Endocrine sequelae and metabolic syndrome in adult long-term survivors of childhood acute myeloid leukemia

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1. Introduction

Five-year survival of childhood acute myeloid leukemia (AML) has increased significantly over the past few decades and is now approximately 65%. Numerous studies have investigated late effects in survivors of childhood acute lymphoblastic leukemia (ALL), however – due to lower patient numbers and lower survival rates – only a small number of studies have been published on late effects in survivors of acute myeloid leukemia (AML). Endocrine late effects, including decreased bone mineral density, infertility, growth retardation, growth hormone deficiency and hypothyroidism and components of the metabolic syndrome (MetS) such as insulin resistance [1,2] and dyslipidemia [3] have been extensively studied in stem cell transplanted (SCT) survivors, especially in those treated with total body irradiation (TBI) [4–12].

Currently, intensive chemotherapy has become the standard treatment in childhood AML and SCT is only advised in less than 20% in most treatment protocols [13]. Consequently, data on long-term outcome after such intensive chemotherapy schedules are scarce. The Nordic AML Late effect Study among 102 AML survivors treated with chemotherapy only between 1984 and 2003, concluded that self-reported health was good and use of health care service was limited [14]. However, Mulrooney et al. reported recurrent cancer, second malignancies and cardiac events exceeding the rates in the normal population in 272 survivors of childhood and adolescent AML, who where treated with chemotherapy only between 1970 and 1986 [15]. Limited data exist on very late endocrine effects and cardiovascular risk factors after intensive treatment for AML with chemotherapy only [3,6,16]. In this case-controlled study we therefore evaluated the effect of chemotherapy only on endocrine sequelae and the components of the MetS in adult long-term survivors of myeloid leukemias.

2. Materials and methods

2.1. Subjects

Fifteen long-term (≥5 years after cessation of treatment) adult survivors of childhood acute myeloid leukemia (AML), treated with chemotherapy only, between 1961 and 2004 in the Erasmus MC-Sophia Children's Hospital, participated in this prospective study. In addition, nine survivors (7 AML, 1 CML, 1 MDS) treated with SCT including TBI in the conditioning regimen, were recruited. Informed consent was obtained according to the Helsinki declaration [27] and the study was approved by the local medical ethical committee.

In addition, a healthy control group, consisting of 60 siblings, friends or neighbors, of the same sex and within an age range of five years of the related survivor, was cross-sectionally recruited. In each individual, all measurements were performed on one and the same day.
Table 1
Baseline characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Chemo-only (N=12)</th>
<th>SCT (N=9)</th>
<th>Controls (N=60)</th>
<th>Survivors not included (N=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male/female</strong></td>
<td>9/3</td>
<td>4/5</td>
<td>33/27</td>
<td>13/9</td>
</tr>
<tr>
<td><strong>Age at follow-up (years)</strong></td>
<td>27.4 (22.0–39.2)</td>
<td>32.4 (23.4–44.5)</td>
<td>32.1 (18.0–61.7)</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Age at diagnosis (years)</strong></td>
<td>5.1 (0.1–15.8)</td>
<td>11.5 (1.1–15.0)</td>
<td>NA</td>
<td>9.6 (0.2–16.8)</td>
</tr>
<tr>
<td><strong>Follow-up time (years)</strong></td>
<td>21.6 (9.1–30.7)</td>
<td>19.0 (11.6–30.0)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Diagnosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>12</td>
<td>7</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>4</td>
</tr>
<tr>
<td>Recurrence or 2nd malignancy</td>
<td>1</td>
<td>2</td>
<td>NA</td>
<td>4</td>
</tr>
<tr>
<td>Allogeneic/Autologous SCT</td>
<td>–</td>
<td>6/3</td>
<td>NA</td>
<td>9/0</td>
</tr>
<tr>
<td>Radiotherapy (Y/N)</td>
<td>0</td>
<td>9</td>
<td>NA</td>
<td>11</td>
</tr>
<tr>
<td><strong>Cumulative dose (Gy)</strong></td>
<td>–</td>
<td>TBI: 8 (4–12)</td>
<td>NA</td>
<td>CRT: 25 (24–26)/TBI: 8 (4–12)</td>
</tr>
<tr>
<td><strong>Chemotherapy (Y/N)</strong></td>
<td>12</td>
<td>9</td>
<td>NA</td>
<td>16</td>
</tr>
</tbody>
</table>

Data expressed as median (range) or as frequencies (N).

SCT, stem cell transplantation; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia; Gy, gray; i.v., intravenous; p.o., per os; TBI, total body irradiation; CRT, cranial radiotherapy; NA, not applicable; ND, not determined.

* Time after cessation of treatment.

† Three survivors were treated with CRT, six with TBI, one with CRT and TBI and one with abdominal RT.

‡ Cumulative doses are unknown.

2.2. Methods

Disease and treatment data were obtained from our local database. Information regarding smoking status, socio-economic status (SES), statins, and antihypertensive medication, hormone supplementation and diabetes was collected using a self-designed questionnaire. Smoking status was defined as non-smoker, former smoker or current smoker. Socio-economic status was defined by the highest level of educational attainment and was obtained in seven categories based on the Dutch educational system. Information about physical activity was collected by a Dutch translation of the Short Questionnaire to Assess Health-enhancing physical activity [17]. Waist circumference was measured at the nearest millimeter using a Harpenden Stadiometer and weight was assessed with underwear only to the nearest 0.1 kg with a standard clinical balance. Body mass index (BMI) was calculated (weight (kg))/[height (m)]² [18]. Waist and hip circumference were measured to the nearest 1 cm, midway between last rib and the iliac crest and at the maximum circumference of the buttocks, respectively [19]. Waist-hip ratio was calculated. Final height standard deviation score (SDS) was calculated using reference values for Dutch adults [20].

Blood pressure was measured with the subject in sitting position after an hour of rest on the right arm with the Dinamap Procare and was defined as the mean of three measurements. Standard deviation scores (SDS) were calculated using the reference value from the Dutch epidemiologic MORGEN-study [21]. Using the National Education Program Adult Treatment Panel Guideline III, participants with at least three of the following components were diagnosed with MetS: waist circumference >102 cm in males or >88 cm in females; triglycerides ≥1.7 mmol/L or treatment for dyslipidemia; high density lipoprotein-cholesterol (HDL-C) <1.03 in males or <1.30 in females; fasting plasma glucose ≥5.6 mmol/L or treatment for type 2 diabetes; blood pressure ≥130/85 mmHg or treatment for hypertension [22,23]. ALT levels were evaluated as a marker for hepatic steatosis [24].

Data on bone mineral density, lean body mass (kg) and percentage of body fat were retrieved from dual energy X-ray absorptiometry (DXA, GE Lunar Prodigy, Madison, WI) and were only available in survivors and values for bone mineral density, lean body mass and total fat percentage were compared with normal Dutch reference values and calculated as standard deviation scores (SDS) as previously reported from our institute [25].

2.3. Laboratory measurements

Fasting blood samples were taken from an intravenous-cannula before 10:00 AM. Serum insulin-like growth factor 1 (IGF-I) (nmol/L), cortisol (nmol/L), ACTH (pmol/L), luteinizing hormone (LH) (U/L), follicle stimulating hormone (FSH) (U/L) and insulin (pmol/L) were measured using a chemi-luminescence-based immunoassay (Immune 2000, Siemens DPC, Los Angeles, CA, USA). IGF-I levels were compared with reference values by using standard deviation scores (SDS) [26]. Testosterone (nmol/L) was measured by coated tube radioimmunoassay (Siemens DPC, New York, NY, USA). Serum AMH levels were determined using an in-house double-antibody enzyme-linked immunosorbent assay (ELISA) commercially available through Beckman-Coulter, Brea, CA [27,28]. Inhibin B levels were measured using kits purchased from Serumet Ltd (Oxford, UK). Thyroid stimulating hormone (TSH) (U/L) and free thyroxine (fT4) (pmol/L) were measured using chemiluminescence assays (Vitros ECI Immunodiagnostic System; Ortho Clinical Diagnostics, Rochester, NY). Triglycerides (mmol/L), high-density lipoprotein-cholesterol (HDL-C) (mmol/L), low-density lipoprotein-cholesterol (LDL-C) (mmol/L), glucose levels (mmol/L) and alanine transaminase (ALT) (U/L) as a marker for liver steatosis were measured using an enzymatic in vitro assay (Roche Diagnostics, Mannheim, Germany). Homeostatic Model Assessment (HOMA) was calculated as a measure of insulin resistance [29].

2.4. Statistics

Statistical analyses were performed with the Statistical Package for Social Sciences (SPSS 17.0, Chicago, IL, USA). Mann–Whitney U-tests were used to compare results between survivors and controls. p < 0.05 (two-tailed) were considered statistically significant. Chi square test was used to evaluate the frequency of MetS and its separate components. Multiple ordinal and linear regression analyses were performed, to correct for relevant confounders. Linear and ordinal regression analyses were all performed using the following strategy: First, all models were corrected for age and sex. Then the effects of the possible confounders socio-economic status, smoking, physical activity, BMI and use of oral contraceptives were evaluated using backward regression modeling. When p < 0.200, the confounders were kept in the subsequent model. Subsequently, the effect of the subgroups treated with SCT (SCT group) and chemotherapy only were evaluated.
3. Results

3.1. Survivors and controls

Of 47 adult survivors of childhood myeloid leukemias in our center, 12 AML patients, treated with chemotherapy only (chemo-only group), and nine survivors treated with SCT for myeloid malignancies (SCT group) participated in this study. Sixteen survivors moved to another outpatient clinic for late effects, two refused to participate and four survivors were lost to follow-up. Two AML survivors were treated with chemotherapy in combination with cranial radiotherapy respectively abdominal radiotherapy and were therefore excluded. One AML survivor with Down syndrome was excluded. One AML survivor was immobilized due to paralyzes [30].

Baseline and treatment characteristics of controls and survivors, as well as representativeness as compared to excluded survivors are shown in Table 1. Treatment protocols included BFM derived AML protocols [31–33]. In addition to chemotherapy, seven AML survivors, one CML survivor and one MDS survivor had been treated with SCT including TBI as conditioning regimen (SCT group) (Table 1). Data of 12 chemo-only survivors were compared with normal healthy controls and with data of SCT survivors. Age at diagnosis, age at follow-up and follow-up time of AML-chemo-only survivors were not significantly different from SCT survivors (p = 0.310, p = 0.193 and p = 0.602 respectively). Numbers of patients receiving specific chemotherapeutic drugs and cumulative doses did not significantly differ between groups, except for cyclophosphamide which total cumulative dose was significantly higher in the SCT group (median dose of 3600 mg/m² vs 500 mg/m² in the chemo-only group).

3.2. Growth and growth hormone deficiency

Height SDS and IGF-I levels of chemo-only survivors were not significantly different from controls (Table 1). None of the chemo-only survivors was GH deficient and one survivor in the SCT group received growth hormone substitution for growth hormone deficiency diagnosed by an insulin tolerance test [34].

3.3. Adrenal and thyroid function

In the chemo-only group, none of the AML survivors had abnormal TSH levels or FT4 levels, whereas five SCT survivors received thyroxin because of primary hypothyroidism. Cortisol levels were significantly higher in the chemo-only group, but after correction for use of oral estrogen supplementation in women, this effect disappeared (β = −0.20, p = 0.900).

3.4. Fertility and gonadal dysfunction

Median testosterone level was 18.3 nmol/L (p = 0.952) in chemo-only survivors and 11.3 in SCT survivors (p = 0.039) as compared to 18.7 in controls.

An Inhibin B level below 100 ng/L was found in 2/11 male chemo-only survivors, in all four SCT survivors and in 2/33 controls (Fig. 1). Median Inhibin B level was 167 ng/L in chemo-only survivors (p = 0.086) and 21 ng/L in SCT survivors (p < 0.001) as compared to 201 ng/L in controls. Inhibin B levels were significantly associated with cumulative dose of cyclophosphamide, but not with cytosine arabinoside dose.

Mean testicular volume was significantly lower in survivors after SCT than in chemo-only survivors (12.25 versus 20 ml, p = 0.004), but after correction for age, BMI, physical activity and cumulative dose of cyclophosphamide, this difference was not significant (β = −3.4, p = 0.387).

Fig. 1. Levels of Inhibin B (ng/L) in controls, chemo-only survivors and SCT survivors. SCT stem cell transplantation.

An AMH level below one was found in 1/3 female chemo-only survivors and in 6/27 controls (Fig. 2). Median AMH level was 1.2 μg/L in chemo-only survivors and 2.3 in controls (p = 0.799), whereas in all 5 SCT survivors AMH level was <0.1 (Table 2). The number of the patients was too small to study the effect of alkylating agents on AMH levels.

3.5. Components of the metabolic syndrome

Three out of 48 controls had the MetS, as compared to 1/12 subjects in the chemo-only group (p = 1.000) and 1/8 subjects in the SCT group (p = 0.507). After correcting for age, sex, smoking and BMI, chemo-only survivors did not have more MetS components than controls (OR = 1.31, p = 0.687), however SCT survivors did have more MetS components (OR = 24.1, p < 0.001).

3.6. Adiposity

High waist circumference was present in 15% of chemo-only survivors (p = 0.569), none of the SCT survivors (p = 0.346) and 13% of controls. BMI, waist and waist hip ratio were not significantly different from controls in chemo-only survivors, whereas in SCT survivors waist hip ratio was significantly higher than in controls (0.95 versus 0.87, p = 0.001).

Total fat percentage was significantly higher compared with healthy Dutch references in both chemo-only survivors and SCT survivors (SDS 1.09, p = 0.010 and SDS 1.19, p = 0.005). Lean body

Fig. 2. Levels AMH (μg/L) in controls, chemo-only survivors and SCT survivors. SCT stem cell transplantation.
mass was significantly lower than in healthy Dutch references in SCT survivors (SDS −1.31, p = 0.024) but not in chemo-only survivors (SDS −0.46, p = 0.248).

3.7. Insulin resistance

Elevated fasting glucose levels or treatment for diabetes were present in 7% of chemo-only survivors (p = 0.437) and 13% of SCT survivors (p = 0.697) as compared to 14% in controls. Fasting glucose, fasting insulin and HOMA were not significantly different between chemo-only survivors and controls (Table 2), while SCT survivors had significantly higher fasting glucose, fasting insulin and HOMA than controls (median 5.2 versus 4.9, p = 0.021, 123 versus 25, p < 0.001 and 2.3 versus 0.5, p < 0.001, respectively).

3.8. Dyslipidemia

In chemo-only survivors, the frequency of dyslipidemia was not significantly different from controls (7% versus 6%, p = 0.953), whereas in SCT survivors, frequency of dyslipidemia was higher than controls (63% vs. 6%, p < 0.001). In chemo-only survivors, triglyceride levels were not different from controls, whereas triglyceride levels were significantly higher in SCT survivors as compared to controls (median 2.5 vs. 0.8, p < 0.001). Moreover, ALT levels were higher in SCT survivors than in controls (median 35 versus median 19, p = 0.004) \( (\beta = 103 \% , p = 0.001) \) [24]. ALT levels were not different between the chemo-only group (median 20, p = 0.392) and controls.

3.9. Blood pressure

The frequency of hypertension was not different in the chemo-only group (21%, p = 0.415) nor in the SCT group (25%, p = 0.391) as compared with controls (15%). Systolic blood and diastolic blood pressure in both therapy groups were not different from controls.

3.10. Bone mineral density

Bone mineral density of the total body (BMD-TB) was not significantly different from Dutch reference values in both chemo-only survivors and SCT survivors (SDS −0.21, p = 0.567, and SDS −1.0, p = 0.122 respectively). One adult chemo-only survivor had a BMD-TB SDS lower than −2, in contrast to four SCT survivors. SDS did not significantly differ between groups in univariate analysis (p = 0.552) nor after adjustment for height.

4. Discussion

After twenty years of follow-up, AML survivors were not found to be at increased risk at endocrinopathies and components of the metabolic syndrome after chemotherapy only. As stem cell transplantation is replaced by intensive chemotherapy for 80% in current treatment protocols for childhood AML, this study focused on endocrine late effects and cardiovascular risk factors in survivors treated with chemotherapy only. In the current study, chemotherapy included cytosine arabinoside in combination with cyclophosphamide, anthracyclins, vincristin and corticosteroids.

Interestingly, we did not find an adverse effect on thyroid function after chemotherapy only, in contrast to hypothyroidism, which was documented in half of the survivors after SCT, treated with TBI. A previous study also found a higher frequency of hypothyroidism among SCT survivors (3/15) as compared with 1/44 of chemo-only survivors at 17 years of follow-up [6]. Similarly, Leahy et al. did not find any endocrinopathies after chemotherapy only in 26 survivors of childhood AML after seven years of follow-up [16], suggesting that hypothyroidism after SCT is due to the effect of total body irradiation as conditioning regimen for SCT.

AMH and Inhibin B levels tended to be lower in chemo-only survivors as compared to controls and 3/14 chemo-only survivors had gonadal dysfunction, indicating that intensive chemotherapy like cyclophosphamide may affect gonadal function, although gonadal dysfunction was much more pronounced in the SCT group [5]. Recently, in adult survivors of childhood non-Hodgkin lymphoma (NHL) survivors, we reported a contribution of cytosine arabinoside to gonadal dysfunction, which may be a previously disguised gonadotoxic drug, masked by the SCT regimens in the past [35]. The present study was underpowered to confirm our previous finding, so larger studies are needed to evaluate this.

Chemotherapy only survivors did not have a higher frequency of components of the Mets than controls. This is in contrast to the higher risk of the MetS after SCT treatment, which is mainly determined by a higher frequency of dyslipidemia. In a study
by Oudin et al., describing both ALL (n = 150) and AML survivors (n = 34) at 15 years of follow-up, frequency of the MetS was higher after SCT treatment compared to chemo-only survivors (18% versus 5%) which was comparable with our findings (13% in SCT survivors versus 8% in chemo-only survivors) [3].

Although the present study is limited by sample size, our results are strengthened by the prospective study design with a cross-sectionally recruited control group. Furthermore, we described the complete spectrum of endocrine functions and components of the MetS in a representative group of AML survivors at very long term follow-up.

As to date most pediatric AML patients are treated with chemotherapy only, larger nationwide prospective epidemiological studies are needed to focus on the effects of intensive chemotherapy later in life.

In conclusion, childhood AML survivors treated with chemotherapy only did not suffer from components of the MetS neither from endocrine late effects at very long term follow up, which is in contrast to childhood AML survivors treated with SCT. Gonadal function should however be evaluated in care in both therapy subgroups.

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**Authors’ contributions**

MvH and SN contributed equally to this manuscript. SN and MvH were the principal investigators and take primary responsibility for the paper. KB and MvW recruited the patients, assessed statistical analysis, interpreted the data and wrote the paper. SN, MvH, RP and AvL critically reviewed the paper. All authors gave their final approval.

**Conflict of interest statement**

The authors reported no potential conflicts of interest.

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**References**


