Impact of Hematopoietic Cell Transplantation on Cardiovascular Risk Factors and Insulin Resistance

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Abstract

Cardiovascular Disease (CVD) contributes to the increased risk of non-relapse late mortality after hematopoietic cell transplant (HCT). HCT survivors have a higher risk for insulin resistance and adverse CVD risk factors but the impact of exposure to HCT preparative conditioning regimens has not been clearly delineated. We enrolled 151 HCT recipients (26.4 ±0.9 years; time since HCT of 2.6-31.5 years) and 92 sibling controls to complete a cardiovascular risk assessment including insulin sensitivity by hyperinsulinemic euglycemic clamp, anthropometry, body composition by dual X-ray absorptiometry, blood pressure, and serum biomarkers. We used linear models to test for mean differences in all continuous outcomes between survivors and siblings, accounting for intra-family correlations with generalized estimating equations. Recipients of HCT were found to have lower insulin sensitivity and more likely to have adverse CVD risk factors in comparison to their healthy siblings. Significantly higher percent fat mass and visceral adipose tissue, and significantly lower lean body mass were noted in HCT recipients than sibling controls despite having a similar body mass index between the two groups. Total body irradiation in the conditioning regimen was one of the strongest factors associated with lower insulin sensitivity, dyslipidemia and abnormal body composition leading to sarcopenic obesity.

Background

The use of hematopoietic cell transplantation (HCT) for the treatment of malignant conditions in children has increased over the past 5 decades.(1) Along with the larger number of children who have undergone HCT, survival after HCT has significantly increased, resulting in a growing population of long-term survivors. As childhood HCT survivors become adults, clinical and epidemiological research is focusing on long-term adverse medical effects from cancer treatment to characterize and understand the ramifications of curative cancer directed therapy,(2) including metabolic syndrome or combinations of cardiovascular disease (CVD) risk factors (obesity, dyslipidemia, hypertension, insulin resistance [IR]). All are known to be potent contributors to the development of premature CVD in adults(3) and represent a leading cause of non-relapse deaths in childhood cancer and HCT survivors.

Previous studies have demonstrated an increased prevalence of metabolic syndrome in both pediatric and adult HCT survivors.(4–8) In a large single-institution study of 1,885 1-year HCT adult survivors treated with total body irradiation (TBI), the prevalence of CVD risk factors was significantly higher among HCT survivors compared with the general population.(4) In a study of cardiovascular risk factors in childhood survivors of HCT who had received total body irradiation (TBI), the cumulative incidence of cardiovascular risk factors, except obesity, increased by 1.7- to 3.2-fold from 5 to 10 years post-TBI.(9)

From indirect measures of insulin resistance, some studies suggest that cancer and HCT survivors may be at higher risk for insulin resistance and subsequent CVD, but the direct relationship to body composition has not been well delineated. This study focused on the early development of CVD risk factors and their relationship to insulin resistance. We undertook a comprehensive assessment in a large population of children and young adult HCT survivors of childhood hematologic malignancies and
compared their CVD risk profiles, insulin resistance (as measured by euglycemic hyperinsulinemic clamp studies), and body composition determined by dual X-ray absorptiometry (DXA) with a cohort of sibling controls.(10)

Methods

The study was approved by the Institutional Review Board and Human Subjects Committee at the University of Minnesota and the Fred Hutchinson Cancer Research Center/Seattle Children's Hospital. Consent and assent were obtained from all participants as appropriate.

Study Participants

Cancer HCT

Eligible subjects were less than age 22 years at cancer diagnosis and were at least 10 years old at the time of entry into this study in order to comply with the study procedures. Full details of the recruitment strategy and participation data have been previously published. Of the 154 recruited patients, 3 patients were found to be ineligible due to previously undiagnosed diabetes (n = 1), severe hypertension (n = 1), or other multiple medical issues (n = 1), leaving a cohort of 151 subjects.

Sibling Controls

The control group consisted of eligible healthy siblings who were ≥ 10 years old at study entry and who had never had a malignancy or HCT. Based on a pre-determined frequency matched enrollment scheme, siblings were recruited with the intent to represent the age and sex distribution of HCT recipients. Selection of the sibling closest in age to the subject was preferred, although not required, and having a sibling was not a requirement for participation. Siblings were chosen as the control population to obtain greater similarity to HCT recipients in genetics, lifestyle, and environment/geographical trends.

Study Procedures

Participants underwent a 2-day examination at either the University of Minnesota Clinical Research Center or the Pediatric Clinical Research Center at Seattle Children's Hospital. Measurements for height and weight were taken at the start of the visit and the body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters-squared (m²). Waist circumference was measured in duplicate midway between the anterior superior iliac spine and the lower rib margin directly over the skin, the method used in all earlier studies from our group considered to be the level of the smallest circumference around the waist. Tanner stage was assessed by study investigators and assigned according to pubic hair development in boys and breast and pubic hair development in girls. Body composition (percent fat mass [PFM], fat mass [FM], visceral adipose tissue [VAT], lean body mass [LBM]) were assessed by DXA (GE Healthcare Lunar Prodigy scanner; Madison, WI, USA) using enCORE software version 9.3. The two GE Healthcare Lunar Prodigy DXA scanners were cross-calibrated using a custom-built phantom that
calibrated bone, fat, and lean body masses. No significant differences were observed between the two scanners. The average of two manual blood pressure measurements using a random zero sphygmomanometer on the right arm of rested, seated subjects was used in analyses.

Hyperinsulinemic euglycemic clamps were conducted after a 10- to 12-hour overnight fast as previously described. Intravenous catheters were inserted in an arm vein for infusion of potassium phosphate, insulin, and glucose and in a contralateral vein for blood sampling. Baseline insulin and glucose levels were determined from samples drawn at -5 and 0 minutes before beginning the insulin and glucose infusions. Insulin infusion was started at time 0 at a rate of 1 mU/kg/min for 3 hours. An infusion of 20% glucose was given and adjusted to maintain euglycemia (serum glucose level of 100 mg/dL [5.6 mmol/L]) with plasma glucose determined every 10 minutes. Insulin sensitivity (M) was determined by the amount of glucose required to maintain euglycemia in the final 40 minutes of the clamp study and expressed as mg/kg/min of glucose with adjustment for lean body mass (M_{lbm}) obtained from DXA. Lower M_{lbm} values are indicative of lower insulin sensitivity (i.e., greater insulin resistance).

Plasma glucose was analyzed at the bedside with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, California). Serum insulin was determined with a chemiluminescence immunoassay (Immulite Insulin DPC, Los Angeles, California). Serum lipid levels were analyzed from fasting blood samples obtained at the time catheters were placed for the clamp, with a Vitros 5600 (Ortho-Clinical Diagnostics, Inc, Rochester, New York). Low-density lipoprotein (LDL) cholesterol was calculated with the Friedewald equation.

**Statistical Analysis**

Fisher Exact tests and t-tests were used to compare demographic characteristics between survivors and siblings. The key outcomes examined in analyses were: M_{lbm}, systolic blood pressure (SBP) (mmHg), diastolic blood pressure (DBP) (mmHg), mean waist circumference (cm), HDL (mg/dL), LDL (mg/dL), triglycerides (mg/dL), blood glucose (mg/dL), insulin (µU/mL), PFM, VAT (g), and LBM (kg), all analyzed as continuous measures. Linear multivariable regression models were used to test for mean differences (MD) in outcomes between survivors (in total and subgroups defined by treatments) and siblings, accounting for intra-family correlations with generalized estimating equations. Among survivors, multivariable linear regression models were employed to compare mean values of continuous outcomes between mutually exclusive treatment groups (total body irradiation and central nervous system irradiation [TBI + CNS], TBI without CNS irradiation [TBI/no CNS], and no TBI nor CNS irradiation [chemo only]). All models were adjusted for age, sex, race/ethnicity, and Tanner stage. An additional model for each non-body size outcomes was estimated with PFM added to the variables in the first model. SAS version 9.4 and R version 3.5.3 were used in these analyses.

**Results**

**Characteristics of Study Participants**
Of the 339 potentially eligible survivors identified, 60 refused participation and 125 could not be contacted (passive refusal). The remaining 154 survivors (45% of those potentially eligible; 72% of those contacted) provided consent to participate along with 92 of their siblings. Three HCT survivors were found to be ineligible at the time of the study because of previously undiagnosed type 2 diabetes mellitus (n = 1), severe hypertension (n = 1), and multiple medical issues (n = 1), all of which required immediate medical attention. This resulted in a final study population of 151 subjects (26.4 ± 0.9 years; range 10-50.5 years; referred to as survivors) and 92 sibling controls (22.2 ± 0.9 years; range 10.5–48 years) at study visit (Table 1). Full details of study recruitment and participation rates have been previously published.(12) Age at most recent HCT ranged from 6 months to 32.6 years, and time since HCT was 2.6–31.5 years. There were no significant differences in age at study, sex distribution, race, Tanner stage or BMI between survivors and sibling controls, though survivors had higher PFM percentiles. Most survivors had an original cancer diagnosis of either acute lymphoblastic leukemia (ALL) (31.1%) or Acute Myeloid Leukemia (AML) (44.3%). On the basis of overall similarities in therapeutic exposures, HCT survivors were grouped into 3 major preparative regimen groups: TBI + CNS radiation (n = 31), TBI/no CNS radiation (N = 85) and chemotherapy only (n = 35). Most survivors had an allogeneic transplant (76.8%) and 76.8% of survivors received TBI and 20.5% of survivors received TBI + CNS.

After adjusting for age, sex, race/ethnicity, and Tanner stage, $M_{\text{lbm}}$ in survivors was significantly lower than sibling controls (9.7 [8.8,10.6] vs 11.6 [10.4,12.7]; p = 0.005) and mean fasting insulin was significantly higher (16.5 [13.6,19.4] vs 9.9 [7.3, 12.4]; p < 0.001; Table 2). Compared to siblings, survivors also had significantly higher levels of total and LDL cholesterol and triglycerides (p < 0.001) and significantly lower levels of HDL cholesterol (p < 0.001). They also had lower height, weight, LBM (p < 0.001), higher PFM (p < 0.001) and VAT (p = 0.014) compared with siblings. SBP, DBP, blood glucose and waist circumference were not significantly different between survivors and siblings.

Multivariate regression models were used to examine differences in radiation treatment exposures between survivors and sibling controls as detailed below and shown in Figs. 1 and 2, with full detailed data in Supplementary Table 1.

**Body Composition:** Compared to sibling controls, survivors exposed to radiation had a lower height (TBI + CNS: mean differences (MD), -11.3; 95% CI [-14.6, -8.0]; p < 0.001; TBI/no CNS: -9.2; 95% CI [-11.6, -6.8]; p < 0.001) and weight (TBI + CNS: -12.3; 95% CI, [-18.3, -6.3]; p < 0.001; TBI/no CNS: -9.7; 95% CI [-18.4, -6.3]; p < 0.001), after adjusting for age-at study, sex, race, and Tanner stage (Supplemental Table 1). BMI and waist circumference were not different between survivors and siblings. However, the mean difference of LBM was significantly lower in those exposed to TBI, particularly TBI + CNS (TBI + CNS: -12.9; 95% CI [-15.9, -9.9]; p < 0.001; TBI/no CNS: -10.3; 95% CI, [-12.3, -8.3]; p < 0.001). Additionally, the mean difference of PFM was significantly higher than sibling controls for all HCT survivors including those with only chemotherapy exposure (TBI + CNS: 5.9; 95% CI [2.6, 9.2]; p < 0.001; TBI/no CNS: 5.0; 95% CI, 2.6–7.5; p < 0.001; chemotherapy only: 4.8; 95% CI [1.2, 8.4]; p = 0.009). Compared to siblings, the mean difference of VAT was significantly higher in survivors who were exposed to TBI (TBI + CNS: 253.2; 95% CI [81.6, 424.8];
p = 0.004; TBI/no CNS: 155.3; 95% CI [20.7, 289.9]; p = 0.024) whereas there was no significant difference in FM.

**Insulin Sensitivity**

Fasting insulin levels were significantly higher in survivors than sibling controls with the greatest impact seen in those who received TBI + CNS (PFM adjusted mean difference, 8.9; 95% CI [4.3, 13.5]; p < 0.001. Supplemental Table 1). While the overall cohort of survivors was less insulin sensitive (more insulin resistant), the most significant impact on insulin sensitivity ($M_{lbm}$) was seen in those who were exposed to TBI + CNS compared to siblings (PFM adjusted mean difference, -4.2; 95% CI [-6.2, -2.3]; p < 0.001).

**Dyslipidemia**

Compared with sibling controls, total cholesterol levels and fasting triglyceride levels were higher in TBI exposed survivors, with the greatest increase in the TBI + CNS group (PFM adjusted total cholesterol mean difference, 36.8; 95% CI [21.0, 52.5]; p < 0.001. Supplemental Table 1b) (PFM adjusted fasting triglyceride mean difference, 134.6; 95% CI [63.1, 206.1]; p < 0.001). HDL cholesterol levels were significantly lower than sibling controls regardless of radiation exposure with the greatest impact was seen in those who received TBI + CNS (PFM adjusted mean difference, -8.4; 95% CI [-12.7, -4.1]; p < 0.001). Additionally, LDL cholesterol levels were found to be significantly higher except in the chemotherapy only group in the PFM adjusted analysis.

**Blood Pressure**

Only the chemo-only survivors were significantly different compared to siblings in SBP (adjusted for PFM mean difference, -5.4; 95% CI [-9.8, -0.9]; p = 0.019). There was no significant difference found in DBP between any of the survivor treatment groups and controls.

**Cardiovascular risk factors comparisons by treatment exposure among survivors**

In pairwise comparisons of mean differences between subgroups of survivors defined by treatment group, those who received TBI + CNS radiation had lower insulin sensitivity ($M_{lbm}$) compared with those who received TBI/no CNS (PFM adjusted mean difference, -3.1, 95% CI [-5.1, -1.1]; p = 0.002) and chemotherapy only (PFM adjusted mean difference, -3.5, 95% CI [-5.6, -1.5]; p = 0.002; Table 3) after adjusting for age, sex, race, Tanner stage and PFM. Survivors who received TBI + CNS radiation had a significantly decreased LBM compared with those who received chemotherapy only (mean difference, -10.3, 95% CI [-15.0, -5.6]; p < 0.001) while no significant difference was observed compared to those who received TBI/no CNS. Total cholesterol and LDL cholesterol were significantly higher, and HDL-cholesterol was significantly lower in the TBI + CNS group compared with survivors who received TBI/no CNS, after adjusting for age, sex, race, Tanner stage and PFM.
Discussion

We showed that HCT survivors have increased CVD risk factors compared to their healthy siblings, these CVD risk factors are associated with TBI conditioning in general, but, in particular to TBI + CNS conditioning and that decreased insulin sensitivity (i.e. higher insulin resistance) is strongly associated with a TBI + CNS conditioning regimen. We also show that significantly higher PFM and VAT, and significantly lower LBM were also noted in HCT survivors than sibling controls despite having a similar BMI between the two groups. Adiposity, usually expressed as an increased BMI, is associated with adverse levels of CVD risk factors and lower insulin sensitivity. However, in this population of HCT survivors, BMI provided a less accurate representation of adiposity, which was defined only after DXA scanning for body composition and determination of lean body and fat masses. We confirmed that one of the strongest factors associated with abnormal body composition leading to sarcopenic obesity (decreased LBM and increased PFM) was the use of TBI in the conditioning regimen. While waist circumference was not significantly different, body composition data showed that survivors exposed to TBI had a higher VAT compared to sibling controls. Additionally, we described that HCT survivors were more likely to have dyslipidemias including higher total cholesterol, LDL cholesterol and triglyceride level and a lower HDL level compared to sibling controls, all indicative of an adverse CV risk profile.

A study by Bizzarri et al. showed that HCT survivors, while not overtly obese by BMI criteria, may develop insulin resistance as measured by indirect approximates of insulin resistance (homeostatic model assessment for insulin resistance [HOMA-IR]) and oral glucose tolerance testing. Similar findings with abnormal body composition reflected in reduced subcutaneous and increased visceral fat distribution, increased total fat mass and reduced LBM have also been described in a smaller cohort of long-term childhood survivors of HCT. Other studies by our group have also shown that childhood cancer survivors have greater adiposity, lower lean body mass, higher total cholesterol, and were less insulin sensitive than sibling controls. By using a direct measure of insulin sensitivity (i.e., hyperinsulinemic euglycemic clamp), we were able to demonstrate decreased insulin sensitivity in HCT survivors compared to sibling controls, even after adjustment for adiposity, suggesting that insulin sensitivity in HCT may be related to cancer and/or conditioning chemotherapy, with the strongest association found with TBI exposure. Insulin resistance has been shown to be related to obesity and elevated cardiovascular risk factors and therefore may be valuable in identifying individuals at greatest risk for future CVD.

This study has some potential limitations. With an overall participation rate of 45%, selection bias cannot be excluded. The population was predominantly white non-Hispanic; therefore, the findings may not be generalizable to other racial/ethnic groups. We also did not collect total radiation exposure data from participants. Determining the impact of any individual therapy becomes very complex and requires very large numbers of subjects. Therefore, the current analysis incorporates the combined impact of the therapeutic exposures on HCT survivors. However, TBI with and without CNS radiation remains a commonly used HCT conditioning regimen today for high-risk leukemia patients.
The results from this study have important implications for the long-term survivorship care of pediatric and young adult HCT survivors. We have demonstrated that HCT survivors have a higher than expected prevalence of multiple CVD risk factors, and early mortality from CVD is one of their leading causes of non-relapse mortality.\(^{4,20}\) We demonstrate that TBI can have long-term impact on body composition including LBM and VAT. It has been shown that CVD risk factors present in children often continue into adulthood on a worsening trajectory.\(^{21–25}\) Our findings support that HCT survivors should be monitored for adverse lipid profiles, body composition and insulin sensitivity and these abnormalities are potential targets for lifestyle and pharmacologic interventions. For example, it is conceivable that similar to other populations, promotion of physical activity focused on improving sarcopenia and reducing fat mass may reduce the magnitude of abnormal cardiometabolic risk factors in HCT survivors. Overall, the presence of CVD risk factors present at such a relatively young age in HCT survivors raises concern for an escalating trajectory of CVD risk and subsequently adverse CVD related events at a younger than expected age. This study confirms the need to screen HCT survivors for cardiometabolic risk factors and identification of patients for early intervention and prevention of CVD related complications.

Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
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<td>CNS</td>
<td>Central nervous system</td>
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<td>CVD</td>
<td>Cardiovascular disease</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<td>DXA</td>
<td>Dual X-ray absorptiometry</td>
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<td>HCT</td>
<td>Hematopoietic cell transplant</td>
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<td>HDL</td>
<td>High-density lipoprotein</td>
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<td>LDL</td>
<td>Low-density lipoprotein</td>
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<tr>
<td>MD</td>
<td>Mean difference</td>
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<td>PFM</td>
<td>Percent fat mass</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>TBI</td>
<td>Total body irradiation</td>
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<td>FM</td>
<td>Fat mass</td>
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<td>LBM</td>
<td>Lean body mass</td>
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<td>VAT</td>
<td>Visceral adipose tissue</td>
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Declarations

Financial Support

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Conflict of Interest

The authors of this Manuscript have no conflict or competing interests to report relevant to this research.

Author Contributions: KSB was responsible for designing the protocol, writing the protocol and report, screening potentially eligible studies, acquisition of data, analyzing data and interpreting results. TGK was responsible for extracting and analyzing data, interpreting results and writing the report. EJC, IHK, SB, DRD, AM, JSS and ARS contributed to writing the report, interpreting results and provided feedback on the report. WML and PG conducted the regression analyses and contributed to the design of the review protocol, writing the report, extracting and analyzing data and interpreting results.

Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

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Tables

Tables 1 to 3 are available in the Supplementary Files section.

Figures
Figure 1

Adjusted mean differences in body size outcomes of height, weight, body mass index (BMI), waist circumference, lean body mass, total fat mass, visceral adipose tissue (VAT) mass, and percent fat mass (PFM) between all survivors, survivor subgroups who received total body irradiation and central nervous system irradiation (TBI+CNS), TBI/no CNS irradiation (TBI), and no TBI nor CNS irradiation (Chemo Only), and siblings (reference). Comparisons adjusted for age at study, sex, race, and Tanner stage.
Figure 2

Adjusted mean differences in continuous cardiovascular outcomes for all survivors, survivor subgroups with total body irradiation and central nervous system irradiation (TBI+CNS), TBI but no CNS irradiation (TBI), and no TBI/no CNS irradiation (Chemo Only) relative to those of siblings (reference): (A) M_{lbm}, fasting blood glucose and fasting insulin; (B) total cholesterol, triglycerides, HDL, and LDL; (C) SBP and DBP. Estimates are adjusted for age-at-study (categorized <18, 18-34.9, 35+), sex, race, and Tanner stage (blue symbols) and these adjustments plus PFM percentiles (categorized <25th, 25th-75th, >75th) (black symbols).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.xlsx
- Table2.xlsx
- Table3.xlsx
- SupplementalTable1.xlsx