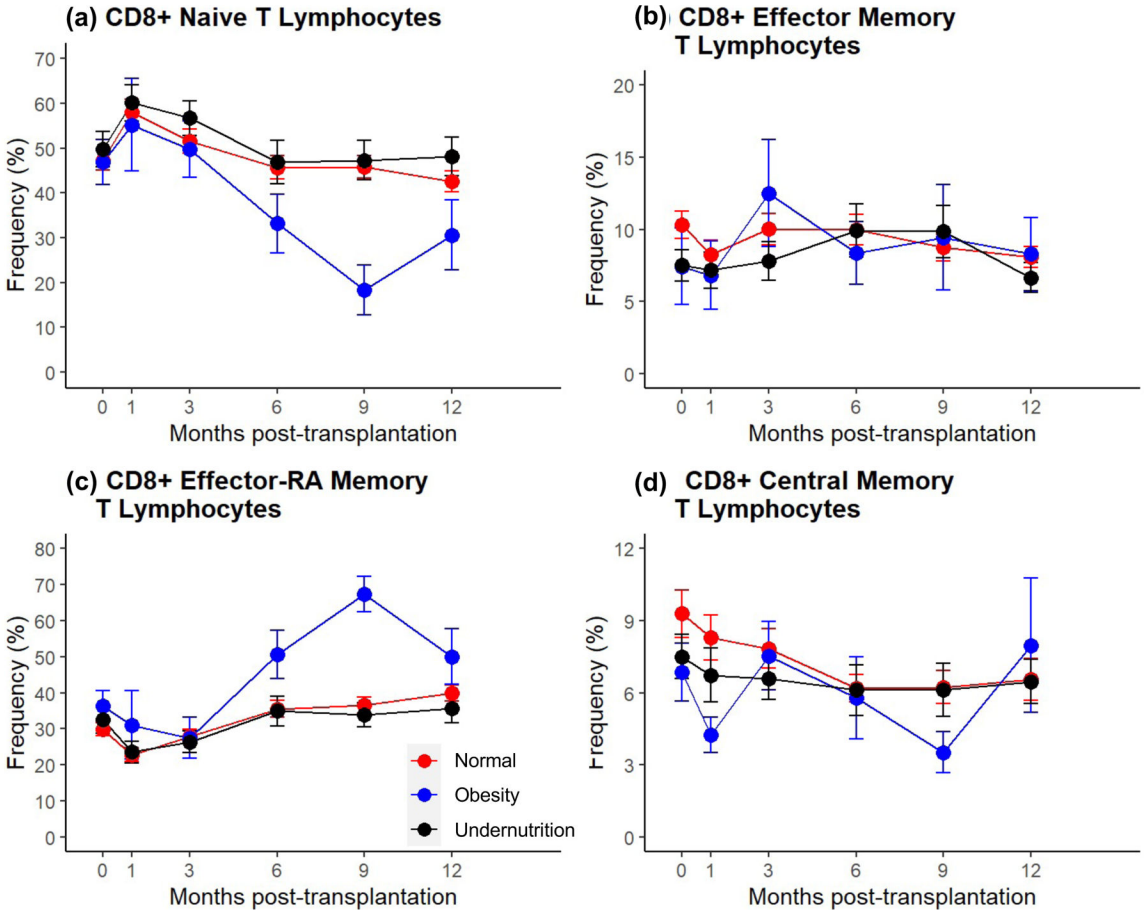

Figure 1: Frequencies of CD8 Tnaive are decreased and CD8 Temra are increased among patients with obesity after kidney transplantation.

(A) A significant interaction effect of time and nutritional status was detected (p=0.007), with patients with obesity having a lower frequency of CD8+ Tnaive at at 9-months post-transplant compared to normal nutritionpatients (95% CI: −38.18% to −12.98%, p<0.0001). **(B)** No significant interaction with time was noted (p=0.186) and no differences between nutritional status groups observed for CD8 Tem (p=0.736). **(C)** A significant interaction between CD8 Temra cells and time was observed (p=0.042) and compared to normal nutritionpatients, patients with obesity on average had 14% higher in Temra cell frequency at 6-months post-transplant (95% CI: 1.02% to 26.98%, p=0.035) and 31.4% higher in mean Temra cell frequency at 9-months (95% CI: 18.57% to 44.22%, p<0.0001). **(D)** No significant interaction with time was noted (p=0.480) and no differences between nutritional status groups observed for CD8 Tem (p=0.841).

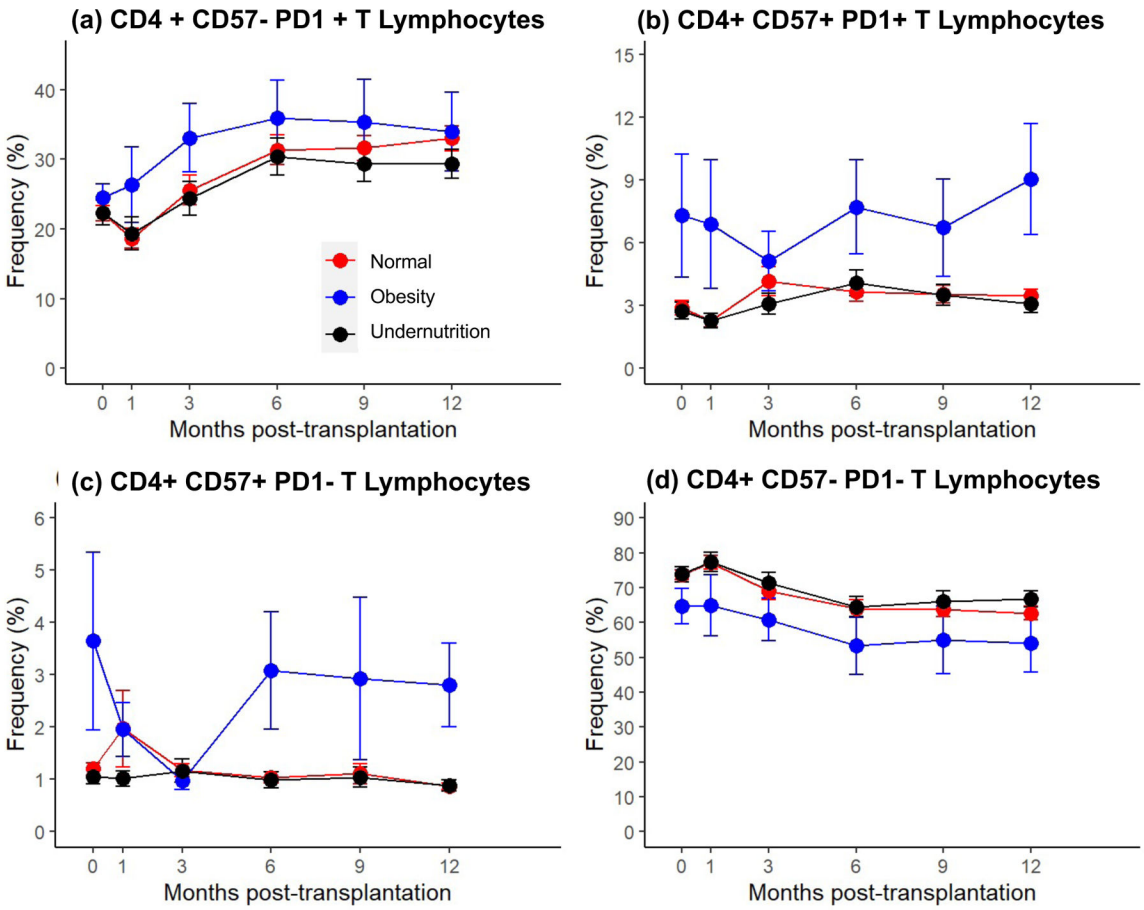

Figure 2: Markers of senescence among CD4 T cells are associated with nutritional status among pediatric transplant recipients.

(A-B) No significant interaction between nutritional status and CD4+ CD57-PD1+ (p=0.948), or CD4+ CD57+PD1+ (p=0.082) T lymphocytes. **(C)** A significant interaction effect of time and nutritional status was detected for CD4 CD57+PD1- (p=0.0004). Compared to normal patients, patients with obesity on average had 1.82% higher in CD4 CD57+PD1- frequency at 12-month post-transplant (95% CI: 0.46% to 3.17%, p=0.0086). **(D)** No significant interaction between nutritional status and CD4+CD57-PD1- T lymphocytes was detected (p=0.504).

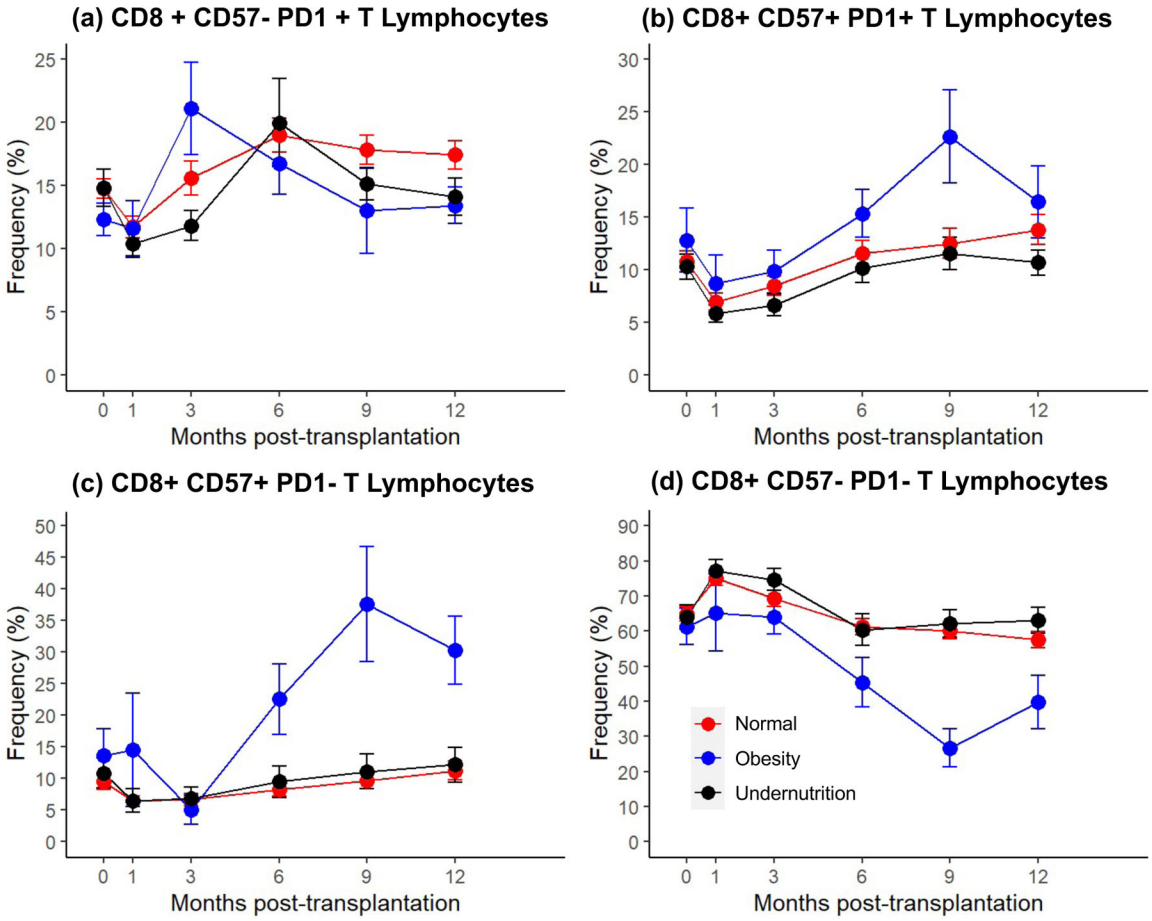

Figure 3: Markers of senescence among CD8 T cells are associated with nutritional status among pediatric transplant recipients.

(A) A significant interaction of time and nutritional status was detected for CD8+CD57-PD1+ T cells (p=0.004). Compared to normal patients, patients with obesity on average had 4.7% lower in CD8+CD57-PD1+ frequency at 12-month post-transplant (95% CI: −8.46% to −0.95%, p=0.014). **(B)** No significant interaction with time was found (p=0.520). On average, frequency of CD8+CD57+PD1+ T cell was higher for patients with obesity compared to both normal growth (95% CI: 0.13% to 7.39%, p=0.0403) and undernourished patients (95% CI: 1.18% to 8.85%, p=0.010). **(C)** No significant interaction with time was found (p=0.059). On average, frequency of CD8+ CD57+PD1- T cells was higher among patients with obesity compared to normal (95% CI: 2.89% to 20.11%, p=0.0089) and under-nourished patients (95% CI: 1.14% to 19.25%, p=0.027). **(D)** A significant interaction of time and nutritional status was detected for CD8+CD57-PD1- T cells (p=0.0035). Compared to normal patients, patients with obesity on average had 34.03% lower in CD8+ CD57-PD1- T cell frequency at 9-month post-transplant (95% CI: −46.21% to −21.84%, p<0.0001) and −20.55% lower at 12-month post-transplant (95% CI: −35.97% to −5.13%, p=0.009).

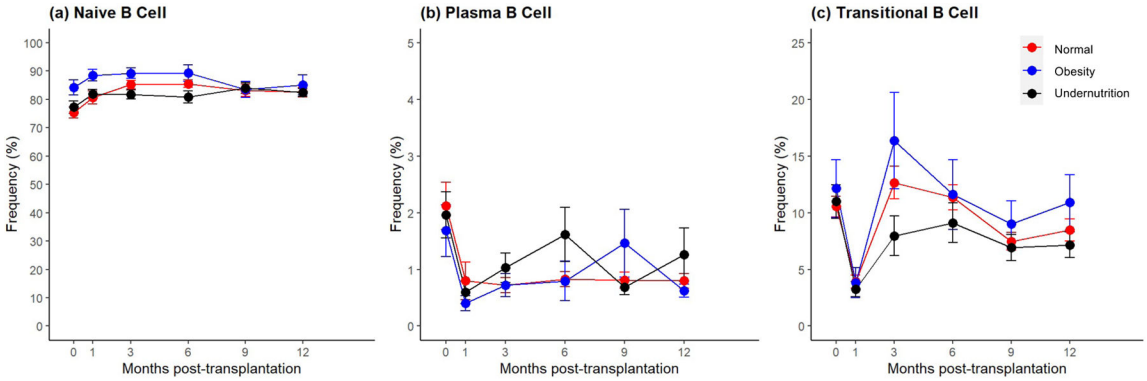

Figure 4: Nutritional status is associated with naïve B cell frequency

(A) A significant interaction between time and naïve B cell frequency was found (p=0.009). Compared to normal growth patients, patients with obesity on average had a higher (8.19%) naïve B cell frequency at baseline (95% CI: 1.68% to 14.70%, p=0.014), 7.23% higher at 1-month (95% CI: 1.35% to 13.11%, p=0.016), and 4.9% higher at 3-month post-transplant (95% CI: 0.32% to 9.51%, p=0.036). **(B & C)** No significant interaction between frequency and time was found for either Plasma cells or Transitional B cells (p=0.716 and p=0.378, respectively). After removing the interaction term, we still did not detect a significant effect of nutritional status on plasma B cell frequency (p=0.455) nor transitional B cell frequency (p=0.189).

Table 1.

Comparison of baseline characteristics between nutritional status groups.

	Normal N = 58	Obesity N = 11	Under-nutrition N = 29	Total N = 98	P-value
Age-Median (IQR)	15.5 (10, 17)	15 (2, 17)	13 (9, 18)	15 (8.2, 17)	0.21
Sex(Female)-n(%)	23 (39.7%)	3 (27.3%)	7 (24.1%)	33 (33.7%)	0.36
Race-n(%)					0.14
Other	5 (8.6%)	2 (18.2%)	7 (24.1%)	14 (14.3%)	
Black or African American	6 (10.3%)	2 (18.2%)	5 (17.2%)	13 (13.3%)	
White	47 (81.0%)	7 (63.6%)	17 (58.6%)	71 (72.4%)	
Ethnicity-n(%)					0.11
Hispanic or Latino	30 (51.7%)	3 (27.3%)	14 (48.3%)	47 (48.0%)	
Not Hispanic or Latino	27 (46.6%)	6 (54.5%)	12 (41.4%)	45 (45.9%)	
Unknown or Not Reported	1 (1.7%)	2 (18.2%)	3 (10.3%)	6 (6.1%)	
Height Z-score - Median (IQR)	−0.8 (−1.2, 0)	−0.5 (−2.4, −0.3)	−2.7 (−3.4, −2.1)	−1.1 (−2.1, −0.2)	
Weight Z-score - Median (IQR)	−0.2 (−1.1, 0.3)	1.8 (0.5, 2)	−2 (−2.7, −1.5)	−0.7 (−1.6, 0.3)	
BMI Z-score - Median (IQR)	0 (−0.7, 0.9)	2.4 (2.2, 2.6)	−0.2 (−1.4, 0.5)	0.1 (−0.7, 1.1)	
CMV Positive -n(%)	29 (50.0%)	8 (72.7%)	13 (44.8%)	50 (51.0%)	0.31
EBV Positive-n(%)	41 (70.7%)	5 (45.5%)	21 (72.4%)	67 (68.4%)	0.33
Site-n(%)					0.18
Site 1	18 (31.0%)	2 (18.2%)	10 (34.5%)	30 (30.6%)	
Site 2	17 (29.3%)	7 (63.6%)	6 (20.7%)	30 (30.6%)	
Site 3	23 (39.7%)	2 (18.2%)	13 (44.8%)	38 (38.8%)	
Immunosuppression					
ATG-n(%)	13 (22.4%)	7 (63.6%)	6 (20.7%)	26 (26.5%)	0.019
Prednisone-n(%)	35 (60.3%)	4 (36.4%)	20 (69.0%)	59 (60.2%)	0.17
Tacrolimus-n(%)	57 (98.3%)	11 (100.0%)	29 (100.0%)	97 (99.0%)	>0.99
Cyclosporine-n(%)	2 (3.4%)	0 (0.0%)	0 (0.0%)	2 (2.0%)	0.65
Azathioprine-n(%)	3 (5.2%)	0 (0.0%)	2 (6.9%)	5 (5.1%)	>0.99
Mycophenolate Mofetil-n(%)	56 (96.6%)	11 (100.0%)	29 (100.0%)	96 (98.0%)	0.65
Sirolimus-n(%)	13 (22.4%)	2 (18.2%)	4 (13.8%)	19 (19.4%)	0.71
Leflunomide-n(%)	1 (1.7%)	0 (0.0%)	0 (0.0%)	1 (1.0%)	>0.99