Non-alcoholic fatty liver disease is associated with early left ventricular dysfunction in childhood acute lymphoblastic leukaemia survivors

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Abstract

Background: Childhood acute lymphoblastic leukaemia (ALL) survivors have an increased risk of metabolic and cardiovascular disease. We aimed to assess the presence of non-alcoholic fatty liver disease (NAFLD) in childhood ALL and if it is associated with early cardiovascular dysfunction.
Methods: In total, 53 childhood ALL survivors and 34 controls underwent auxological evaluation, biochemical assay, liver, heart and vascular ultrasound study.
Results: NAFLD was more frequent in ALL patients than in controls (39.6% vs 11.7%, $P<0.01$). Patients with NAFLD were more obese and insulin resistant than patients without NAFLD. Flow-mediated dilatation and interventricular septum were lower in the ALL group than those in the control group ($P<0.001$ for both). The patients with NAFLD showed lower left ventricular ejection fraction than those without NAFLD ($P=0.011$). In ALL survivors, BMI-SDS and subcutaneous fat were the strongest predictors of NAFLD, whereas preperitoneal adipose tissue and C-reactive protein were the strongest predictors of left ventricular ejection fraction.
Conclusions: Childhood ALL survivors had higher prevalence of NAFLD than healthy controls, which is associated with early left ventricular impairment. In the case of fatty liver, a comprehensive heart evaluation is mandatory. We strongly recommend to prevent visceral adiposity in ALL survivors, to search for metabolic syndrome or its components and to reinforce the need of intervention on diet and lifestyle during the follow-up of these patients.

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Introduction

Over the last decades, the progress in multiagent therapy and supportive care have improved the survival rates of children with acute lymphoblastic leukaemia (ALL) (1, 2), but several long-term complications have been described in survivors (3, 4). Late treatment-related effects such as secondary malignancies, cardiotoxicity, neurotoxicity, endocrinopathies, obesity, infertility and psychosocial effects (5, 6, 7, 8, 9, 10) have been reported early after the stop therapy as many years or decades later (2). From the metabolic point of view, ALL survivors are prone to develop visceral obesity, glucose intolerance, dyslipidaemia, insulin resistance (IR) and metabolic syndrome (MetS) (11, 12, 13, 14, 15, 16), which represent risk factors for cardiovascular disease (CVD).

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Insulin resistance has been reported as being strongly associated with hepatic steatosis, both in non-obese and lean subjects (17, 18). The reduced insulin sensitivity in adipose tissue seems to determine the increase in lipolysis and thus an increased flux of free fatty acids to the liver, with a subsequent accumulation of triglycerides in the hepatocytes. Triglycerides accumulated in the liver lead to the non-alcoholic fatty liver disease (NAFLD), which is potentially progressive and is considered the hepatic expression of the MetS (19). NAFLD is an alcohol-like liver disease, which occurs in the absence of alcohol abuse, and spans from simple steatosis to non-alcoholic steatohepatitis (NASH) and cirrhosis. This is the most common cause of chronic liver disease worldwide, with a considerably high rate of morbidity and mortality (20, 21). Its prevalence increases with age (22, 23) and appears to be less than 10% in children (24), but above 50% in obese children (25), and higher in adults, with a prevalence of 36.8% in men and 25.7% in women older than 30 years in our Mediterranean area (26). NAFLD is a multisystem disease that affects many extra-hepatic systems, including the heart and the vascular system (27). Even though most of papers have focused on the relationship between liver steatosis and cardiovascular impairment in adults, some interesting data have been reported in childhood and adolescence, as well. In particular, alteration of markers of subclinical atherosclerosis (28, 29), myocardial IR (30), impairment of heart metabolism (31) and abnormal left ventricular structure with impairment of diastolic function have been reported in adults (32, 33).

Although it is well acknowledged that NAFLD can be considered the liver manifestation of MetS, which is a possible complication in childhood ALL survivors, there are no studies searching for fatty liver in these patients. In this study, we aimed to evaluate the presence of ultrasound (US)-assessed NAFLD in childhood ALL and if it is associated with US signs of early cardiovascular dysfunction. Furthermore, we also aimed to evaluate whether there are auxological and metabolic parameters accounting for liver and cardiovascular alterations in these patients.

Subjects and methods

Patients

Childhood ALL survivors who received chemotherapy according to the ongoing international ALL protocols of the Italian Association for Pediatric Hematology and Oncology (AIEOP), University Hospital of Bari were recruited between February 2014 and January 2015. Inclusion criteria were (a) age 4–20 years; (b) end of antineoplastic therapy since at least 3 months and (c) complete remission of ALL. Exclusion criteria were (a) endocrine and/or metabolic (including liver diseases) and/or cardiovascular disorders prior to ALL diagnosis and (b) genetic syndromes (Down syndrome, etc.). The study protocol was approved by the Local Ethic Committee. Written informed consent was signed by both parents or by patients aged older than 18 years. All the procedures used were in accordance with the guidelines of the Helsinki Declaration on Human Experimentation. Fifty-three patients (19 males and 34 females), aged 9.7 ± 4.1 years, who met the inclusion criteria accepted to join the study. All the patients had ended chemotherapy after 28.5 months (range 3–102 months). Six patients received chemotherapy according to high risk, 29 to intermediate risk and 18 to standard risk AIEOP-BFM ALL protocols (Table 1). Nine patients were treated with 18 Gy cranial irradiation for central nervous system prophylaxis. The control group consisted of 34 healthy subjects, pair matched by age (10.9 ± 4.0 years), sex (17 males and 17 females), recruited from patients attending the Paediatric Clinic of the University of Bari for minor trauma (first aid) or allergologic screening. All the subjects underwent a complete physical examination, including anthropometric parameters (height, weight, body mass index-standard deviation score – BMI-SDS) and assessment of pubertal stage according to Tanner criteria, blood sampling for biochemistry, liver, heart and vascular US studies. Both systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured from the right brachial artery using a mercury gauge in the supine position prior to examination after resting for a minimum of 5 min.

Biochemistry

After an overnight fast, blood samples were taken for the evaluation of lipid profile, i.e. total cholesterol, triglycerides (TG), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, fasting insulin, glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT) and high-sensitivity C-reactive protein (hsCRP). Insulin sensitivity was measured using homeostasis model assessment of insulin resistance (HOMA-IR), calculated as insulin (U/mL) × blood glucose (mmol/L)/22.5 (34). Total adiponectin and multimeric

Table 1 Details of radiotherapy and chemotherapy for ALL patients ($N = 53$ pts, 49 *B-lineage ALL*, 4 *T-ALL*).

Protocol	Protocol risk	Patients (*N*)	Cranial irradiation (18 Gy)	Chemotherapy according to different treatment phases			
				Induction	Consolidation	Reinduction	Maintenance
AIEOP-BFM ALL 2000	Standard	*15*	No	63 days: PDN, VCR, DNR, L-ASP, CPM, ARA-C, 6MP	56 days; MTX, 6MP	49 days: DXM,VCR, ADM, L-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP
	Intermediate	*22*	No	63 days: PDN, VCR, DNR, L-ASP, CPM, ARA-C, 6MP	56 days: MTX, 6MP	2 × 28 days: DXM, VCR, ADM, L-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP
		4 (T-ALL)	Yes				
	High	*1*	No	63 days: PDN, VCR, DNR, L-ASP, CPM, ARA-C, 6MP	Block 1, 2, 3 (3 × 6 days): DXM, VCR, VDS, MTX, IFO, ARA-C, CPM, L-ASP, DNR, VP16	2 × 49 days: DXM, VCR, ADM, L-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP
		4	Yes				
AIEOP-BFM ALL 2009	Standard	*3*	No	63 days: PDN, VCR, DNR, PEG-ASP, CPM, ARA-C, 6MP	56 days: MTX, 6MP	49 days: DXM, VCR, ADM, PEG-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP
	Intermediate	*3*	No	63 days: PDN, VCR, DNR, PEG-ASP, CPM, ARA-C, 6MP	56 days: MTX, 6MP	49 days: DXM,VCR, ADM, PEG-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP
	High	*1*	No	63 days: PDN, VCR, DNR, PEG-ASP, CPM, ARA-C, 6MP	Block 1, 2, 3 (3 × 6 days): DXM, VCR, VDS), MTX, IFO, ARA-C, CPM, PEG-ASP, DNR, VP16	2 × 49 days: DXM, VCR, ADM, PEG-ASP, CPM, ARA-C, 6TG	Up to 24 months of treatment: oral MTX and 6MP

6MP, mercaptopurine; 6TG, thioguanine; ADM, Adriamycin; ARA-C, cytarabine; CPM, cyclophosphamide; DNR, daunorubicin; DXM, dexamethasone; L-ASP, l-asparaginase; MTX, methotrexate; PDN, prednisone; PEG-ASP, pegylated asparaginase; VCR, vincristine.

high-molecular-weight (HMW) subfraction were measured by a commercial ELISA (ELISA 47-ADPH-9755; ALPCO Diagnostics, Salem, Vermont).

Liver US examination

Patients and controls underwent liver US to detect the presence of NAFLD. US is currently the most common method for screening NAFLD (35, 36). Its sensitivity to detect steatosis decreases with a degree of fat infiltration less than 30% (37). It was performed in all subjects by a single trained and experienced operator using a scanner Philips/ATL HDI 5000. The operator was unaware of the clinical course and laboratory details of the patients. Steatosis was classified as (36): grade 1 – mild (liver attenuation slightly less than spleen), grade 2 – moderate (more pronounced difference between liver and spleen and intrahepatic vessels not seen or slightly higher attenuation than liver) and grade 3 – severe (markedly reduced liver attenuation with sharp contrast between liver and intrahepatic vessels) (38). The US measurements of intra-abdominal ('visceral') and subcutaneous fat were taken using a convex (C5-40R) probe and linear array probe L12-5 respectively. US-determined subcutaneous fat was defined as the distance between the skin and external face of the recto abdominis muscle, and visceral fat was defined as the distance between the internal face of the same muscle and the anterior wall of the aorta.

Echocardiography and vascular US studies

Conventional 2-dimensional echocardiographic M-mode pulsed Doppler and tissue Doppler imaging were all performed according to international guidelines (39, 40). We performed vascular US measurements at Cardiology Department. All patients underwent duplex scan examination of brachial artery to evaluate the flow-mediated vasodilation (FMD): this parameter evaluates the dilation of brachial artery after a pressure stress. Temperature, food, stress, drugs and sympathetic stimuli affect FMD. Therefore, the examination was performed on fasting subjects in the previous 8–12 h, in a quiet

room with the temperature maintained between 22°C and 24°C. The measurement was performed on the right brachial artery using high-resolution US instrument, and we used a linear array transducer with a minimum frequency of 7 MHz to acquire images of brachial artery with a good resolution. A baseline image was acquired; thereafter, arterial occlusion was created by cuff inflation to suprasystolic pressure. The sphygmomanometric cuff was inflated at least 50 mmHg above systolic pressure to occlude arterial inflow for a standardised length of time. The image of the artery was continuously recorded from 30 s before to 2–3 min after cuff deflation. The baseline was the average of the measurements obtained in the first minute. The reactive increase of flow was calculated as the ratio between the maximum diameter obtained after the deflation of the cuff and the basal diameter of the artery. FMD was therefore defined as the percentage increase in the diameter of the brachial artery, after application of a pressure stimulation (41). All patients underwent duplex scan examination of carotid vessels, and the intima-media thickness of common right and left carotids arteries (cIMT) were measured using Philips Sonos 5500 with a 7.5 MHz high-resolution probe. cIMT was revealed as a hypoechoic band that did not project into the arterial lumen and was measured during the final phase of diastole according to the method described by Pignoli *et al*. (42). According to literature, the cIMT is defined as the distance between the edge of the lumen-intima and media-adventitia of the vessel. In this way, the cIMT is defined as the distance between the first hyperechoic line and the second line, separated by a hypoechoic space. The measurement was performed bilaterally, for three times, in the proximal direction 1 cm from the carotid bulb, and cIMT was calculated as the arithmetic mean of the three measurements in 3 different positions (43). The value of cIMT considered for statistical analysis was represented by the average of right and left measurements. Another vascular parameter that we measured was the APAO (antero-posterior abdominal aorta diameter). This measurement was obtained with an US scanner high-resolution Philips 5500 equipped with an electronic probe from 3 MHz. The patients were fasted for at least 8 h to reduce the air bowel and to improve the acquisition of the image. The APAO was defined as the maximum outer diameter of the infrarenal abdominal aorta, calculated as the distance between the near and far walls of the abdominal aorta. The measurements were performed 1 cm above and distal to the navel and in centimetres (44).

Statistical analysis

Data were given as mean ± standard deviation (s.d.). BMI was expressed in Standard Deviation Score (SDS) with the GrowthCalculator3 in keeping with Italian growth charts. After evaluating the normal distribution of the variables, the analysis of variance (ANOVA) test was used to compare the mean values between groups. The Chi-square test was used to assess the statistical difference between categorical variables. The Spearmen's linear correlation coefficient was used to study the relationship between the continuous variables. The prediction model was run for LVRF and NAFLD by means of linear regression analysis and then multiple linear regression analysis with a stepwise method fitted by least squares with the variables that resulted correlated with them. The statistical analysis was run with SPSS, version 20.0 for Mac OS. A P value <0.05 was considered statistically significant.

Results

No significant differences were found between ALL children and controls according to age, sex, pubertal stage, weight, height, BMI-SDS, SPB and DBP. Metabolic and biochemical data of patients and controls are reported in Table 2. US findings, baseline clinical characteristics and metabolic markers are listed in Table 2. US signs of NAFLD were observed in 21 of 53 patients (39.6%) and classified as mild in 17 (32%), moderate in 3 (5.7%) and severe in 1 (1.9%). US signs of NAFLD were found in 4/34 (11.7%) of the control group ($P<0.01$), classified as mild. The prevalence of fatty liver in the ALL subjects was not different on the basis of gender, puberty, chemotherapy risk protocol and radiotherapy treatment (Table 2). Subcutaneous and preperitoneal adipose tissue were significantly higher in the ALL patients with NAFLD than those in the patients without NAFLD ($P=0.039$ and $P=0.021$ respectively). The ALL patients with NAFLD had significantly higher BMI-SDS, HOMA-IR and waist/height ratio than the patients without NAFLD (Table 2). On the other hand, HDL cholesterol was higher in non-NAFLD than in NAFLD patients. No significant differences were found for the other metabolic parameters.

Vascular and heart findings

FMD and IVS were significantly lower in the ALL group than in the control group ($P<0.001$ for both) (Table 3). The patients with NAFLD showed a statistically lower

Table 2 Clinical and biochemical characteristics of study population. Data are expressed as mean ± S.D.

	Controls	ALL patients	*P**	US-negative hepatic steatosis	US-positive hepatic steatosis	*P***
Subjects (males/females)	34 (17/17)	53 (19/34)	0.191	32 (13/19)	21 (6/15)	0.371
Age at recruitment (years)	10.9 ± 4.0	9.7 ± 4.1	0.173	9.2 ± 3.7	10.4 ± 4.8	0.293
Age at diagnosis (years)	–	5.4 ± 3.8	–	5.1 ± 3.6	5.9 ± 4.2	0.440
Radiotherapy (no/yes)	–	44/9	–	26/6	18/3	0.672
Prepubertal/pubertal		36/17		24/8	12/9	0.173
BMI-SDS	0.6 ± 1.0	0.9 ± 0.9	0.101	0.6 ± 0.8	1.3 ± 0.9	**0.003**
Waist/height ratio	–	0.5 ± 0.1	–	0.51 ± 0.06	0.55 ± 0.06	**0.028**
Sistolic BP (mmHg)	96 ± 5	106 ± 10	**<0.001**	105 ± 10	107 ± 11	0.400
Diastolic BP (mmHg)	65 ± 8	66 ± 8	0.763	65 ± 8	67 ± 6	0.301
HOMA-IR	1.7 ± 0.5	2.6 ± 2.0	**0.011**	2.1 ± 1.2	3.4 ± 2.7	**0.029**
Triglycerides (mg/dL)	59 ± 35	69 ± 31	0.160	69 ± 31	68 ± 30	0.844
Total cholesterol (mg/dL)	128 ± 13	151 ± 24	**0.003**	156 ± 23	144 ± 25	0.084
LDL cholesterol (mg/dL)	59 ± 15	87 ± 18	**<0.001**	89 ± 17	84 ± 19	0.351
HDL cholesterol (mg/dL)	61 ± 7	50 ± 10	**<0.001**	53 ± 9	47 ± 10	**0.021**
hsCRP (mg/dL)	1.8 ± 1.0	5.2 ± 10.3	**<0.001**	3.8 ± 2.9	7.3 ± 16.0	0.232
AST (IU/mL)	20 ± 5	22 ± 6	0.839	23 ± 5	21 ± 7	0.076
ALT (IU/mL)	24 ± 7	23 ± 6	0.781	23 ± 4	23 ± 9	0.872
GGT (IU/mL)	18 ± 6	16 ± 5	0.690	15 ± 5	17 ± 4	0.279
Adiponectin	8.3 ± 3.3	7.8 ± 2.9	0.489	8.1 ± 2.8	7.3 ± 3.1	0.319
HMW adiponectin	5.7 ± 1.8	4.5 ± 2.5	0.010	4.6 ± 2.4	4.2 ± 2.7	0.572
Subcutaneous adipose tissue	n.a.	2.3 ± 6.8	–	0.7 ± 0.6	4.6 ± 10.4	**0.039**
Visceral adipose tissue	n.a.	8.7 ± 11.5	–	7.2 ± 8.5	10.9 ± 15.0	0.266
Preperitoneal adipose tissue	n.a.	0.7 ± 0.4	–	0.6 ± 0.4	0.8 ± 0.5	**0.021**

*Controls vs patients; **US-negative vs US-positive hepatic steatosis. n.a. not assessed.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, gamma-glutamyl transpeptidase; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; hsCRP, high-sensitive C-reactive protein; US, ultrasound.

LVEF than those without NAFLD (62.0 ± 7.7 vs 56.0 ± 8.7 respectively, $P=0.011$, Fig. 1), without any difference for FMD (9.9 ± 4.5 vs 9.7 ± 4.5%), cIMT (0.46 ± 0.06 vs 0.46 ± 0.07 cm), APAO (9.7 ± 1.8 vs 10.5 ± 2.2 mm) and IVS (7.8 ± 1.9 vs 8.5 ± 2.0 mm). Metabolic, heart and vascular US parameters were not different between patients treated with CT+RT and with CT alone or on the basis of chemotherapy risk protocol (data not shown).

Correlations analysis

HOMA-IR was correlated with BMI-SDS ($r^2=0.581$, $P=<0.001$), preperitoneal adipose tissue ($r^2=0.318$, $P=0.02$) and waist/height ratio ($r^2=0.531$, $P<0.001$). The correlation analysis showed that the LVEF was negatively correlated with age at diagnosis ($r^2=-0.343$, $P=0.012$), age at recruitment ($r^2=-0.306$, $P=0.026$), preperitoneal adipose tissue ($r^2=-0.487$, $P<0.001$) and hsCRP ($r^2=-0.333$, $P=0.015$). The IVS was correlated with BMI-SDS ($r^2=0.364$, $P=0.007$) and waist/height ratio ($r^2=0.453$, $P=0.001$). FMD was correlated with adiponectin ($r^2=0.370$, $P=0.008$) and HMW adiponectin ($r^2=0.373$, $P=0.007$).

Regression analysis

In ALL survivors group, we investigated the predictors of NAFLD and LVEF correlated with clinical and metabolic characteristics. BMI-SDS, subcutaneous adipose tissue, waist/height ratio, preperitoneal adipose tissue and LDL cholesterol were independently associated with NAFLD (Table 4). When we considered all the variables with a stepwise analysis, only BMI-SDS and subcutaneous adipose tissue were significantly associated with NAFLD severity (adjusted $r^2=0.243$; $P<0.001$). Preperitoneal adipose tissue, age at diagnosis

Table 3 Heart and vascular ultrasound findings in patients and controls.

	ALL group	Control group	*P*
LVEF (%)	59.6 ± 8.6	60.7 ± 5.7	0.527
FMD (%)	9.8 ± 4.5	14.6 ± 4.3	<0.001
C-IMT (cm)	0.46 ± 0.06	0.48 ± 0.07	0.13
APAO (mm)	10.1 ± 2.0	10.7 ± 0.9	0.075
IVS (mm)	8.1 ± 1.9	11.9 ± 0.7	<0.001

APAO: antero-posterior abdominal aorta diameter; c-IMT, common right and left carotids arteries; FMD, flow-mediated vasodilation; IVS, interventricular septum; LVEF, left ventricular ejection fraction.

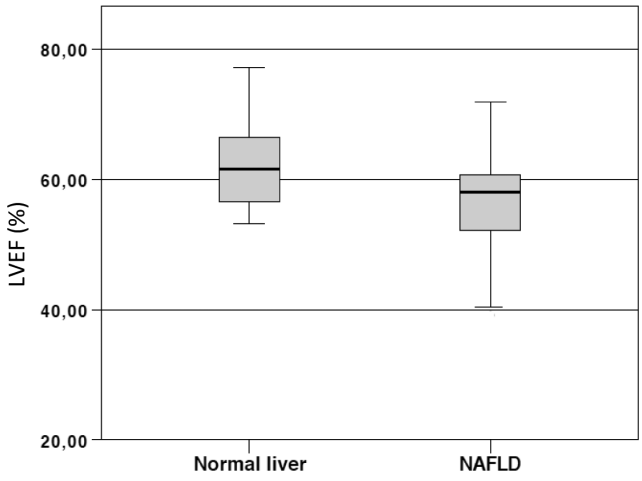

Figure 1

Boxplot pictures of left ventricular ejection fraction (LVEF) in ALL patients with and without non-alcoholic fatty liver disease (NAFLD). The difference between the mean values is statistically significant ($P=0.011$).

and at recruitment, hsCRP and NAFLD severity were independently associated with LVEF (Table 4). Considering all the variables, preperitoneal adipose tissue and hsCRP were associated with LVEF (adjusted $r^2=0.286$; $P<0.001$).

Discussion

The most important findings of our paper are that NAFLD was detected in nearly 40% of childhood ALL survivors, more frequently than in age-, sex- and BMI-matched healthy subjects and that in ALL subjects, NAFLD was associated with a reduction of the LVEF compared to normal liver patients. The latter finding is in keeping with the recent finding that NAFLD is seemingly a further independent factor playing a role in the reduction of ejection fraction in NAFLD patients (45). NAFLD was associated also with an increased cardiovascular risk and correlated with alterations in cardiac geometry and function with a reduction of LVEF (46). To the best of our knowledge, NAFLD in ALL survivors was reported only in two case reports, and in both of them, the steatosis occurred after total body irradiation in association with severe insulin resistance and metabolic impairment (47). No observational studies were run in these patients despite they may develop MetS or its components more frequently than the general population and despite NAFLD, which has become increasingly frequent, is considered the hepatic expression of MetS.

It is acknowledged that childhood ALL survivors have an increased risk of MetS and its components, and radiotherapy further increases the risk of occurrence. The aetiology is likely multifactorial and might include not only direct and indirect effect of cancer therapy on metabolism but also reduced physical activity plays an important role (48, 49, 50). On the other hand, fatty liver is strongly associated with MetS (51) and with its diagnostic elements (52), but it is not yet clear if it is a cause or a consequence. These disorders share common pathophysiological mechanisms, suggesting that liver steatosis could be considered the hepatic manifestation of MetS (53, 54). Liver biopsy represents the gold standard for the diagnosis of NAFLD, even though US is currently the most common noninvasive method for the screening of asymptomatic patients, providing for at least 30% of steatosis (55, 56). US is also recommended as first-line investigation due to its availability and low cost and is recommended as first-line investigation due to its availability and low cost (57). Furthermore, an association

Table 4 NAFLD and LVEF in ALL survivors. Association among liver steatosis (NAFLD), left ventricular ejection fraction (LVEF), and other main parameters in linear regression analysis. Data are regression coefficients with adjusted r^2 in parentheses.

Variables	Predictors	*B* (r^2)	*P*
NAFLD presence	BMI-SDS	0.402 (0.145)	*0.003*
	Subcutaneous adipose tissue	0.369 (0.119)	*0.007*
	Waist/height circumference	0.347 (0.103)	*0.012*
	Preperitoneal adipose tissue	0.327 (0.089)	*0.017*
	LDL-cholesterol	−0.323 (0.086)	*0.020*
	HOMA-IR	0.314 (0.081)	*0.022*
LVEF	Preperitoneal adipose tissue	−0.487 (0.222)	*<0.001*
	Age at diagnosis	−0.343 (0.100)	*0.012*
	NAFLD severity	−0.337 (0.096)	*0.014*
	hsCRP	−0.333 (0.094)	*0.015*
	Age at recruitment	−0.306 (0.076)	*0.026*

between insulin resistance and NAFLD ultrasound scoring has been demonstrated in pubertal obese children (58), suggesting that US of the liver is useful in the routine check-up of the children with insulin resistance to allow the detection of NAFLD at an early stage.

In our paper, we showed that the prevalence of NAFLD in the patients was higher than that in healthy controls. As BMI was not different between patients and controls, we argue that this finding is likely related to changes in lifestyle, with reduced physical activity (3, 4), but we cannot exclude that the treatment with antineoplastic agents partially account for the fat accumulation. In fact treatment of ALL, beyond its acknowledged effect on IR, has been reported to cause derangement of lipid profile, in particular during steroids and asparaginase administration (59, 60, 61), and even though this toxic effect seems to be transient, long-term follow-up of patients with history of severe hypertriglyceridemia is recommended to better understand additional therapy-related risk factors for the development of MetS and CVD (60). The patients with NAFLD presented lower HDL cholesterol and higher indexes of adiposity (BMI, waist circumference/height ratio, subcutaneous and peritoneal fat) and IR than those with normal liver, and these factors are clustered in the definition of MetS. The common connection between these metabolic disorders is abnormal lipid traffic and its consequences, such as visceral adiposity and insulin resistance, which plays a central role in their pathogenesis (62). In our sample, IR increase was paralleled by BMI-SDS and visceral adiposity increase, whereas NAFLD was associated with higher degree of IR and visceral obesity, supporting this mechanism. The fat accumulation in the liver is an independent risk factor for MetS, and thus, the patients with NAFLD should initiate a strict weight and dietary control to prevent the occurrence of MetS and its consequences. The role of NAFLD as a potential independent factor for CVD, the most frequent cause of mortality in patients with fatty liver, has been widely reported over the last years (20, 21, 28). An independent increase of cardiovascular events in patients with US-diagnosed fatty liver has been reported (63, 64, 65). However, the fat accumulation in the liver is a slow and progressive condition with a wide spectrum of severity. NASH is an advanced stage of the disease, which features an inflammatory component in addition to fat accumulation and shows an increased cardiovascular risk (66, 67). In our study, we showed that there are no statistically significant differences between the 2 groups regarding ejection fraction while about endothelial function, there is a statistically significant difference. It is possible that the link between NAFLD and endothelial dysfunction could be represented by an altered nitric oxide (NO) balance. NO produced by endothelial NO synthase (eNOS) plays a key role in liver physiology and pathophysiology, contributing to the maintenance of liver homeostasis (68). On the contrary, NO derived from inducible-NO synthase (iNOS) is produced under pathological conditions and may modify many structural liver proteins. In several pathological conditions, NO production is shifted from eNOS to iNOS derived, with consequent increase in reactive nitrogen species and free radicals. Another study demonstrated a significant reduction in endothelium-dependent vasodilation evaluated by strain-gauge plethysmography in hypertensives with associated NAFLD, compared with hypertensives without NAFLD (69). These data endorse the close link between NAFLD and endothelial function and support our data. We show a light reduction of endothelial function in NAFLD patients compared to control group though within the normal range, perhaps by increasing the sample could emerge statistically significant differences between the 2 groups regarding the ejection fraction. In addition, higher levels of hsCRP and preperitoneal adipose tissue mass correlated with lower left ventricular function. HsCRP, synthesised and secreted primarily but not exclusively by the liver in response to cytokines, is used clinically as a marker of inflammation. In the setting of obesity and other chronic inflammatory conditions, there is a persistent modest elevation in hsCRP production. A modest elevation in hsCRP in the circulation has been reported to damage the endothelium resulting in a risk factor for insulin resistance, hypertension and cardiovascular disease (70). The regression analysis confirmed the role of these elements on the LVEF and showed that NAFLD severity is predictive of left ventricular dysfunction. An impairment of left ventricular function has been reported and confirmed in patients with MetS (71, 72) and more recently, also in asymptomatic NAFLD subjects (32, 33), whereas the association of NAFLD with carotid plaque and increased intima-media thickness is on debate (73). The association of steatosis and left ventricular dysfunction is likely due to insulin resistance, which affects both fat accumulation in the liver and left ventricle mass and function (74). The heart dysfunction in patients with NAFLD seems to be preceded by abnormalities in myocardial metabolism, secondary to myocardial IR, which leads to an increase of the left ventricular mass. The excessive lipid traffic causes cardiac toxicity, impairs

the heart metabolism and leads to cardiac dysfunction. The subclinical inflammation usually associated with visceral fat mass accumulation and advanced stage of fatty liver disease contributes to increase the CVD risk (27, 73). These data from literature support our findings. We also showed that visceral adipose tissue, i.e. preperitoneal adipose tissue, leads to IR, common background for the occurrence of NAFLD and left ventricular dysfunction. Our study has some limitations that deserve comments. First, this is a cross-sectional study and thus we cannot establish both the temporality and the causality of the observed associations. However, our aim was not to assess the causality of this association but to detect whether our patients were prone to develop NAFLD and its metabolic consequences. The ongoing longitudinal study will give further insights into the mechanisms that interplay among visceral adiposity, IR, liver steatosis and CVD. Second, the diagnosis of NAFLD was based on US imaging. It is less sensitive than the liver biopsy, but it is suggested as first-line examination to screen NAFLD. Moreover, conventional US is neither sensitive nor specific to reveal fibrosis clues, except in advanced stages where signs of cirrhosis are evident. The US degree of severity of steatosis might suggest the execution of biopsy to predict the risk of progression to steatohepatitis and fibrous tissue accumulation. In conclusion, we showed that childhood ALL survivors have an increased prevalence of NAFLD, a marker of insulin resistance and visceral adiposity. Fatty liver is associated with a left ventricular dysfunction, suggesting that even in younger patients, after rather a short follow-up period, an early impairment of cardiovascular function may be detected. There is a growing body of evidence showing that NAFLD contributes to progressive cardiometabolic risk, thus fat accumulation in the liver represents a good marker of increased CVD in these patients also. We suggest performing routine abdomen US, with a particular attention to steatosis and visceral fat mass, which seems to be predictive of fatty liver and left ventricular dysfunction. In the case of fatty liver, a comprehensive heart evaluation is mandatory. NAFLD is a reversible condition that has to be promptly diagnosed and treated with a dietary and lifestyle approach, the only one currently available. We strongly recommend to prevent visceral adiposity in ALL survivors, to search for metabolic syndrome or its components, to reinforce the need of weight loss in overweight patients or addressing them to a paediatric endocrinology with an expertise in metabolic disorders.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Authors contribution statement

M D wrote the study design, performed the statistical analysis and edited the manuscript; P M researched data, edited the manuscript and recruited the patients; M M performed the liver ultrasound; G L recruited the patients and edited part of the manuscript; C N recruited patients and followed up the patients; F V recruited the control subjects and edited part of the manuscript; E S wrote the manuscript and contributed to data analysis; A Z performed the cardiological evaluation; M M C wrote the cardiological section and gave substantial contribution the data interpretation; V L M gave substantial contribution to data interpretation, literature research and data collection; N S is the supervisor of the oncological follow-up; P G contributed to write and to edit the paper and supervised the study; M F F joined to conceive the study and gave substantial contribution to data interpretation; she is the guarantor of the paper.

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