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GALLSTONES IN PEDIATRIC HEMATOPOIETIC CELL TRANSPLANT SURVIVORS WITH UP TO 40 YEARS OF FOLLOW-UP

Paul A. Hoffmeister, MPH¹, Barry E. Storer, PhD¹, George B. McDonald, MD^{1,3}, and K. Scott Baker, MD, MS^{1,2}

¹Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

²Department of Pediatrics, University of Washington School of Medicine, Seattle, WA, USA

³Department of Medicine, University of Washington School of Medicine, Seattle, WA, USA

Abstract

Purpose—To determine risk factors for the development of gallstones and the prevalence of related cholecystectomy in children following hematopoietic cell transplantation (HCT).

Patients and Methods—A retrospective chart review of 1,343 patients aged <18 years old who survived at least one year after HCT from 1969–2011 was performed. Multivariate Cox regression models were used to estimate the hazard ratio (HR) of risk factors associated with gallstones.

Results—Gallstones developed in 91 patients, a median of 3.5 (0.1 to 30.9) years after HCT at 16.3 (0.8 to 44.2) years of age, with a 40-year cumulative incidence of 11%. At initial diagnosis, 61 (67%) patients were symptomatic and 30 (23%) had incidental gallstones. Risk factors associated with gallstones included autologous transplant (HR = 2.7, $p = .02$), unrelated donor (HR=2.0, $p=.05$), grade 3–4 acute graft-versus-host disease (GVHD) (HR = 2.2, $p = .03$), chronic GVHD (HR = 2.0, $p = .05$), second transplant (HR = 2.3, $p = .03$), diabetes (HR = 2.2, $p = .05$) and estrogen therapy (HR = 1.8, $p=.03$). Fifty-six patients underwent cholecystectomy. The prevalence of cholecystectomy among 853 surviving patients was 5.2%.

Conclusions—Childhood HCT patients have an increased risk of developing gallstones.

Keywords

hematopoietic cell transplantation; gallstones; cholecystectomy; graft-versus-host disease; children

INTRODUCTION

The number of long-term survivors of childhood hematopoietic cell transplantation (HCT) is growing. Survivors may develop a variety of late effects including chronic health conditions, endocrine sequelae, and other medical problems.(1) Although gallstones have been reported

Correspondence: K. Scott Baker, MD, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. N. (D5-280), PO Box 19024, Seattle, WA 98109-1024, Phone: (206) 667-4348, Fax: (206) 667-5899, ksbaker@fhcrc.org.

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to occur in 3.5% in one study of childhood HCT patients, very little is known about the risk factors for patients developing gallstones.(2)

In the general population, risk factors associated with gallstones include older age, female gender, pregnancy, estrogen therapy, family history, obesity and genetics, and other factors such as rapid weight loss, diabetes, dyslipidemia, cirrhosis, cholestasis, total parental nutrition (TPN), ceftriaxone, decreased physical activity, Crohn's disease, and hemolysis.(3) In childhood cancer, gallstones have been associated with ileal conduit, parenteral nutrition, abdominal surgery, and abdominal radiation therapy.(4) In one study of childhood HCT patients, gallstones were more frequent in children treated for bone marrow failure than malignancy.(2) In HCT patients, virtually all patients who receive myeloablative pre-transplant conditioning therapy develop biliary sludge (microliths) which may promote the development of gallstones.(5) In addition, calcineurin inhibitors (such as cyclosporine and tacrolimus) used for immunosuppression have been associated with an increased incidence of gallstones after kidney and heart transplantation.(6;7)

The purpose of this study was to determine the incidence and risk factors for the development of gallstones in over 1,300 childhood HCT patients and to describe the prevalence of a history of cholecystectomy among long-term survivors.

MATERIALS AND METHODS

Patient Selection

Between 1969 and January 1, 2011, 1,365 consecutive children less than 18 years of age received transplant conditioning therapy and survived at least one year after HCT at the Fred Hutchinson Cancer Research Center (FHCRC), Seattle, WA. Excluded from the analysis were 22 patients who did not consent to follow-up or who were incarcerated. The remaining 1,343 patients or their responsible guardians consented to follow-up under Protocol 999.2 and the data were reviewed under Protocol 1782 approved by the FHCRC Institutional Review Board. Eighteen patients who had developed gallstones or undergone cholecystectomy before HCT were excluded from the analysis and the remaining 1,325 were analyzed.

Transplant preparative regimen, source of stem cells, supportive care, and follow-up

Transplant preparative regimens included either chemotherapy or chemoradiotherapy as previously described.(8–13) Most chemotherapy-only regimens utilized cyclophosphamide 50 mg/kg/day for 4 days alone or combined with busulfan 4 mg/kg/day for 4 days.(9;11) Most total body irradiation (TBI) regimens included cyclophosphamide 60 mg/kg/day for 2 days. From 1969–2000, TBI was delivered from dual opposing cobalt-60 sources at a dose rate in air of 5–8 cGy/min as either 9.2–10.0 Gy single-exposure TBI or fractionated TBI with exposures of 2.0–2.75 Gy for 6–7 consecutive days or hyper-fractionated exposures of 1.2 Gy 2–3 times daily for 4 consecutive days (total dose 12.0 – 15.75 Gy).(8;9;12;13) TBI in reduced intensity regimens was given as 2.0 Gy 1–2 times daily for 1–2 days. From 2001 onward, TBI was delivered by linear accelerator.

Allogeneic transplant recipients received either bone marrow, peripheral blood, or umbilical cord blood stem cells harvested from related or unrelated donors. All allogeneic transplant recipients received prophylaxis for acute graft-versus-host disease (GVHD) depending on the type of donor and protocol in use at time of HCT and generally included methotrexate and a calcineurin inhibitor (cyclosporine or tacrolimus), but less often included corticosteroids, anti-thymocyte globulin, mycophenolate mofetil, or monoclonal antibodies as additional prophylactic agents.(14–16) Acute and chronic GVHD were diagnosed, graded, and treated as previously described.(17–20)

All patients received antibacterial prophylaxis, and some also received antifungal and antiviral prophylaxis as well as use of laminar airflow rooms, growth factors, and intravenous immunoglobulin according to the standard of practice at the time of transplant. Most patients had indwelling central venous catheters and received nutritional support with hyperalimentation. Ursodeoxycholic acid (ursodiol) prophylaxis for hepatic complications was given from 2003 onwards at 12 mg/kg/day starting approximately 2 weeks before conditioning and continuing until day +60, +90, and +180 for autologous transplants, myeloablative, and nonmyeloablative regimens of allogeneic transplants, respectively.

All surviving patients had an evaluation for engraftment and chronic GVHD at 80–100 days after transplant. Patients returned to FHCRC for long-term follow-up at one year and electively thereafter. Follow-up after one year consisted of annual contact with referring physicians from 1969 to 1990, and by questionnaires mailed annually to patients and their primary medical provider from 1991 to the present. Eighty percent of patients had been contacted within 3 years of the study date.

Study data

Data were obtained from the FHCRC clinical information database, transplant flow-sheets, and long-term follow-up records. Study data were collected through March 1, 2013. Gallstones were defined as incidental or symptomatic based on whether or not the patient had signs and symptoms of biliary disease at the time diagnostic scans were performed.

Body Mass Index (BMI, kg/m²) was calculated based on patient height and weight at last follow-up (except for those with gallstones whose BMI at time of gallstone diagnosis was used). Obesity was defined as a BMI 95th percentile based on age and gender in patients <18 years old and a BMI 30 in patients 18 years old. (21;22) Abdominal surgery was defined as any surgery involving the peritoneal cavity, excluding hernia surgery. Abdominal irradiation was defined as local irradiation given before or shortly after HCT to the abdomen, pelvis, renal, adrenal, retroperitoneal, or lower spine. Estrogen therapy was defined as oral contraceptive use or estrogen replacement therapy. Pregnancy included both miscarriages and childbirth.

Study endpoints, variables, and statistical methods

For the purpose of analysis, transplant diagnoses were categorized into 6 groups: acute lymphoblastic leukemia (ALL); acute myelogenous leukemia (AML), myelodysplastic syndrome (MDS), and juvenile myelomonocytic leukemia (JMML), and myeloproliferative disorders (AML/MDS/JMML/MPD); other hematologic malignancies; other malignancies;

aplastic anemia; and other non-malignant disorders. Other hematologic malignancies included chronic myelogenous leukemia (CML), Hodgkin disease, and Non-Hodgkin lymphoma. Other malignancies included neuroblastoma (n=105), brain tumors (n=14), Ewing sarcoma (n=12), Wilms tumor (n=6), germ cell (n=5), and retinoblastoma (n=1). Other non-malignant disorders included Histiocytic diseases (10 hemophagocytic lymphohistiocytosis and 2 langerhans cell histiocytosis), primary immunodeficiency (n=51), red cell dysplasia (n=22), metabolic disorders (n=7), and other miscellaneous diseases (n=14).

Other baseline variables selected for analysis included: gender, race/ethnicity (non-Hispanic white vs. other race/ethnicity), age at transplantation (<9 vs. 9 to <18 years), year of transplant (1969–1989 vs. 1990–2011), TBI (none, low dose, high dose), donor type (sibling vs. other related vs. unrelated vs. autologous), HLA-matching between donor and recipient (matched or mismatched defined as one or more antigen mismatches at HLA-A, -B, or –DRB1), acute GVHD prophylaxis (MTX-based, cyclosporine-based, tacrolimus/other-based, none), acute GVHD grade (0 vs. 1–2 vs. 3–4), abdominal surgery (yes / no), local abdominal irradiation (yes / no), and ursodiol prophylaxis (yes/no). In addition, the following time-dependent variables were included: clinical extensive chronic GVHD, calcineurin inhibitors for prophylaxis or treatment, calcineurin inhibitors for chronic GVHD therapy, second HCT, obesity, diabetes, estrogen therapy, and pregnancy.

Statistical analysis was performed for symptomatic gallstones and repeated for both symptomatic and incidental gallstones. Cumulative incidence curves for the development of gallstones were estimated by standard methods, treating death without gallstones and cholecystectomy performed for conditions unrelated to gallstones as competing risks. Cox regression was used for hazard ratio analysis with adjustment for covariates. Post-transplant events were entered as time-dependent covariates at the time the events occurred. Overall tests of significance reflect likelihood ratio tests; comparisons of individual levels to the reference level reflect Wald tests. All p-values are 2-sided.

The point prevalence of cholecystectomy among surviving patients was calculated using the formula [patients with a history of cholecystectomy / total surviving patients at risk] as of March 1, 2013.

RESULTS

Patient population

Patient and transplant characteristics are presented in Table 1. Median follow-up was 20.4 (1.0 to 40.1) years. Ninety-one patients developed gallstones, a median of 3.5 (0.1 to 30.9) years after first HCT at 16.3 (0.8 to 44.2) years of age.

Among the 91 patients who developed gallstones, 79 had received allogeneic transplants. Among these 79 patients, gallstones developed in 7 patients before day 100 (3 before onset of acute GVHD, 2 after acute GVHD, and 2 who did not develop acute GVHD). Of the remaining 72 patients, 35 did not develop chronic GVHD, 2 developed gallstones before chronic GVHD, and 35 developed gallstones after chronic GVHD. Among the 35 patients

who developed gallstones after chronic GVHD, 23 had been treated with calcineurin inhibitors (14 cyclosporine, 5 tacrolimus, and 4 both cyclosporine and tacrolimus), 11 received prednisone alone (n=6) or combined with azathioprine (n=3), cyclophosphamide and azathioprine (n=1), or rapamycin (n=1), and 1 patient was untreated. Eight patients developed gallstones a median of 8.0 years (0.2 – 20.8) after second transplant.

Gallstone diagnosis, treatment, and pathology

Initial diagnosis—At initial diagnosis of gallstones, 9 (10%) had received ursodiol prophylaxis and 13 (14%) had received ceftriaxone during the first 100 days after transplant. Among 27 patients who developed gallstones less than one year after transplant, 10 were receiving parental nutrition at the time of diagnosis of gallstones and 17 had completed TPN. Among all 91 patients, 30 had evidence of biliary sludge on diagnostic scans performed on or before the diagnosis of gallstones, 9 had diabetes, 6 were receiving treatment for dyslipidemia (2 statin and fibrate, 2 statin, 1 niacin, 1 ezetimibe and fibrate), 1 had liver cirrhosis, and none of the patients had reported Crohn's disease. Among 40 female patients, 15 were on estrogen therapy and 6 had stopped estrogen therapy a median of 5 (1 to 12) years earlier. Seven patients had prior pregnancies (5 childbirths and 2 miscarriages). Among the 91 patients who developed gallstones, 61 (67%) patients were symptomatic and 30 (23%) had incidental stones at initial diagnosis.

Symptomatic gallstones—Symptomatic stones were found in 61 patients (49 with hematologic malignancies and 12 non-malignant disorders) a median of 6.3 (0.1 to 30.9) years after HCT. After initial diagnosis, 45 (74%) patients underwent cholecystectomy (32 within 60 days of diagnosis, 13 a median of 3.8 (0.2–25.7) years later), 5 died with persistent stones, 5 were alive with persistent stones, and 6 had resolution of their stones. Resolution of gallstones occurred spontaneously (n=3) and following treatment with ursodiol (n=3). One 42-year old patient died of congestive heart failure and myocardial infarction within two days following cholecystectomy.

Incidental gallstones—Incidental stones were found in 30 patients (17 with hematologic malignancies, 6 non-hematologic malignancies, and 7 non-malignant disorders) a median of 2.4 (0.1 to 29.4) years after HCT. Incidental stones were discovered on diagnostic scans performed in 11 patients for cancer staging, 8 liver abnormalities (4 hepatitis C virus work-up, 2 elevated liver function tests, 1 liver GVHD, and 1 hepatoma), 5 kidney abnormalities (3 kidney stones, 1 renal cyst, 1 fungal disease), and 6 miscellaneous reasons. After initial diagnosis, 10 patients became symptomatic and underwent cholecystectomy a median of 2.4 (0.4–11.3) years later and 1 had an elective cholecystectomy. Nineteen patients remained asymptomatic. Of these, 10 had ongoing incidental stones a median of 6.7 (0.8 – 19.9) years later and 9 patients had died (3 disease relapse, 3 secondary malignancies, 3 other causes).

Pathology—Among the 56 patients who underwent cholecystectomy, pathology reports were available for 20 patients. Sixteen cases had chronic cholecystitis and 4 had acute and chronic cholecystitis. Gallstones were reported in 14 cases. Three cases had 1 to 2 gallstones, 7 had multiple gallstones, and 4 had an unspecified number. Stone composition by color was cholesterol (yellow/green, n=6), pigmented (black/brown, n=4), mixed-type

(n=2), and unspecified (n=2). One case with a cholesterol gallstone also had gallbladder cholesterosis. Two stones were impacted in the cystic duct.

Risk factors for gallstones

In univariate analysis, we found no significant difference between patients with and without gallstones with respect to gender ($p = 0.67$), race ($p = 0.84$), ursodiol prophylaxis ($p = .74$), and obesity ($p = .58$). The results of univariate analysis are presented in Table 2. All of the variables significant ($p < 0.05$) in univariate analysis were entered into the multivariate model.

The results of multivariate analysis are shown in Table 3. Gallstones were significantly associated with autologous transplant ($HR = 2.73$, $p = .02$), unrelated donor ($HR = 1.95$, $p = .05$), grade 3–4 acute GVHD ($HR = 2.20$, $p = .03$), clinical extensive chronic GVHD ($HR = 1.98$, $p = .05$), second transplant ($HR = 2.28$, $p = .03$), diabetes ($HR = 2.16$, $p = .05$), and estrogen therapy ($HR = 1.77$, $p = .03$).

Because of concern that inclusion of incidental gallstones may have affected the results, multivariate analysis was repeated including only symptomatic gallstones ($n=71$). Excluding incidental stones, symptomatic gallstones remained significantly associated in multivariate analysis with unrelated donor ($HR = 2.22$, $p = .02$), clinical extensive chronic GVHD ($HR = 2.49$, $p = .01$), and second transplant ($HR = 2.49$, $p = .03$). Mismatched donor transplants became more significant ($HR = 1.89$, $p = .04$), whereas autologous transplant ($p = .15$), grade 3–4 acute GVHD ($p = .13$), diabetes ($p = .11$), and estrogen therapy ($p = .10$) became less significant. Pregnancy, which was previously not included in the multivariate model, was significantly associated with symptomatic gallstones ($HR 3.75$, $p = 0.006$).

As shown in Figure 1, the cumulative incidence of gallstones (symptomatic and incidental) was 11% at 40 years after HCT. Figure 2 shows the cumulative incidence of gallstones by donor type.

Prevalence of cholecystectomy

Among the 91 gallstone patients, 28 had died. Among the remaining 63 patients, 44 had undergone cholecystectomy. Prevalence data is shown in Table 4. The overall prevalence of cholecystectomy was 5.2% (44 / 853) among current survivors. Compared to the general population(23), the prevalence of cholecystectomy among male survivors was roughly 2 to 4 fold higher at every age category below 50 years of age, whereas female survivors had a decreased prevalence of cholecystectomy at every age category except 30–39 year olds for which the prevalence was nearly double.

DISCUSSION

This is the first study to report the frequency of the development of incidental and symptomatic gallstones in a large population following childhood HCT. Our study reports a much higher incidence of gallstones than previous studies of childhood cancer and HCT patients. In our study, among 1,325 patients with up to 40 years of follow-up, 91 (6.9%) patients developed gallstones, which is 2-fold higher than the 3.5% (20/575) previously

reported among childhood HCT patients.(2) Furthermore, the cumulative incidence of gallstones appears to rise with increasing length of follow-up reaching 11% at 40 years after transplant compared to 1.03% in childhood cancer patients at 18 years after diagnosis.(4) Our cumulative incidence of gallstones is comparable to a 9.7% history of gallstones reported in one study of long-term childhood and adult HCT survivors treated for CML.(24) The major finding of this study was an association of gallstone formation with multiple risk factors including autologous transplant, unrelated donor, grade 3–4 acute GVHD, clinical extensive chronic GVHD, second transplant, diabetes, and estrogen therapy.

Biliary sludge is found by ultrasound in approximately 70%(25) and at autopsy in 100% of HCT patients following myeloablative conditioning.(26) In one study of HCT patients, biliary sludge consisted primarily of unmeasurable residue, calcium bilirubinate, and a calcium-binding protein.(26) Biliary sludge may serve as nucleating material for the development of gallstones during the immediate period following transplantation. However, over time, most of the sludge is emptied from the gallbladder as patients resume eating. As the risk from biliary sludge induced gallstones diminishes, our findings suggest that other risk factors or combination of risk factors contribute to gallstone formation.

Autologous transplant was associated with a two-fold increased risk of gallstones. It is unlikely that autologous transplantation itself should be associated with gallstone formation, but may be a surrogate measure of other risk factors. In our study population, a majority of autologous patients were transplanted for neuroblastoma and other solid tumors which frequently are treated with abdominal surgery and/or abdominal irradiation, which were associated with a non-significant increased risk of gallstones in univariate analysis. In addition, non-hematologic malignancy patients undergo frequent restaging scans and are thus more likely to have incidental gallstones identified.

Among allogeneic transplants, unrelated donors had a significant increased risk of gallstones. The increased risk is likely attributable to a higher risk of developing acute and chronic GVHD compared to related donors. Both grade 3–4 acute GVHD and clinical extensive chronic GVHD were associated with a two-fold increase in gallstone formation. Acute GVHD of the gastrointestinal tract is associated with bile acid malabsorption.(27) Bile acid malabsorption may lead to an increase in biliary cholesterol supersaturation and cholesterol crystal formation, which in turn may lead to cholesterol gallstone formation.(28) In addition, the risk associated with acute GVHD may be attributable to other factors that promote gallstone formation, including increased use of parental nutrition, narcotics, and immunosuppressive agents, as well as, weight loss and decreased physical activity. Whereas acute and chronic GVHD were associated with gallstone formation, we found that treatment of GVHD with calcineurin inhibitors was not associated with gallstones in multivariate analysis. Our findings suggest that calcinurien inhibitors are not a significant risk factor for gallstone formation in HCT patients, unlike kidney and heart transplant patients in which long-term cyclosporine- and tacrolimus-based immunosuppression has been associated with an increased risk of gallstones.(6;7)

After the initial transplant period, development of gallstones was associated with a second transplant, developing diabetes, and estrogen therapy. Second transplant was associated with

a two-fold increased risk of gallstones likely reflecting all of the risk factors associated with a first transplant. Diabetes is a well-known risk factor for gallstones(3) and childhood HCT patients have an increased risk of developing diabetes(29). Estrogen therapy in females was associated with an increased risk of gallstones as has been described in the general population.(3) Gonadal failure is common in female childhood HCT survivors, usually related to busulfan or TBI exposure, and generally requires long-term estrogen replacement therapy.(1)

In addition to the aforementioned risk factors, individual patients had various conditions that may have contributed to gallstone formation, including TPN use, ceftriaxone exposure, and liver cirrhosis. Childhood HCT patients have an increased risk of developing dyslipidemia as part of the metabolic syndrome(30), which may also contribute to gallstone formation. The majority of gallstones available for analysis were cholesterol stones suggesting that dyslipidemia may contribute to gallstone formation in our patients. Interestingly, some patients developed gallstones despite being on statin therapy.

We compared the prevalence of a history of cholecystectomy in our HCT patients to that in the general population.(23) As expected, the prevalence of cholecystectomy for men in our study was roughly 2 to 4 times higher reflecting the relatively low rate of gallstone development among men in the general population. Similarly, the prevalence of cholecystectomy for women 30–39 years old in our study was nearly double, although the prevalence among other female age groups was lower than the general population possibly reflecting the higher rate of gallstone development in women in the general population.

The medical management of gallstones after HCT in children is poorly defined. In the general population, the natural history of gallstones results in cholecystectomy in 22% of patients.(31) In one study of 235 pediatric HCT patients, 15% of patients with gallstones underwent cholecystectomy due to acute cholecystitis, whereas the majority of asymptomatic patients were managed non-operatively.(2) In our study, 62% of patients with gallstones underwent cholecystectomy (74% with symptomatic gallstones and 37% with incidental gallstones). The reason for the four-fold increased rate of cholecystectomy is likely due to longer follow-up and the development of chronic cholecystitis or biliary obstruction. Our findings suggest that incidental gallstones may be followed non-operatively, although a third of patients will become symptomatic. Most symptomatic patients required cholecystectomy, however, in a few cases, gallstones resolved without surgical intervention. Although cholecystectomy is generally considered a safe surgical procedure, one patient died of complications following cholecystectomy.

Our study had several limitations. This was a retrospective study and the identification of cases of gallstones was dependent on patients and/or their physicians in providing follow-up information. Our data is heavily biased towards reports of symptomatic gallstones with an underreporting of incidental gallstones, likely understating the true incidence of gallstones in our population as discussed above. Because of the potential that incidental gallstones may have affected our results, we performed the multivariate analysis with and without incidental gallstones. In general, the results for symptomatic gallstones were similar, although some variables were less significant presumably due to a smaller sample size and autologous

transplantation in particular was less significant most likely due to its association with incidental gallstones as discussed above. We were also limited in our ability to assess certain potential risk factors including family history and genetics, rapid weight loss, diet, dyslipidemia, decreased physical activity, and hemolysis. We were also unable to assess the role of statins in preventing gallstones. Lastly, other factors such as the impact of TPN could not be assessed since most HCT patients receive TPN in the weeks following HCT, but the total duration of TPN therapy is not routinely collected.

CONCLUSION

Long-term survivors of childhood HCT are at risk for gallstones and gallstone complications in the years following transplant, especially those who had an autologous transplant, unrelated donor, grade 3–4 acute GVHD, clinical extensive chronic GVHD, second transplant, diabetes, and females treated with estrogen therapy.

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Figure 1.
Cumulative incidence of symptomatic and incidental gallstones in 91 of 1,365 pediatric hematopoietic cell transplant patients

Figure 2.
Cumulative incidence of symptomatic and incidental gallstones by donor type in 91 of 1,365 pediatric hematopoietic cell transplant patients

Table 1**Patient data**

	Gallstones	Total
Number of patients	91	1325
Age at transplant – years mean (range)	11.1 (0.4–17.9)	9.0 (0.3–17.9)
Gender		
Female	40 (44%)	549 (41%)
Male	51 (56%)	776 (59%)
Race/Ethnicity		
Non-Hispanic White	74 (81%)	1049 (79%)
Other race/ethnicity	17 (19%)	276 (21%)
Diagnosis at Transplant		
Hematologic Malignancy		
ALL	26 (29%)	427 (32%)
AML	18 (20%)	261 (20%)
CML	12 (13%)	98 (7%)
JMML / MDS / MPD	8 (9%)	71 (5%)
Non-Hodgkin's Lymphoma	1 (1%)	32 (2%)
Hodgkin's Disease	1 (1%)	23 (2%)
Non-Hematologic Malignancy		
Neuroblastoma and other solid tumors *	6 (7%)	143 (11%)
Non-Malignant Disorder		
Aplastic Anemia	13 (14%)	164 (12%)
Histiocytic Disorders	2 (2%)	12 (1%)
Other non-malignant *	4 (4%)	94 (7%)
Donor type at first HCT		
Autologous	12 (13%)	207 (15%)
Sibling / twin	41 (45%)	669 (50%)
Related (Parent or relative)	11 (12%)	126 (9%)
Unrelated	27 (30%)	323 (24%)
HLA matching at first HCT		
Matched	56 (62%)	898 (68%)
Mismatched	23 (25%)	220 (17%)
Total Body Irradiation at first HCT		
Fractionated 12–15.75 Gy	66 (73%)	864 (65%)
Low-dose 2–8 Gy	4 (4%)	70 (5%)
Non-TBI containing regimens	21 (23%)	391 (30%)
Methotrexate for acute GVHD prophylaxis		
Cyclosporine-based	43 (47%)	599 (45%)
Tacrolimus-based	4 (4%)	115 (9%)
Methotrexate only	29 (32%)	358 (27%)
None	12 (13%)	232 (18%)

	Gallstones	Total
Other	3 (3%)	21 (2%)
Acute GVHD grades		
0–II	53 (59%)	920 (69%)
III–IV	23 (25%)	191 (14%)
Not evaluable **	3 (3%)	10 (1%)
Second transplant	8 (9%)	143 (11%)
Clinical Extensive Chronic GVHD (yes / evaluable) ***	42 / 71 (59%)	401 / 1101 (36%)
Survivors	63 (69%)	853 (64%)
Follow-up – years median (range)	20.4 (1.5–40.1)	15.4 (1.0–40.1)

* Other diagnosis = See text

** Three patients developed gallstones before acute GVHD and 7 were not evaluable for acute GVHD.

*** Excluded were 7 patients who developed gallstones before day 100, autologous only HCT recipients, and 10 patients who were unevaluable.

ALL indicates Acute Lymphoblastic Leukemia; AML, Acute Myelogenous Leukemia; CML, Chronic Myelogenous Leukemia; GVHD, Graft versus Host Disease; HCT, Hematopoietic Cell Transplantation; HLA, Human Leukocyte Antigen; JMML, Juvenile Myelomonocytic Leukemia; MDS, Myelodysplastic Syndrome; MPD, Myeloproliferative disorder.

Table 2

Univariate analysis

	HR (95% CI)	P
Age at transplant		
<9 years	1.0	
9 to <18 years	1.50 (1.0–2.3)	0.06
Year of transplant		
1969–1989	1.0	
1990–2011	1.57 (1.0–2.5)	0.05
Diagnosis		
ALL	1.0	
AML/MDS/JMML	1.11 (0.6–1.9)	0.72
Other heme malignancy*	1.45 (0.7–2.8)	0.28
Other malignancy*	0.99 (0.4–2.3)	0.99
Aplastic anemia	0.78 (0.4–1.5)	0.47
Other nonmalignant*	1.05 (0.4–2.5)	0.92
Total body irradiation		
None	1.0	
Low dose (2 – 8 Gy)	2.07 (0.7–6.1)	0.19
High dose (12–15.75 Gy)	1.51 (0.9–2.5)	0.10
Abdominal surgery		
No	1.0	
Yes	1.58 (0.9–2.8)	0.12
Abdominal irradiation		
No	1.0	
Yes	1.47 (0.7–2.9)	0.28
Donor		
Sibling/twin	1.0	
Unrelated	2.59 (1.5–4.3)	0.0003
Other related (parent or relative)	1.82 (0.9–3.6)	0.08
Autologous	1.91 (1.0–3.7)	0.06
HLA matching		
Matched	1.0	
Mismatched	2.09 (1.3–3.4)	0.003
Acute GVHD prophylaxis		
Methotrexate-based	1.0	
Cyclosporine-based	1.43 (0.9–2.3)	0.16
Tacrolimus/Other-based	1.45 (0.6–3.4)	0.39
None	1.23 (0.6–2.4)	0.56

	HR (95% CI)	P
Acute GVHD grade^I		
0	1.0	
1–2	1.87 (1.1–3.3)	0.03
3–4	3.29 (1.8–6.1)	0.0002
Clinical extensive chronic GVHD^I		
No	1.0	
Yes	2.13 (1.3–3.4)	0.001
Calcineurin inhibitor for prophylaxis or treatment^I		
No	1.0	
Yes	1.20 (0.7–1.9)	0.45
Calcineurin inhibitor for chronic GVHD^I		
No	1.0	
Yes	1.87 (1.1–3.1)	0.01
Second transplant^I		
No	1.0	
Yes	2.06 (1.0–4.3)	0.05
Diabetes^I		
No	1.0	
Yes	2.50 (1.2–5.4)	0.02
Estrogen therapy (females only)^I		
No	1.0	
Yes	2.46 (1.2–5.0)	0.01
Pregnancy (females only)^I		
No	1.0	
Yes	2.38 (0.9–6.4)	0.09

^I Included as a time-dependent variable.

ALL indicates Acute Lymphoblastic Leukemia; AML, Acute Myelogenous Leukemia; GVHD, Graft versus Host Disease; HLA, Human Leukocyte Antigen; JMML, Juvenile Myelomonocytic Leukemia; MDS, Myelodysplastic Syndrome.

Table 3

Multivariate analysis

	HR (95% CI)	P
Donor		
Sibling/twin	1.0	
Unrelated	1.95 (1.0–3.7)	0.05
Other related (parent or relative)	1.11 (0.5–2.4)	0.78
Autologous	2.73 (1.2–6.1)	0.02
HLA match		
Matched	1.0	
Mismatched	1.49 (0.8–2.6)	0.17
Acute GVHD grade^I		
0	1.0	
1–2	1.44 (0.8–2.7)	0.25
3–4	2.20 (1.1–4.5)	0.03
Clinical extensive chronic GVHD^I		
No	1.0	
Yes	1.98 (1.0–3.9)	0.05
Calcineurin inhibitor for chronic GVHD^I		
No	1.0	
Yes	0.60 (0.3–1.3)	0.19
Diabetes^I		
No	1.0	
Yes	2.16 (1.0–4.7)	0.05
Second transplant^I		
No	1.0	
Yes	2.28 (1.1–4.9)	0.03
Estrogen therapy (females only)^I		
No	1.0	
Yes	1.77 (1.0–3.0)	0.03
Year of transplant		
1969–1989	1.0	
1990–2011	0.89 (0.5–1.6)	0.68

^I Included as a time-dependent variable.

GVHD, Graft versus Host Disease; HLA, Human Leukocyte Antigen.

Table 4

Prevalence of cholecystectomy

Sex, age group (yr)	Patients		Cholecystectomy	
	N=	N=	HCT Survivors (%)	U.S. Population (%)
Total patients	853	44	5.2	--
<u>Men (n = 490)</u>				
<20	152	5	3.3	0.0
20–29	135	5	3.8	0.8
30–39	114	4	3.5	1.5
40–49	72	8	11.1	4.4
50–59	19	0	--	8.1
<u>Women (n = 363)</u>				
<20	133	1	<1.0	2.1
20–29	87	3	3.5	4.9
30–39	87	13	14.9	7.5
40–49	48	4	8.3	13.1
50–59	8	1	12.5	16.7

Prevalence of cholecystectomy among 853 pediatric hematopoietic cell transplant (HCT) survivors compared to 14,238 persons in U.S. general population from Everhart JE, reference (23).