1    **Title page**

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3    **Pulmonary dysfunction after treatment for childhood cancer – Comparing multiple-breath**

4    **washout with spirometry**

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38  Conception and design of the study: PL, NVDW, CSR; acquisition of data: CS, JU, RK, BF, SZ, RJ,

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**Abstract**

**Rationale:** Childhood cancer survivors are at risk of long-term pulmonary dysfunction, but we lack sensitive outcome measures to detect early pulmonary damage.

**Objective**: To assess the ability of nitrogen multiple-breath washout ($N_2$MBW) for detecting pulmonary dysfunction compared to spirometry in long-term survivors of childhood cancer.

**Methods:** We analyzed cross-sectional data from long-term (≥ 5-year) survivors of childhood cancer, aged ≤16 years at cancer diagnosis, ≥16 years at study (assessment period 2015-2019). We categorized survivors by risk: high risk for those having had pulmotoxic chemotherapy, chest radiation, thoracic surgery, and/or hematopoietic stem cell transplantation, and standard risk for other cancer therapies. Primary outcomes were the global lung clearance index (LCI) and acinar ventilation inhomogeneity index ($S_{ACIN}$) from $N_2$MBW, and forced expiratory volume in one second ($FEV_1$) and functional vital capacity (FVC) from spirometry. We calculated $z$ scores for $N_2$MBW and spirometry parameters and compared pulmonary dysfunction between risk groups. Pulmonary dysfunction was defined as $z$ score +1.64 for $N_2$MBW and -1.64 for spirometry.

**Results:** We studied 46 survivors, median age at diagnosis 10 years (interquartile range [IQR] 4-14), median age at study 30 years (IQR 25-40). Thirty-seven percent were at high risk and 63% at standard risk for pulmonary dysfunction. LCI and $S_{ACIN}$ were higher in the high risk group compared to the standard risk group (mean LCI $z$ scores 2.09, standard deviation [SD] 2.39 vs 0.95, SD 2.81; mean $S_{ACIN}$ $z$ scores 2.45, SD 3.29 vs 0.65, SD 2.79). $FEV_1$ and FVC were lower in the high risk compared to the standard risk group (mean $FEV_1$ $z$ scores -0.94, SD 1.39 vs -0.10, SD 1.07; mean FVC $z$ scores -1.14, SD 1.23 vs 0.15, SD 1.61). Overall, LCI, $S_{ACIN}$, $FEV_1$, and FVC were abnormal in 60%, 53%, 33%, and 33% of high risk patients compared to 23%, 21%, 0%, and 4% of standard risk patients.

**Conclusions:** $N_2$MBW identified more cases of pulmonary dysfunction in long-term survivors of childhood cancer than spirometry, even in patients who had cancer therapy not specifically known

84  as being pulmotoxic. $N_2$MBW could be a complementary screening tool for early pulmonary damage

85  after treatment for childhood cancer.

86  ClinicalTrials.gov (identifier: NCT02730767).

87  **Number of words in the abstract: 350/350 words**

**Text**

**Introduction**

Pulmotoxic cancer treatment can cause long-term pulmonary damage (1, 2) leading to a 15-fold increased mortality compared to the general population (3). There is clear evidence for pulmotoxicity for some treatments such as bleomycin and chest radiation for which guidelines recommend surveillance (4). A number of other chemotherapies are suspected of damaging the lungs (5-9), but solid data are lacking. Because symptomatic disease occurs relatively late due to the large functional reserve of the lungs and a long silent period (10), early screening for functional changes is necessary.

Spirometry currently is recommended for screening childhood cancer survivors (4) even though it mainly measures changes in the large airways and is insensitive to small airway damage potentially caused by cancer treatment. Increasingly, though, nitrogen multiple-breath washout ($N_2MBW$) is being used to measure ventilation inhomogeneity and small airway disease (11). Its main outcome indices are the lung clearance index (LCI), a marker for global ventilation inhomogeneity, and the acinar ventilation inhomogeneity index ($S_{ACIN}$), which measures global and acinar ventilation inhomogeneity. $N_2MBW$ is more sensitive than spirometry for the detection of early pulmonary disease in children with cystic fibrosis (12, 13), in pediatric patients undergoing hematopoietic stem cell transplantation (HSCT) (14) and lung transplantation (15), and in adults after HSCT (14, 16). It has not yet been used with childhood cancer survivors.

Pulmotoxic chemotherapy (17), chest radiation (18), and HSCT can damage the alveolar, vascular, and parenchymal lung compartments. The histopathological process involves inflammation where cytokines and growth factors stimulate collagen production by fibroblasts, leading to lung fibrosis (Figure 1) (18). Initially, this damage occurs in the small airway compartments, resulting in reduced ventilation of the lung periphery and impaired diffusion. The $N_2MBW$ could be a sensitive test to assess ventilation inhomogeneity resulting from early fibrotic damage. As the fibrosis progresses, also larger airways may be damaged, resulting in airway obstruction and restriction, which can be measured with spirometry. Previous studies in childhood

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cancer survivors have shown signs of reduced lung volumes and reduced oxygen diffusion capacity, which are both indicative for fibrosis, but only very few survivors had signs of airway obstruction (19). To the best of our knowledge, no previous study in childhood cancer survivors has used the $N_2MBW$ test to assess early pulmonary damage. Since lung fibrosis starts in the smaller airways, we hypothesized that $N_2MBW$ would detect more cases of pulmonary dysfunction than spirometry. We further hypothesized that high risk survivors would show more pulmonary dysfunction than standard risk survivors of childhood cancer. This study measured pulmonary function in adult survivors of childhood cancer, and it compared $N_2MBW$ and spirometry results in high risk survivors exposed to confirmed pulmotoxic treatment and standard risk survivors treated with other cancer therapies.

**Methods**

**Study design and study population**

We enrolled childhood cancer survivors participating in the SURfit study (20). SURfit is a randomized controlled, physical activity intervention study conducted between 2015 and 2019 at the University Children's Hospital Basel, Switzerland. Participants were recruited through the Swiss Childhood Cancer Registry, a nationwide registry of all patients diagnosed with leukemia, lymphoma, central nervous system tumors, malignant solid tumors, or Langerhans cell histiocytosis before age 21 years in Switzerland (21). Inclusion criteria for SURfit were age ≤16 years at cancer diagnosis, survival of five years or more since cancer diagnosis, and age at study ≥16 years. Participants were randomized 1:1 to an intervention group with 2.5 hours of physical activity per week additional to individual baseline activity, and a control group with continuation of individual baseline activity.

We conveniently sampled participants from two risk groups for pulmonary dysfunction: 1) high risk for pulmonary dysfunction due to exposure to established pulmotoxic cancer treatments including busulfan, bleomycin, carmustine, lomustine, chest radiation, thoracic surgery, and/or HSCT (4), and 2) standard risk for pulmonary dysfunction due to other chemotherapies (5-9, 22).

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142    Within these groups, we recruited SURfit participants who agreed to undergo additional pulmonary

143    function assessment. Pulmonary function was measured cross-sectionally three months after

144    randomization in the SURfit study for organizational reasons. We did not expect any change of

145    pulmonary function in survivors who increased their physical activity levels due to the intervention.

146    Nevertheless, we adjusted for study group in a sensitivity analysis.

147    Ethics approval was granted by the Swiss Ethics Committee on research involving humans

148    (Ethikkommission Nordwest- und Zentralschweiz [EKNZ], reference number: EKNZ-2015-169), and

149    the SURfit study was registered at ClinicalTrials.gov (identifier: NCT02730767).

150

**Nitrogen multiple-breath washout**

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152    Pulmonary function was measured by one experienced technician in a specialized pulmonary

153    function laboratory at the University Children's Hospital Basel, Switzerland. The technician was

154    blinded to the risk group of survivors. All $N_2$MBW measurements were performed according to the

155    European Respiratory Society and American Thoracic Society consensus statement (23) on the

156    same commercially available device (Exhalyzer D, Spiroware 3.1.6, Eco Medics AG). Main $N_2$MBW

157    indices were LCI, conductive ventilation inhomogeneity index ($S_{COND}$), $S_{ACIN}$, and functional residual

158    capacity (FRC). We calculated $z$ scores using published reference values from healthy adults (24).

159

**Spirometry**

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161    Spirometry was performed after $N_2$MBW using the same Jaeger MasterScreen (CareFusion,

162    Hochberg, Germany) device according to the European Respiratory Society and American Thoracic

163    Society consensus statement (25). Main spirometry indices were forced expiratory volume in one

164    second ($FEV_1$), forced vital capacity (FVC), and the Tiffenau index ($FEV_1$/FVC). We calculated $z$

165    scores using the Global Lung Function Initiative (GLI) reference equations (26).

166

**Risk group stratification**

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168   We categorized survivors by risk: high risk for those having had pulmotoxic chemotherapy

169   (bleomycin, busulfan, cardmustine, lomustine), HSCT, chest radiation (mediastinal/lung radiation,

170   cranio-spinal radiation, and total body irradiation), and/or thoracic surgery (4), and standard risk for

171   other cancer therapies. We further stratified high risk survivors into survivors with and without

172   HSCT.

173

174   **Defining covariates**

175   We assessed demographic characteristics at study entry and collected information on cancer

176   diagnosis and treatment from medical records. Anthropometric measures were collected at the time

177   of pulmonary function assessment as previously reported (20).

178

179   **Statistical analysis**

180   Data were expressed in mean ± standard deviation (SD) or median and interquartile range (IQR) as

181   appropriate. Upper limits of normality (ULN) were defined as $z$ score +1.64 for LCI, FRC, $S_{COND}$, and

182   $S_{ACIN}$. Lower limits of normality (LLN) were defined as $z$ score -1.64 for $FEV_1$ and FVC, and as <0.7

183   for $FEV_1$/FVC (26). We used chi-squared and t-tests to compare demographic and clinical

184   characteristics, and pulmonary function as appropriate in high vs standard risk survivors. With

185   univariable and multivariable linear regression models, we investigated the association between

186   pulmonary risk groups (high vs standard risk) and pulmonary function parameters, controlling for

187   potential confounders. We adjusted for age, sex, weight, height, and active smoking status. In a

188   sensitivity analysis, we added intervention group and time since diagnosis to the multivariable model

189   to investigate the possible effects of increased physical activity or time since diagnosis on

190   pulmonary function parameters. We used STATA software (Version 15.1, StataCorporation, Austin,

191   TX).

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193

194   **Results**

**Study population**

The SURfit study included 162 survivors overall, 46 of whom (28%) were recruited for pulmonary function assessment. Complete characteristics of the assessed survivors are presented in Table 1, and the flowchart in Figure E1 illustrates the allocation of survivors into the high and standard risk groups. Among the assessed survivors, median age at diagnosis was 10 years (interquartile range [IQR] 4–14), median age at study 30 years (IQR 25–40), and median time since diagnosis 20 years (IQR 15–32). Over half of the survivors had been treated for leukemia and one-quarter for lymphoma, all but two survivors (96%) had received chemotherapy, and half of the 46 assessed survivors had undergone radiotherapy. Seventeen of the assessed survivors (37%) belonged to the high risk and 29 (63%) to the standard risk group. In the high risk group, 15 survivors (88%) had received chest radiation with a median cumulative dose of 20 Gray, nine survivors (53%) received mediastinal/lung radiation, two survivors (12%) cranio-spinal radiation, and four survivors (24%) total body irradiation. The high and standard risk groups differed by age at diagnosis and by weight at lung function, and 11 high risk and 13 standard risk patients were in the physical intervention group. At the time of study, none of the survivors reported asthma or any other pre-existing pulmonary disease and none had chronic respiratory symptoms.

Table E1 (online supplement) compares the characteristics of all SURfit participants, with and without pulmonary function assessment. Survivors without pulmonary function assessment were younger at cancer diagnosis (median age 6 years), and a higher proportion in the assessment group had leukemia (25 of 46 survivors, 54%) than did those in the group with no pulmonary function assessment (32 of 116, 28%).

**N$_2$MBW parameters**

After quality control, N$_2$MBW indices were available for 15 of 17 high risk survivors (15 LCI and 15 S$_{ACIN}$ indices) and 26 of 29 standard risk survivors (26 LCI and 24 S$_{ACIN}$ indices) (Figure E1, online supplement). Overall, childhood cancer survivors had increased mean $z$ scores for LCI, FRC, and S$_{ACIN}$ compared to reference values, and high risk patients had higher $z$ scores than standard risk patients (Table 2). N$_2$MBW indices tended to be increased as well in standard risk patients with

223     mean $z$ scores of 0.95 for LCI, 0.20 for FRC, and 0.65 for $S_{ACIN}$. Absolute LCI and $S_{ACIN}$ values

224     (Figures 2 and 3) illustrate that a considerable proportion of high and standard risk patients had

225     pulmonary function indices above the ULN.

226        LCI and $S_{ACIN}$ were abnormal in 60% (9 of 15 survivors) and 53% (8 of 15 survivors) of

227     participants at high risk and in 23% (6 of 26 survivors) and 21% (5 of 24 survivors) at standard risk

228     (p<0.050) (Table 3). $N_2MBW$ detected any abnormal value in 63% (26/41) of patients, 80% (12/15)

229     in the high risk and 54% (14/26) in the standard risk group (p=0.094).

230

231     **Spirometry parameters**

232     After quality control, spirometry indices were available for 15 of 17 high risk survivors (15 $FEV_1$ and

233     15 FVC indices) and 23 of 29 standard risk survivors (23 $FEV_1$ and 23 FVC indices) (Figure E2,

234     online supplement). Childhood cancer survivors overall and high risk survivors in particular had

235     decreased mean $z$ scores for $FEV_1$ (overall -0.43, high risk -0.94) and FVC (overall -0.36, high risk -

236     1.14) and preserved $FEV_1/FVC$ ratios (overall 0.07, high risk 0.36) (Table 2). Spirometry parameters

237     were normal in standard risk patients, with mean $z$ scores of -0.10 for $FEV_1$, 0.15 for FVC, and -0.12

238     for $FEV_1/FVC$.

239        $FEV_1$ and FVC were abnormal in 33% of participants in the high risk group and in 0% and 4%

240     of participants in the standard risk group (p<0.050) (Table 3). As expected, standard risk survivors

241     had a prevalence of abnormal spirometry comparable to the healthy reference population (5% given

242     a defined LLN of mean $z$ score -1.64). Overall, spirometry detected less cases of pulmonary

243     dysfunction than $N_2MBW$ with any abnormal parameter in 18% (7/38) of patients, 33% (5/15) in the

244     high risk and 9% (2/23) in the standard risk group (p=0.055). The proportion of participants who had

245     both an abnormal spirometry and an abnormal $N_2MBW$ test was n=4 (24%) in the high risk group,

246     and n=1 (4%) in the standard risk group.

247

248     **Association between pulmotoxic exposure and pulmonary function parameters**

249     In a linear regression adjusting for the possible confounders sex, age, weight, height, and active

250     smoking status at pulmonary function assessment, we investigated the change of lung function

251     indices when comparing high risk vs standard risk (reference) survivors (Table E2, online

252     supplement). LCI and $S_{ACIN}$ were higher in survivors exposed to pulmotoxic cancer treatment – LCI

253     by 1.110 units and $S_{ACIN}$ by 0.036 units. $FEV_1$ and FVC were lower in survivors exposed to

254     pulmotoxic cancer treatment – $FEV_1$ by 0.239 L and FVC by 0.778 L. The physical activity

255     intervention had no apparent effect on pulmonary function parameters. We also observed no effect

256     of time since diagnosis on pulmonary function parameters (data not shown).

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258     **Pulmonary function parameters in high risk survivors after HSCT**

259     Table E3 (online supplement) shows demographic and clinical characteristics, and pulmonary

260     function abnormalities of the 17 high risk survivors, among whom five had undergone allogeneic

261     HSCT. Four of these five HSCT survivors received total body irradiation with 12 Gray and one

262     survivor received pulmotoxic chemotherapy with busulfan. Pulmonary function assessment was

263     complete in four of the five HSCT survivors. All four HSCT survivors with available $N_2$MBW results

264     had at least one abnormal value, whereas only one had abnormal spirometry results (Table E3,

265     online supplement). All four of these HSCT survivors had abnormal $S_{ACIN}$ parameters and two had

266     abnormal LCI parameters, but only one had decreased $FEV_1$ and FVC (Figures 2 and 3).

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268

269     **Discussion**

270     $N_2$MBW has to date been investigated only in pediatric cancer patients undergoing HSCT (14). This

271     is the first study that shows results of $N_2$MBW tests in long-term survivors of childhood cancer. We

272     found that more than half of childhood cancer survivors had some signs of pulmonary dysfunction.

273     $N_2$-MBW detected more cases of abnormal pulmonary function than spirometry. LCI and $S_{ACIN}$ in

274     particular were abnormal in patients considered to be at no increased risk because they had

275     received chemotherapy other than busulfan, bleomycin, or nitrosoureas or radiotherapy other than

276     to the chest.

277        Our results are in line with other studies suggesting that $N_2MBW$ might be a sensitive and

278    complementary marker of pulmonary damage. A study of adult HSCT recipients from our group

279    showed that LCI correlated well with increasing grades of chronic graft-versus-host-disease

280    (cGvHD), a pulmonary complication after HSCT (16). LCI and $S_{ACIN}$ were more sensitive than

281    spirometry in detecting abnormal pulmonary function; 74% of patients had abnormal LCI, but only

282    36% had abnormal $FEV_1$. In the current study, 60% of high risk patients had abnormal LCI and 33%

283    abnormal $FEV_1$. A publication on lung transplant recipients reported that LCI increased with severity

284    of bronchiolitis obliterans syndrome (27). A significant proportion of patients had abnormal LCI but

285    not $FEV_1$ values, which also suggests LCI to be more sensitive than spirometry in the early

286    detection of pulmonary disease (27). In the only study that assessed $N_2MBW$ in pediatric HSCT

287    recipients who were still under active cancer treatment (14), 28 children underwent pulmonary

288    function assessment before and after HSCT. Again, LCI was a sensitive marker for cGvHD and was

289    associated with persisting pulmonary symptoms, but LCI measured at HSCT was not predictive for

290    the development of pulmonary cGvHD within one year after HSCT (14).

291        For spirometry indices, we identified only two studies that used the LLN to define abnormal

292    spirometry as we did in our study and as recommended in the literature (26). In a Danish study of 94

293    leukemia survivors not exposed to pulmotoxic cancer treatment, with a median age at study of 16

294    years, abnormal $FEV_1$, FVC, and $FEV_1$/FVC were observed in 8%, 15%, and 1% of survivors

295    compared to 0%, 4%, and 4% in our standard risk group (22). In the other study of 41 Hodgkin and

296    non-Hodgkin lymphoma survivors exposed to pulmotoxic cancer treatment, with a median age at

297    study of 21 years, 27%, 27%, and 10% had abnormal $FEV_1$, FVC, and $FEV_1$/FVC, which compares

298    to 33%, 33%, and 7% in our high risk patients (7). One additional study also used the LLN with a

299    lower cut-off (*z* score -2.00) (28). All other studies that included abnormal spirometry indices used

300    %-predicted to define abnormality and results are therefore not directly comparable to our findings

301    (Table E4, online supplement).

302        As the first study to investigate $N_2MBW$ in childhood cancer survivors, its strength derives

303    from its standardized assessment of pulmonary function performed by a specialized, experienced

304    pulmonary function laboratory. We included only high quality data after rigorous quality control, and

305 technicians for pulmonary function assessment were blinded to the survivor's risk group. We

306 collected detailed cancer treatment information for all patients.

307       One of the limitations of our study is that the study population was small and included a mix

308 of different underlying diagnoses and pulmotoxic cancer treatments. However, the fact that we

309 found some evidence for a benefit of $N_2MBW$ in this heterogeneous group is encouraging and

310 should stimulate further studies including larger numbers of participants. The small number of

311 participants in our study did not allow to study treatment modalities separately, and also limits the

312 interpretation of the differences found between HSCT and no-HSCT survivors. However, the fact

313 that all HSCT survivors had abnormal $S_{ACIN}$ parameters is worthwhile noticing and may suggest that

314 total body irradiation and intensive conditioning regimens may have caused alveolar damage.

315 Replication of our results and refined assessment in larger studies are needed before the place of

316 $N_2MBW$ in the clinical follow-up of childhood cancer survivors becomes clearer. Furthermore, we

317 assessed pulmonary function only once in a cross-sectional fashion and did not have information on

318 baseline pulmonary function before initiation of cancer treatment. Therefore, we cannot exclude

319 preexisting preclinical pulmonary dysfunction. However, as survivors were asymptomatic and not

320 aware of any pulmonary disease, this should not have substantially changed the results. Also the

321 reference populations used for establishing normal values for lung function tests might contain

322 subjects with subclinical disease, that has resulted neither in symptoms nor in a diagnosis.

323 Longitudinal studies assessing pulmonary function before, during, and after treatment will be useful

324 to investigate whether abnormal $N_2MBW$ indices predict future pulmonary morbidity and mortality in

325 childhood cancer survivors. These studies should also measure diffusion capacity of the lungs for

326 carbon monoxide (DLCO), which is another sensitive measure of early lung damage after chest

327 radiation (28) and chemotherapy with bleomycin (29), but was not included in the current study.

328 Further, there is no objective reference standard available, which represents early fibrotic changes

329 in the lung, and against which we could assess performance of $N_2MBW$ and spirometry. $N_2MBW$

330 and spirometry are proxy measures for different anatomical abnormalities with $N_2MBW$ measuring

331 ventilation homogeneity of the whole lung and spirometry primarily measuring obstruction of the

332 larger, proximal airways. Therefore, conclusions on the superiority of either measure cannot be

333    drawn at this stage. Finally, follow-up time since cancer diagnosis was variable in our participants;

334    however, we found no association between the length of follow-up time and pulmonary function

335    parameters.

336          Pulmotoxic cancer treatment leads to inflammatory and fibrotic changes of the small airways.

337    While busulfan, bleomycin, and nitrosoureas are currently recognized as pulmotoxic

338    chemotherapeutic agents, cyclophosphamide (6), methotrexate (9), and cisplatin (5, 6) also have

339    been implicated in pulmonary damage. This is consistent with our finding that a considerable

340    proportion of the standard risk group (48%) had abnormal $N_2MWB$ results that suggest some

341    damage to the small airways.

342          Mortality due to pulmonary diseases following treatment for childhood cancer is particularly

343    elevated (3). Yet early detection of pulmonary damage enables medical and lifestyle interventions

344    that possibly improve pulmonary outcomes (30, 31). Our observation that spirometry was normal in

345    standard risk patients stands in contrast to the pulmonary damage in half of this group that was

346    suggested by $N_2MBW$. Because $N_2MBW$ also is a tidal breathing test, the administration of which

347    can be largely independent of the age and clinical condition of patients, $N_2MBW$ can monitor

348    pulmonary function during childhood.

349          We conclude that further study of childhood cancer survivors is needed, particularly

350    longitudinal assessments, along with more sensitive surveillance of the pulmonary function of

351    patients previously perceived as facing standard risk. Since $N_2MBW$ identified more cases of

352    pulmonary dysfunction than spirometry, we believe $N_2MBW$ could be a complementary technique—

353    for patients of all ages—for the screening of childhood cancer survivors for pulmonary damage.

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448

449 **Table 1.** Characteristics of childhood cancer survivors participating in the SURfit study and

450 undergoing a pulmonary function assessment

| | Total<br>N=46 (100%)[*] | High risk[†]<br>N=17 (37%)[*] | Standard risk[‡]<br>N=29 (63%)[*] | P[§] |
|---|---|---|---|---|
| **Demographic characteristics** | | | | |
| Male sex | 24 (52%) | 9 (53%) | 15 (52%) | 0.936 |
| Age at study, median [IQR], years | 30 [25–40] | 31 [22–36] | 28 [25–41] | 0.559 |
| **Clinical characteristics** | | | | |
| Age at diagnosis, median [IQR], years | 10 [4–14] | 12 [9–14] | 6 [4–12] | 0.016 |
| Time since diagnosis, median [IQR], years | 20 [15–32] | 18 [13–25] | 24 [18–32] | 0.083 |
| Weight at study, median [IQR], kg | 68 [60–78] | 64 [52–71] | 74 [62–79] | 0.029 |
| Height at study, median [IQR], cm | 169 [163–177] | 174 [161–179] | 168 [163–174] | 0.851 |
| Active smoking | 10 (22%) | 2 (12%) | 8 (28%) | 0.209 |
| ICCC-3 cancer diagnosis | | | | |
|   I   Leukemia | 25 (54%) | 6 (35%) | 19 (66%) | 0.156 |
|   II  Lymphoma | 11 (24%) | 8 (47%) | 3 (10%) | |
|   III  CNS tumor | 2 (4%) | 1 (6%) | 1 (4%) | |
|   IV  Neuroblastoma | 1 (2%) | 0 (0%) | 1 (4%) | |
|   V  Retinoblastoma | 1 (2%) | 0 (0%) | 1 (4%) | |
|   VIII Bone tumor | 4 (9%) | 2 (13%) | 2 (7%) | |
|   IX  Soft tissue sarcoma | 1 (2%) | 0 (0%) | 1 (3%) | |
|   XIII LCH | 1 (2%) | 0 (0%) | 1 (4%) | |
| History of relapse | 2 (4%) | 1 (12%) | 1 (4%) | 0.130 |
| Any chemotherapy | 44 (96%) | 17 (100%) | 27 (93%) | 0.237 |
| Any radiotherapy | 23 (50%) | 15 (88%) | 8 (28%) | <0.001 |
| Chest radiation[∥] | 15 (33%) | 15 (88%) | 0 (0%) | <0.001 |
|     Median, [IQR], Gray | 20 [12–35] | 20 [12–35] | NA | NA |
|   Mediastinal/lung radiation | 9 (20%) | 9 (53%) | 0 (0%) | <0.001 |
|     Median, [IQR], Gray | 27 [20–39] | 27 [20–39] | NA | NA |
|   Cranio-spinal radiation | 2 (4%) | 2 (12%) | 0 (0%) | 0.059 |
|     Median, [IQR], Gray | 22 [20–23] | 22 [20–23] | NA | NA |
|   Total body irradiation | 4 (9%) | 4 (24%) | 0 (0%) | 0.059 |
|     Median, [IQR], Gray | 12 [12–12] | 12 [12–12] | NA | NA |
| HSCT | 5 (11%) | 5 (29%) | 0 (0%) | 0.002 |

451
452 Abbreviations: CNS, central nervous system; IQR, interquartile range; N, number; HSCT, hematopoietic stem cell
453 transplantation; LCH, Langerhans cell histiocytosis; ICCC-3, International Classification of Childhood Cancer, 3rd edition
454
455 [*] Column percentages are given

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456    † High risk = pulmotoxic cancer treatment including busulfan, bleomycin, nitrosureas, chest radiation, thoracic surgery,
457     HSCT
458    ‡ Standard risk = no pulmotoxic cancer treatment
459    § P-values comparing high risk and standard risk patients calculated from chi-squared tests for categorical variables and
460     from t-tests for continuous variables
461    ‖ Including mediastinal/lung radiation, cranio-spinal radiation, and total body irradiation
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**Table 2.** $N_2MBW$ (LCI, FRC, $S_{COND}$, $S_{ACIN}$) and spirometry ($FEV_1$, FVC, $FEV_1/FVC$) indices in childhood cancer survivors, median age 30 years (N=46)

| | Reference population* | Total N=46 | High risk† N=17 | Standard risk‡ N=29 | P§ |
|---|---|---|---|---|---|
| **$N_2MBW$‖** | | | | | |
| **LCI** | | | | | |
| mean (SD) | 6.94 (0.61) | 7.77 (1.64) | 8.22 (1.46) | 7.52 (1.71) | 0.195 |
| z score, mean (SD) | NA | 1.37 (2.69) | 2.09 (2.39) | 0.95 (2.81) | |
| **FRC L** | | | | | |
| mean (SD) | 3.21 (0.81) | 3.38 (1.10) | 3.38 (1.13) | 3.38 (1.10) | 0.998 |
| z score, mean (SD) | NA | 0.21 (1.35) | 0.21 (1.39) | 0.20 (1.36) | |
| **$S_{COND}$** | | | | | |
| mean (SD) | 0.028 (0.026) | 0.018 (0.016) | 0.017 (0.013) | 0.018 (0.0170) | 0.782 |
| z score, mean (SD) | NA | -0.40 (0.60) | -0.44 (0.51) | -0.38 (0.65) | |
| **$S_{ACIN}$** | | | | | |
| mean (SD) | 0.058 (0.028) | 0.095 (0.090) | 0.127 (0.092) | 0.076 (0.078) | 0.076 |
| z score, mean (SD) | NA | 1.34 (3.08) | 2.45 (3.29) | 0.65 (2.79) | |
| **Spirometry¶** | | | | | |
| **$FEV_1$** | | | | | |
| mean (SD) | 4.46 | 3.57 (0.83) | 3.38 (1.00) | 3.70 (0.69) | 0.247 |
| z score, mean (SD) | NA | -0.43 (1.26) | -0.94 (1.39) | -0.10 (1.07) | |
| **FVC L** | | | | | |
| mean (SD) | 5.32 | 4.35 (1.12) | 3.98 (1.10) | 4.59 (1.09) | 0.101 |
| z score, mean (SD) | NA | -0.36 (1.58) | -1.14 (1.23) | 0.15 (1.61) | |
| **$FEV_1/FVC$** | | | | | |
| mean (SD) | 0.85 | 0.83 (0.09) | 0.85 (0.08) | 0.82 (0.10) | 0.305 |
| z score mean (SD) | NA | 0.07 (1.24) | 0.36 (1.14) | -0.12 (1.29) | |

Abbreviations: FRC, functional residual capacity; $FEV_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; HSCT, hematopoietic stem cell transplantation; LCI, lung clearance index; NA, not applicable; $N_2MBW$, nitrogen multiple-breath washout; N, number; SD, standard deviation; $S_{ACIN}$, acinar ventilation inhomogeneity index; $S_{COND}$, conductive ventilation inhomogeneity index

* Expected mean values from published reference populations: Husemann, Eur Respir J, 2014 (21) for $N_2MBW$; Quanjer, Eur Respir J, 2012 (22) for spirometry

† High risk = pulmotoxic cancer treatment including busulfan, bleomycin, nitrosureas, chest radiation, thoracic surgery, HSCT

‡ Standard risk = no pulmotoxic cancer treatment

§ P-values comparing high and standard risk patients calculated from t-tests

‖ $N_2MBW$ indices included after quality check; total survivors: 41 $N_2MBW$ (41 LCI, 39 $S_{ACIN}$), high risk survivors: 15 $N_2MBW$ (15 LCI, 15 $S_{ACIN}$), standard risk survivors: 26 $N_2MBW$ (26 LCI, 24 $S_{ACIN}$); see also Figure E1

¶ Spirometry indices included after quality check; total survivors: 38 spirometry (38 $FEV_1$, 38 FVC), high risk survivors: 15 spirometry (15 $FEV_1$, 15 FVC), standard risk survivors: 23 spirometry (23 $FEV_1$, 23 FVC); see also Figure E1

**Table 3.** Prevalence of abnormal N$_2$MWB parameters (above the upper limit of normality) and

spirometry parameters (below the lower limit of normality) in 46 childhood cancer survivors, median

age 30 years, stratified into high and standard risk for pulmonary dysfunction

| | Reference population* | Total N=46 | High risk† N=17 | Standard risk‡ N=29 | P§ |
|---|---|---|---|---|---|
| **N$_2$MBW‖** | | | | | |
| LCI, ULN | 5% | 15/41 (37%) | 9/15 (60%) | 6/26 (23%) | **0.018** |
| FRC L, ULN | 5% | 6/41 (15%) | 2/15 (13%) | 4/26 (15%) | 0.858 |
| S$_{COND}$ L$^{-1}$, ULN | 5% | 0/39 (0%) | 0/14 (0%) | 0/25 (0%) | NA |
| S$_{ACIN}$ L$^{-1}$, ULN | 5% | 13/39 (33%) | 8/15 (53%) | 5/24 (21%) | **0.036** |
| Any abnormal N$_2$MBW value | NA | 26/41 (63%) | 12/15 (80%) | 14/26 (54%) | 0.094 |
| **Spirometry ¶** | | | | | |
| FEV$_1$, LLN | 5% | 5/38 (13%) | 5/15 (33%) | 0/23 (0%) | **0.003** |
| FVC L, LLN | 5% | 6/38 (16%) | 5/15 (33%) | 1/23 (4%) | **0.017** |
| FEV$_1$/FVC, LLN | 5% | 2/38 (5%) | 1/15 (7%) | 1/23 (4%) | 0.754 |
| Any abnormal spirometry value | NA | 7/38 (18%) | 5/15 (33%) | 2/23 (9%) | 0.055 |

Abbreviations: LCI, lung clearance index; FEV$_1$, forced expiratory volume in 1 second; FVC, forced vital capacity; FRC, functional residual capacity; LLN, lower limit of normality; N, number; NA, not applicable; N$_2$MBW, nitrogen multiple-breath washout; S$_{ACIN}$, acinar ventilation inhomogeneity index; S$_{COND}$, conductive ventilation inhomogeneity index; ULN, upper limit of normality

* Expected prevalence of abnormal pulmonary function parameters based on definitions of ULN = $z$ score + 1.64 (for N$_2$MBW) and LLN = $z$ score - 1.64 (for FEV$_1$ and FVC) and <0.7 for FEV$_1$/FEV
† High risk = pulmotoxic cancer treatment including busulfan, bleomycin, nitrosoureas, chest radiation, thoracic surgery, HSCT
‡Standard risk = no pulmotoxic cancer treatment
§ P-values comparing high and standard risk patients calculated from chi-squared tests
‖ Included N$_2$MBW indices after quality check; total survivors: 41 N$_2$MBW (41 LCI, 39 S$_{ACIN}$), high risk survivors: 15 N$_2$MBW (15 LCI, 15 S$_{ACIN}$), standard risk survivors: 26 N$_2$MBW (26 LCI, 24 S$_{ACIN}$)
¶ Included spirometry indices after quality check; total survivors: 38 spirometry (38 FEV$_1$, 38 FVC), high risk survivors: 15 spirometry (15 FEV$_1$, 15 FVC), standard risk survivors: 23 spirometry (23 FEV$_1$, 23 FVC)
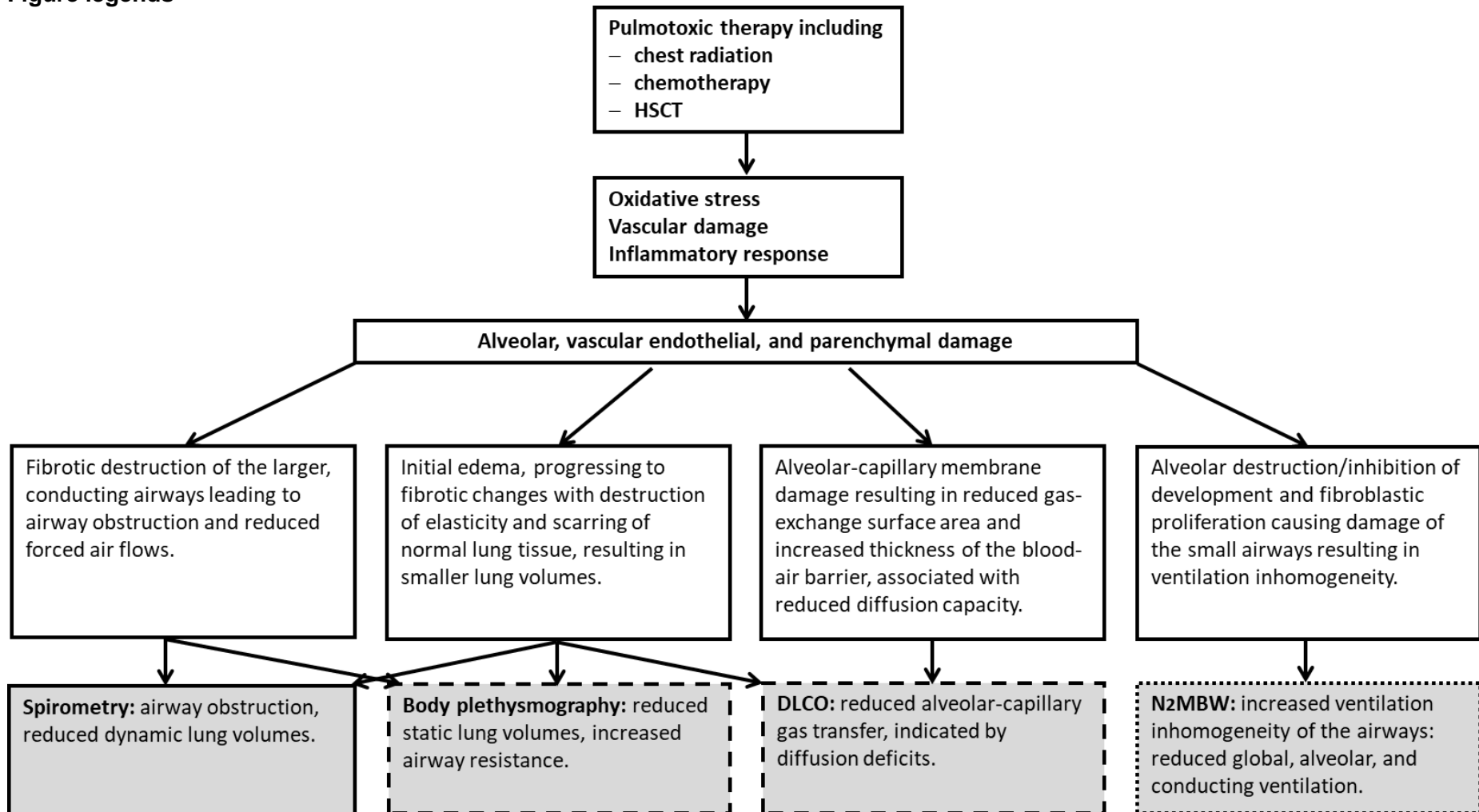
**Figure 1: Model of pathophysiological mechanisms underlying pulmotoxic effects of cancer therapy and relationship with lung function outcome measures:**

The white boxes describe mechanisms of cancer-treatment-related lung injury. Ionizing radiation from chest radiation, chemotherapy, and hematopoietic stem cell transplantation induce release of oxygen radicals, cause vascular damage, and promote inflammation in the lung. Persistent inflammation results in pathological changes in the alveolar, vascular endothelial, and parenchymal departments, which is associated with pathologic immune cell infiltration, capillary permeability, and pulmonary edema. The lung damage may be acute and reversible—presenting as pneumonitis, or chronic and irreversible—presenting as pulmonary fibrosis.

The shaded boxes describe the outcome measures described in different types of lung function tests. The box framed with continuous lines describe lung function parameters assessed in previous studies and in this study; boxes framed in dashed line represents outcomes assessed in previous studies but not in this study; the box framed with a pointed line describes outcomes assessed only in this study.

Abbreviations: DLCO, diffusing capacity of the lung for carbon monoxide; HSCT, hematopoietic stem cell transplantation; $N_2MBW$, nitrogen multiple-breath washout.
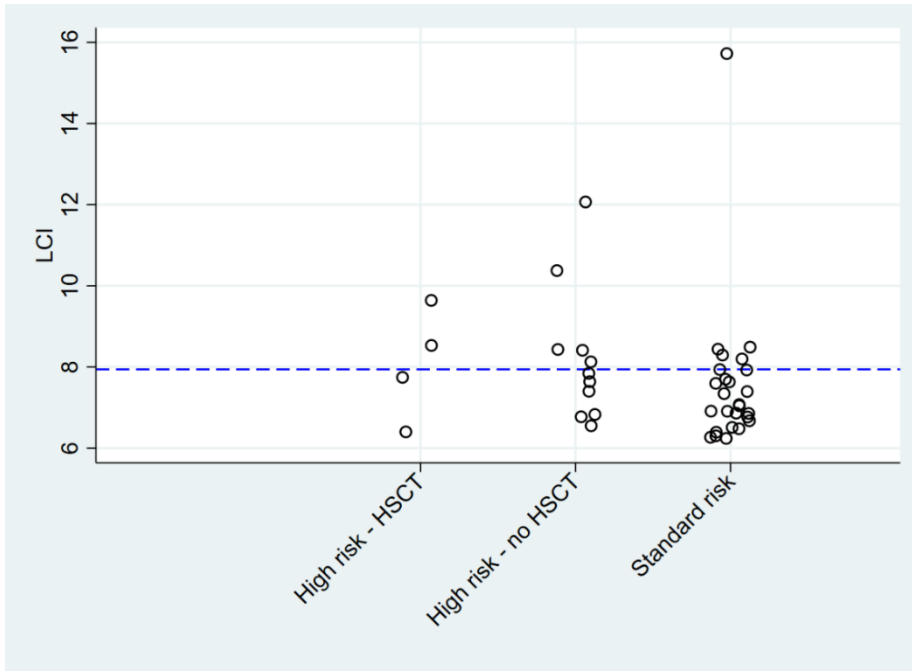
**Figure 2.** LCI in 46 adult childhood cancer survivors stratified into high risk – with and

without HSCT – and standard risk for pulmonary dysfunction. Dashed blue line = ULN = 7.94

(21)

Abbreviations: HSCT, hematopoietic stem cell transplantation; LCI, lung clearance index; ULN, upper limit of
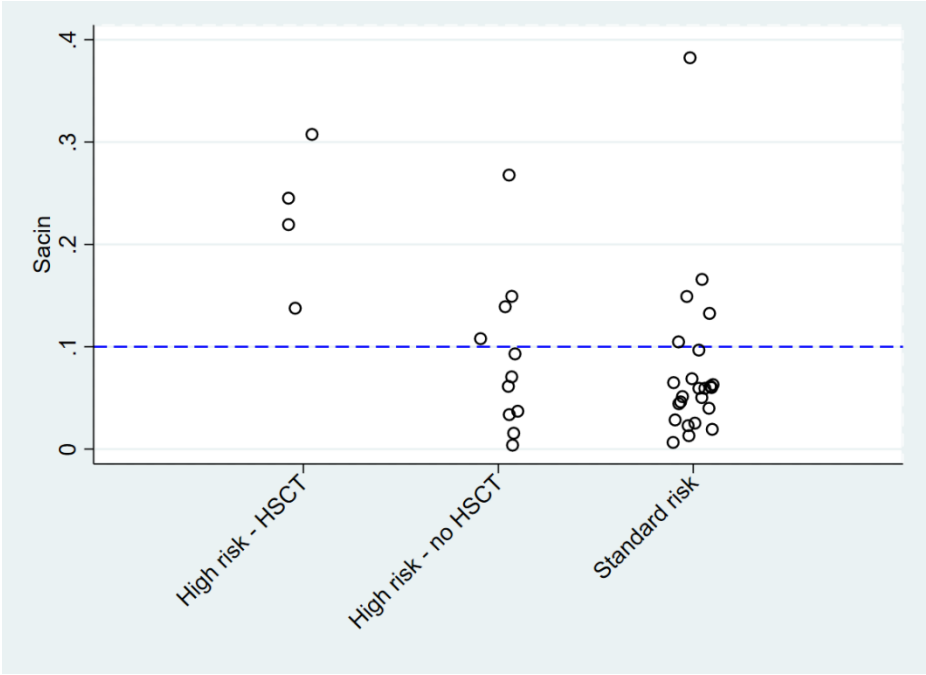
normality

**Figure 3.** $S_{ACIN}$ in 46 adult childhood cancer survivors stratified into high risk, with and without HSCT, and standard risk for pulmonary dysfunction. Dashed blue line = ULN = 0.10 (21)

Abbreviations: HSCT, hematopoietic stem cell transplantation; $S_{ACIN}$, acinar ventilation inhomogeneity index; ULN, upper limit of normality