Uniwersytet Medyczny w Białymstoku

DZIEDZINA NAUKI MEDYCZNE I NAUKI O ZDROWIU
DYSCYPLINA NAUKI MEDYCZNE

ROZPRAWA DOKTORSKA

Ocena stężenia wybranych markerów gospodarki węglowodanowej i lipidowej oraz ich związek z wystąpieniem nadwagi i otyłości u dzieci i młodzieży po leczeniu przeciwnowotworowym

Katarzyna Konończuk

Promotor: prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan

Promotor pomocniczy: dr hab. n. med. Eryk Latoch

Klinika Pediatrii, Onkologii i Hematologii

Kierownik jednostki: prof. dr hab. n. med. Maryna Krawczuk-Rybak

Rozprawa doktorska została zrealizowana w ramach kształcenia w Szkole Doktorskiej UMB

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Składam serdeczne podziękowania

Prof. dr hab. n. med. Katarzynie Muszyńskiej-Rosłan
Za możliwość rozwoju naukowego, umożliwienie przeprowadzenia badań, wsparcie merytoryczne i cenne uwagi

Dr hab. n med. Erykowi Latochowi

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Rodzicom, rodzeństwu i przyjaciołom

Za wiarę w moje umiejętności i wsparcie

SPIS TREŚCI

1. WYKAZ PUBLIKACJI STANOWIĄCYCH ROZPRAWĘ DOKTORSKĄ

Prace oryginalne

1. Konończuk, Katarzyna, Eryk Latoch, Beata Żelazowska-Rutkowska, Maryna Krawczuk-Rybak, and Katarzyna Muszyńska-Rosłan. "Increased Levels of Adipocyte and Epidermal Fatty Acid-Binding Proteins in Acute Lymphoblastic Leukemia Survivors." *Journal of Clinical Medicine* 10, no. 8 (April 8, 2021): 1567. DOI: 10.3390/jcm10081567. **IF – 4.964, MNiSW – 140 pkt.**
2. Konończuk, Katarzyna, Katarzyna Muszyńska-Rosłan, Karolina Konstantynowicz-Nowicka, Maryna Krawczuk-Rybak, Adrian Chabowski, and Eryk Latoch. "Biomarkers of Glucose Metabolism Alterations and the Onset of Metabolic Syndrome in Survivors of Childhood Acute Lymphoblastic Leukemia." *International Journal of Molecular Sciences* 23, no. 7 (March 28, 2022): 3712. DOI: 10.3390/ijms23073712. **IF – 5.600, MNiSW – 140 pkt.**

2. ZESTAWIENIE DOROBKU NAUKOWEGO

Rodzaj publikacji	Liczba	Impact Factor	Punktacja MNiSW
Prace włączone do rozprawy doktorskiej	2	10.564	280
Prace, które nie zostały włączone do rozprawy doktorskiej	11	47.325	1375
Streszczenia zjazdowe	11	-	-
Razem	24	57.889	1655

3. WSTĘP

Nowotwory stanowią jedną z głównych przyczyn zgonów w populacji pediatrycznej. W ostatnich latach nastąpił znaczący rozwój metod diagnostycznych i terapeutycznych, w tym leczenia wspomagającego oraz stosowania wielu nowych leków, co przyczyniło się do zwiększenia wyleczalności nowotworów wieku dziecięcego [1,2]. Aktualnie, w krajach wysokorozwiniętych, 5-letnie przeżycie wolne od niekorzystnych zdarzeń wynosi około 80 % [3].

Stosowane w dzieciństwie skojarzone leczenie przeciwnowotworowe często prowadzi do licznych powikłań zdrowotnych. Pacjenci są narażeni na niekorzystne skutki zarówno podczas intensywnego leczenia, jak i wiele lat po jego zakończeniu. Dane literaturowe wskazują, że około 60% pacjentów rozwija co najmniej jedno przewlekłe schorzenie, a 27,5% rozwija ciężkie schorzenia zagrażające życiu. Ozdrowieńcy, w porównaniu do rówieśników, wykazują 3,3 razy większe ryzyko występowania chorób przewlekłych [4]. Badania przeprowadzone nad ozdrowieńcami w Polsce wskazują prawidłową funkcję wszystkich narządów jedynie u 11.75% badanych, przy czym najczęściej występują powikłania ze strony układu sercowo-naczyniowego (31,7%) [1].

Ryzyko rozwoju otyłości i cukrzycy jest znacząco wyższe u ozdrowieńców w porównaniu do rówieśników. Nakładające się zaburzenia metaboliczne – otyłość, nieprawidłowa glikemia, dyslipidemia oraz nadciśnienie tętnicze – prowadzą do rozwoju zespołu metabolicznego (MetS), co w konsekwencji zwiększa umieralność z przyczyn sercowo-naczyniowych w tej grupie pacjentów [5].

Intensywne leczenie choroby nowotworowej sprzyja ograniczeniu aktywności fizycznej i nieprawidłowym nawykom żywieniowym pacjentów. W zależności od intensywności stosowanego leczenia i zaawansowania choroby nowotworowej, nasilenie dolegliwości i powikłań zdrowotnych jest zróżnicowane. Spośród wszystkich nowotworów wieku dziecięcego do rozwoju otyłości predysponują najczęściej ostra białaczka limfoblastyczna i guzy ośrodkowego układu nerwowego. Przyczyną może być radioterapia ośrodkowego układu nerwowego i duże dawki steroidów stosowanych w leczeniu tych nowotworów. Dotychczas nie ustalono jednoznacznie czy przebyte w dzieciństwie leczenie przeciwnowotworowe wpływa trwale na zaburzenia szklaków metabolicznych w późniejszym życiu. Coraz więcej dowodów naukowych wskazuje jednak, że ryzyko występowania zaburzeń przemiany lipidów i węglowodanów jest

większe wśród ozdrowieńców niż w populacji ogólnej. W związku z powyższym poszukuje się biomarkerów tych zaburzeń, które wskażą na wczesne zaburzenia metaboliczne jeszcze przed wystąpieniem pełnoobjawowej choroby [6,7].

Białka wiążące kwasy tłuszczowe (fatty acid biding protein, FABP) pełnią funkcje regulacyjne metabolizmu lipidów i węglowodanów. Adipocytarne FABP koordynuje transport kwasów tłuszczowych na poziomie komórkowym, a jego wyższe stężenie we krwi występuje w stanach nadwagi i otyłości. Badania przeprowadzone w grupie dzieci z nadwagą wykazały dodatnią korelację pomiędzy zwiększonym stężeniem A-FABP, a wystąpieniem zespołu metabolicznego [8,9]. Epidermalne FABP, w mniejszym stopniu niż A-FABP jest wydzielane z tkanki tłuszczowej, jednak bierze udział w patogenezie insulinooporności (IR). Ponadto wykazano dodatnią korelację pomiędzy wyższym stężeniem E-FABP a częstszym występowaniem czynników MetS [10,11].

Zaburzenia gospodarki węglowodanowej prowadzą do rozwoju otyłości i insulinooporności. Dotychczas głównymi badanymi markerami tych zaburzeń były: C-peptyd, grelina, GIP (gastric inhibitory peptide, glukozozależny peptyd insulinotropowy), glukagon, insulina, PAI-1 (plasminogen activator inhibitor-1, inhibitor aktywatorów plazminogenu typu 1), rezystyna, leptyna i wisfatyna. Podwyższone stężenie C-peptydu występuje w stanach insulinooporności i we wczesnym etapie rozwoju cukrzycy typu II [12]. Grelina jest peptydowym hormonem produkowanym głównie przez komórki żołądka, który odgrywa kluczową rolę w regulacji apetytu i homeostazy energetycznej poprzez stymulowanie uczucia głodu. W populacji dzieci otyłych obserwowano obniżone stężenie greliny i jej negatywną korelację z BMI [13-15]. W dotychczas prowadzonych badaniach obserwowane były wyższe stężenia glukagonu w otyłości i IR [16,17]. Hiperinsulinemia przyczynia się do rozwoju otyłości na drodze hamowania lipolizy [18]. Podwyższone stężenie GIP może wpływać na nadmierną akumulację tkanki tłuszczowej i rozwój insulinooporności, poprzez zwiększenie lipogenezy w tkance tłuszczowej. Długotrwała nadaktywność GIP jest powiązana z pogorszeniem homeostazy glukozy i lipidów, co sprzyja rozwojowi i progresji zespołu metabolicznego [19,20]. PAI-1 jest powiązany z insulinoopornością, jednym z kluczowych komponentów zespołu metabolicznego, poprzez wpływ na szlaki sygnalizacyjne związane z metabolizmem glukozy i lipidów [21,22]. Innym markerem wydzielanym przez komórki tłuszczowe jest rezystyna, która odgrywa istotną rolę w regulacji procesów zapalnych i metabolizmu glukozy. Podwyższone stężenia rezystyny

występują w IR [23]. Leptyna, hormon peptydowy wydzielany przez adipocyty bierze udział w regulacji homeostazy energetycznej, apetytu i masy ciała. U osób otyłych występująca oporność na leptynę prowadzi do osłabienia jej mechanizmów zmniejszających apetyt i zwiększających metabolizm [24]. Ponadto, zwiększona masa trzewnej tkanki tłuszczowej prowadzi do wyższej produkcji wisfatyny, która poprzez zaburzenia w metabolizmie glukozy zwiększa wydzielania insuliny oraz stymuluje receptory insulinowe. Nadmierne wydzielanie wisfatyny może także przyczyniać się do rozwoju insulinooporności, ponieważ jej przewlekle podwyższone poziomy są związane z zaburzeniami sygnalizacji insulinowej [25,26]. Dodatkowo większość z opisanych markerów bierze również udział w powstawaniu przewlekłego procesu zapalnego i w konsekwencji może prowadzić do powikłań sercowo-naczyniowych.

W związku z coraz lepszymi efektami leczenia nowotworów wieku dziecięcego liczba ozdrowieńców znacząco wzrasta. Populacja ta jest jednak szczególnie narażona na występowanie wielu odległych następstw leczenia, co stanowi istotne wyzwanie współczesnej medycyny. Monitorowanie stanu zdrowia ozdrowieńców oraz wczesna diagnostyka problemów zdrowotnych w tym m.in. zaburzeń metabolicznych istotnie wpływa na poprawę ich jakości i długości życia.

4. OMÓWIENIE PRAC WCHODZĄCYCH W SKŁAD ROZPRAWY DOKTORSKIEJ

4.1. INCREASED LEVELS OF ADIPOCYTE AND EPIDERMAL FATTY ACID-BINDING PROTEINS IN ACUTE LYMPHOBLASTIC LEUKEMIA SURVIVORS

Cele pracy:

1) Ocena stężenia A-FABP i E-FABP u dzieci po zakończonym leczeniu ostrej białaczki limfoblastycznej.

2) Określenie zależności pomiędzy stężeniami A-FABP i E-FABP, a nadwagą i otyłością oraz czynnikami zespołu metabolicznego u dzieci po zakończonym leczeniu ostrej białaczki limfoblastycznej.

Materiały i metody:

W badaniu wzięło udział 62 byłych pacjentów Kliniki Pediatrii, Onkologii i Hematologii Dziecięcej Uniwersyteckiego Dziecięcego Szpitala Klinicznego w Białymstoku, leczonych z powodu ALL (śr. wiek w dniu badania 12.41 ± 4.98 lat). Grupa badana została podzielona ze względu na wartość BMI zgodnie z siatkami centylowymi OLA/OLAF dla wieku i płci na podgrupy z prawidłową masą ciała oraz z nadwagą i otyłością [27,28]. Czynniki zespołu metabolicznego dla dzieci poniżej 16 roku życia zostały określone na podstawie wytycznych IDF: obwód talii (WC) ≥ 90c, trójglicerydy (TG) ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL. Pacjenci w wieku 16 lat lub starsi zostali ocenieni według wytycznych IDF dla dorosłych: WC ≥ 94 cm dla mężczyzn and WC ≥ 80 cm dla kobiet, TG ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL dla mężczyzn i < 50 mg/dL dla kobiet, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL [29].

Stężenie A-FABP i E-FABP w surowicy oceniono za pomocą dostępnego zestawu ELISA (BioVendor Laboratorni Medicina a.s., Brno, Czech Republic). Analizę statystyczną wykonano w programie STATA v. 12.1. (StatCorp, College Station, Texas, USA).

Wyniki:

Grupa badana prezentowała wyższe stężenia A-FABP (25.57 ± 14.46 vs. 15.13 ± 7.61 ng/mL; $p < 0.001$) w porównaniu do grupy kontrolnej, natomiast stężenie E-

FABP (12.07 ± 7.37 vs. 10.12 ± 3.21 ng/mL; p = 0.325) nie różniło się istotnie statystycznie w powyższych grupach. Pacjenci z nadwagą i otyłością wykazywali wyższe stężenia A-FABP (32.02 ± 17.10 vs. 20.33 ± 9.24 ng/mL; p = 0.006) w porównaniu do grupy z prawidłowym BMI. W grupie badanej 53.23% pacjentów spełniało przynajmniej jedno kryterium zespołu metabolicznego. A-FABP (AUC 0.72) okazał się lepszym predyktorem występowania cech MetS niż E-FABP (AUC 0.62). Dzieci po zakończonym leczeniu z co najmniej jedną cechą MetS wykazywały wyższe stężenie A-FABP (30.63 ± 15.91 vs. 20.14 ± 10.52 ng/mL; p = 0.003), a ze spełnionymi co najmniej dwiema cechami MetS wyższe stężenia A-FABP (33.62 ± 17.16 vs. 20.27 ± 10.35 ng/mL; p = 0.018) i E-FABP (13.37 ± 3.62 vs. 10.19 ± 4.02 ng/mL; p = 0.026) w porównaniu do grupy dzieci, które nie spełniały żadnych kryteriów MetS, jak i w porównaniu do grupy kontrolnej (A-FABP - 33.62 ± 17.16 vs. 15.13 ± 7.61 ng/mL; p = 0.001; E-FABP - 13.37 ± 3.62 vs. 10.12 ± 3.21 ng/mL; p = 0.021). Pacjenci powyżej 5 lat od zakończonego leczenia prezentowali wyższe stężenia A-FABP (27.85 ± 14.22 vs. 22.10 ± 14.44 ng/mL; p = 0.045) niż pacjenci z krótszym czasem obserwacji.

Wnioski:

W badanej grupie pacjentów po zakończonym leczeniu ALL wykazano istotne zaburzenia w metabolizmie lipidów. Zwiększone stężenie FABP w grupie badanej może świadczyć o zwiększonym ryzyku zaburzeń w gospodarce lipidowej w wyniku stosowania leczenia przeciwnowotworowego w dzieciństwie. Nadwaga i otyłość przyczyniają się do wzrostu ryzyka zaburzeń metabolicznych w tej grupie pacjentów.

4.2. BIOMARKERS OF GLUCOSE METABOLISM ALTERATIONS AND THE ONSET OF METABOLIC SYNDROME IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

Cele pracy:

1) Ocena stężenia wybranych markerów gospodarki węglowodanowej u pacjentów po zakończonym leczeniu ostrej białaczki limfoblastycznej wieku dziecięcego.
2) Ocena zależności pomiędzy dziewięcioma biomarkerami gospodarki węglowodanowej a nadwagą i otyłością, insulinoopornością i występowaniem czynników zespołu metabolicznego po zakończonym leczeniu ALL.

Materiały i metody:

Do badania włączono 56 byłych pacjentów Kliniki Pediatrii, Onkologii i Hematologii Dziecięcej Uniwersyteckiego Dziecięcego Szpitala Klinicznego w Białymstoku, leczonych z powodu ALL w wieku dziecięcym. Średni wiek uczestników badania wynosił 12.36 ± 5.15 lat. Nadwaga i otyłość w grupie badanej została określona na podstawie wartość BMI zgodnie z siatkami centylowymi OLA/OLAF dla wieku i płci [27,28]. HOMA-IR został wyliczony zgodnie ze wzorem: stężenie insuliny (μIU/mL) x stężenie glukozy (mmol/L) / 22.5. Czynniki zespołu metabolicznego dla dzieci poniżej 16 roku życia zostały zdefiniowane na podstawie wytycznych IDF: obwód talii (WC) ≥ 90c, trójglicerydy (TG) ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL. Pacjenci w wieku 16 lat lub starsi zostali ocenieni według wytycznych IDF dla dorosłych: WC ≥ 94 cm dla mężczyzn and WC ≥ 80 cm dla kobiet, TG ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL dla mężczyzn i < 50 mg/dL dla kobiet, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL [29].

Stężenie wybranych markerów gospodarki węglowodanowej w surowicy oceniono za pomocą dostępnego pakietu Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA, USA). Analizę statystyczną wykonano w programie STATA v. 12.1. (StatCorp, College Station, Texas, USA).

Wyniki:

Grupa badana wykazywała wyższe stężenie GIP (1050.12 pg/mL (592.44; 1479.55) vs. 417.67 pg/mL (280.34; 741.02); $p = 0.026$), glukagonu (394.94 pg/mL

(234.92; 612.39) vs. 237.62 pg/mL (140.22; 324.11); p = 0.001), leptyny (5219.36 pg/mL (1329.38; 12551.94) vs. 1846.23 pg/mL (765.72; 3361.22); p = 0.022) i PAI-1 (4914.04 pg/mL (3638.52; 6040.11) vs. 3936.78 pg/mL (3091.16; 4900.93); p = 0.047) oraz niższe stężenie greliny (224.07 pg/mL (161.76; 356.32) vs. 634.33 pg/mL (377.65; 1070.13); p < 0.001) w porównaniu do grupy kontrolnej. Dzieci leczone z powodu ALL z nadwagą i otyłością prezentowały wyższe stężenia glukagonu (572.58 ± 327.20 pg/mL vs. 363.39 ± 230.83 pg/mL; p = 0.006) i leptyny (9180.43 ± 6989.29 pg/mL vs. 4952.19 ± 4642.43 pg/mL; p = 0.034) niż pacjenci z prawidłowym BMI. Natomiast w porównaniu ozdrowieńców z prawidłowym BMI do grupy kontrolnej uzyskano wyższe stężenia GIP (4993.09 ± 10750.06 pg/mL vs. 487.86 ± 278.73 pg/mL; p = 0.005) i niższe stężenie greliny (296.64 ± 232.40 pg/mL vs. 764.97 ± 557.20 pg/mL; p < 0.001) w podanej podgrupie badanej. Pacjenci powyżej pięciu lat od zakończonego leczenia wykazywali wyższe stężenia PAI-1 (5524.84 ± 2338.48 pg/mL vs. 3711.59 ± 2360.43 pg/mL; p < 0.001) i rezystyny (12247.92 ± 7238.51 pg/mL vs. 5182.93 ± 3620.10 pg/mL; p = 0.002) w porównaniu do krótszego czasu obserwacji. W analizie porównującej pacjentów ze spełniony z przynajmniej jednym kryterium MetS z grupą bez spełnionych kryteriów MetS, zaobserwowano wyższe stężenia C-peptydu (792.42 pg/mL (444.15; 1046.09) vs. 419.15 pg/mL (258.64; 727.40); p = 0.028), leptyny (6999.47 pg/mL (3347.83; 16562.53) vs. 3613.77 pg/mL (664.69; 6269.79); p = 0.003) i PAI-1 (5305.50 pg/mL (3814.72; 6898.52) vs. 4478.74 pg/mL (3409.32; 5383.39); p = 0.034) w grupie z czynnikami MetS. Nieprawidłową wartość HOMA-IR wykazało 10.7% pacjentów, prezentowali oni wyższe stężenia C-peptydu (1150.13 ± 626.88 pg/mL vs. 425.84 ± 528.95 pg/mL; p = 0.005), glukagonu (625.22 ± 355.41 pg/mL vs. 284.69 ± 177.93 pg/mL; p = 0.042) i leptyny (9930.65 ± 6458.18 pg/mL vs. 2906.16 ± 4843.62 pg/mL; p = 0.016) w porównaniu do podgrupy z prawidłowym HOMA-IR. Glukagon (AUC 0.71; p = 0.003) i leptyna (AUC 0.67; p = 0.026) okazały się najlepszymi predyktorami nadwagi i otyłości w grupie badanej.

Wnioski:

Pacjenci po zakończonym leczeniu ALL w dzieciństwie wykazują zaburzenia w gospodarce węglowodanowej, które mogą predysponować do wystąpienia zespołu metabolicznego i chorób sercowo-naczyniowych. Z tego powodu zasadna wydaje się

stała kontrola i monitorowanie pacjentów po zakończonym leczeniu nowotworu w dzieciństwie w kierunku rozwoju chorób metabolicznych.

5. PUBLIKACJE STANOWIĄCE ROZPRAWĘ DOKTORSKĄ

Journal of *Clinical Medicine*

MDPI

Article

Increased Levels of Adipocyte and Epidermal Fatty Acid-Binding Proteins in Acute Lymphoblastic Leukemia Survivors

Katarzyna Konończuk [1,*], Eryk Latoch [1,*], Beata Żelazowska-Rutkowska [2], Maryna Krawczuk-Rybak [1] and Katarzyna Muszyńska-Rosłan [1]

[1] Department of Pediatric Oncology and Hematology, Medical University of Bialystok, 15-274 Bialystok, Poland; rybak@umwb.edu.pl (M.K.-R.); kmroslan@post.pl (K.M.-R.)
[2] Department of Pediatric Laboratory Diagnostic, Medical University of Bialystok, 15-274 Bialystok, Poland; zelazowskab@wp.pl
* Correspondence: kononczukk@gmail.com (K.K.); eryklatoch@gmail.com (E.L.); Tel.: +48-85-745-0846 (E.L.)

Abstract: Childhood cancer survivors are highly exposed to the development of side effects after many years of cessation of anticancer treatment, including altered lipid metabolism that may result in an increased risk of overweight and metabolic syndrome. Adipocyte (A-FABP) and epidermal (E-FABP) fatty acid-binding proteins are expressed in adipocytes and are assumed to play an important role in the development of lipid disturbances leading to the onset of metabolic syndrome. The aim of this study was to investigate the association between serum A-FABP and E-FABP levels, overweight, and components of the metabolic syndrome in acute lymphoblastic leukemia survivors. Sixty-two acute lymphoblastic leukemia (ALL) survivors (34 females) were included in the study. The mean age at the time of the study was 12.41 ± 4.98 years (range 4.71–23.43). Serum levels of A-FABP and E-FABP were analyzed using a commercially available ELISA kit. The ALL survivors presented statistically higher A-FABP levels in comparison with the healthy controls (25.57 ± 14.46 vs. 15.13 ± 7.61 ng/mL, $p < 0.001$). The subjects with body mass index (BMI) above the normal range (18 overweight, 10 obese) had a greater level of A-FABP compared to the ALL group with normal BMI (32.02 ± 17.10 vs. 20.33 ± 9.24 ng/mL, $p = 0.006$). Of all participants, 53.23% had at least one risk factor of metabolic syndrome; in this group, only the A-FABP level showed a statistically significant difference compared to the healthy control group (30.63 ± 15.91 vs. 15.13 ± 7.61 ng/mL, $p < 0.001$). The subjects with two or more metabolic risk factors (16.13%) presented higher levels of both A-FABP (33.62 ± 17.16 vs. 15.13 ± 7.61 ng/mL, $p = 0.001$) and E-FABP (13.37 ± 3.62 vs. 10.12 ± 3.21 ng/mL, $p = 0.021$) compared to the controls. Univariable regression models showed significant associations between BMI and systolic blood pressure with the A-FABP level (coeff. 1.02 and 13.74, respectively; $p < 0.05$). In contrast, the E-FABP level was only affected by BMI (coeff. 0.48; $p < 0.01$). The findings reported herein suggest that the increased levels of A-FABP and E-FABP may be involved in the pathogenesis of overweight and the onset of metabolic syndrome in acute lymphoblastic leukemia. However, further longitudinal, prospective studies of fatty acid-binding proteins and their potential role in the pathogenesis of obesity and metabolic syndrome in ALL survivors remain to be performed.

Keywords: FABP; A-FABP; E-FABP; overweight; obesity; children; metabolic syndrome; ALL; acute lymphoblastic leukemia; CCS; childhood cancer survivors

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1. Introduction

Acute lymphoblastic leukemia (ALL) constitutes over 25% of all childhood cancers, which qualifies it to be the most common childhood malignancy. Due to significant advances in the diagnosis and treatment of ALL in recent years, the improvement in survival rate up to 90% has been reached depending on the risk group [1–3]. As a result, the number of survivors increases dramatically with the improvement of treatment results.

On the other hand, many studies indicate that this population is exposed to numerous treatment-related complications in later life [4–6]. Therefore, there has recently been a growing interest in the detection and prevention of late sequelae of anticancer treatment among this population.

Over the past two decades, multiple reports have been published that indicate childhood cancer survivors (CCS) are at risk of premature aging and develop many diseases earlier than the general population [7]. The most common complications include diseases of civilization such as obesity, diabetes mellitus, metabolic syndrome, heart diseases, osteoporosis or second cancers [8,9]. To a large extent, this may be related to the treatment applied in this group of patients; however, the underlying processes are multifactorial and still not fully explained. To date, cranial radiation has been noted to play an important role in the development of metabolic syndrome (MetS) and insulin resistance (IR) [10]. Other adverse factors include the use of anticancer agents, glucocorticosteroids, as well as decreased physical activity and poor eating habits, which further result in the difficulty of reversing unhealthy habits among survivors many years after the treatment.

All of these factors boost the risk of cardiovascular complications and overweight in ALL survivors than their peers [11]. Moreover, the available studies have demonstrated that anticancer treatment may alter lipid metabolism; however, prognostic markers that can be used in clinical practice are limited [12].

Fatty acid-binding proteins (FABPs) are a family of proteins involved in the regulation of lipid and glucose metabolism. As lipid chaperones, they can bind to saturated and unsaturated long-chain fatty acids, which is important to coordinate lipid migration to different cellular compartments. FABP-4 is also known as adipocyte-FABP (A-FABP), which is produced in adipocytes. Some authors indicate that overweight individuals have a higher concentration of A-FABP in the bloodstream, which is strongly correlated with body fat mass [13].

Epidermal FABP (E-FABP) is released mostly by epidermal cells of the skin as well as by adipocytes and other cells and tissues, i.e., macrophages, lens, lung, and brain [14]. Both of these FABPs also play a significant role in the development of insulin resistance and atherosclerosis [15,16].

This study aimed at investigating the association between the A-FABP and the E-FABP levels, overweight, and components of the metabolic syndrome in acute lymphoblastic leukemia survivors.

2. Materials and Methods

The study included 62 childhood cancer survivors (28 male and 34 female) of the Department of Pediatric Oncology and Hematology of the Medical University of Bialystok. All the patients were treated for acute lymphoblastic leukemia. The mean age at the time of the study was 12.41 $\pm$ 4.98 years (range 4.71–23.43). The characteristics of the patients are presented in Table 1. International protocols (The International Berlin-Frankfurt-Münster Group—I-BFM) approved by Polish Pediatrics Leukemia and Lymphoma Group were used in the treatment. Hematological stem cell transplantation (HSCT) was performed in 6 participants. All the patients were in complete continuous remission. Written informed consent was obtained from all the subjects. The control group consisted of 25 healthy peers (10 female) born on time, with normal body weight and BMI during the examination and did not take any medication, who were offspring of the department's employees. The study was approved by the Ethics Committee of the Medical University of Bialystok in accordance with the Declaration of Helsinki (permission number: R-I-002/463/2016).

Data on age, sex, type of diagnosis, and treatment were collected from the medical records. All the patients underwent a clinical examination and anthropometric measurements during the follow-up visit. A Martin anthropometer was used to measure height and a digital scale to weigh (Seca, Germany). Body mass index (BMI) was calculated as weight in kilograms divided by height in squared meters (kg/m^2). The subjects were divided into overweight and obesity groups and a group with BMI in a normal range based on

the OLA/OLAF growth charts of BMI for age and sex. Overweight was defined as BMI values +1 SD, while obesity was defined as +2 SD [17,18]. The waist-to-height ratio (WHtR) was calculated by dividing waist circumference by height, assuming abdominal obesity as WHtR $\geq$ 0.5 [19]. Blood pressure was measured using a standardized sphygmomanometer (performed three times at 1–2 min intervals); before the measurement, the participant rested peacefully for 5 min. Hypertension (HT) was defined as a mean systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) level $\geq$ 95th percentile adjusted for age, sex, and height [20].

Table 1. Descriptive characteristics of the study group.

	Number (%)	Median (IQR) [a]
Patients	62 (100%)	
Male	28 (45.2%)	
Female	34 (54.8%)	
Age at diagnosis (years)		4.13 (3.03–6.45)
Age at study (years)		12.39 (8.17–16.08)
Follow-up after treatment (years)		7.05 (2.24–9.07)
Overweight	18 (29%)	
Obese	10 (16%)	
Glucocorticoids:		
Cumulative corticosteroid dose (mg/m^2) [c]	62 (100%)	3087 (3087–3087) [b]
Prednisone (cumulative dose in mg/m^2)	62 (100%)	1680 (1680–1680) [b]
Dexamethasone (cumulative dose in mg/m^2)	62 (100%)	210 (210–210) [b]
Radiotherapy	9 (14.5%)	
Cranial radiotherapy (CRT) (cumulative dose in Grey)	8 (12.9%)	12 (12–12) [b]
Total body irradiation (TBI)	2 (3.23%)	12 (12–12) [b]
No	53 (85.5%)	
HSCT	6 (9.7%)	
Metabolic derangements		
1 Metabolic risk factor	23 (37.1%)	
2 Metabolic risk factors	5 (8.1%)	
3 Metabolic risk factors	4 (6.5%)	
4 Metabolic risk factors	1 (1.6%)	

[a] Interquartile range (IQR). [b] Most patients received the same dosage of anticancer agents according to the treatment protocol; therefore, the first and third quartiles did not differ from the median. [c] Calculated as prednisone equivalents.

The components of metabolic syndrome in children under the age of 16 were defined by the International Diabetes Federation (IDF) recommendations as waist circumference (WC) $\geq$ 90th centile, triglycerides $\geq$ 150 mg/dL ($\geq$1.7 mmol/L), high-density lipoprotein (HDL) cholesterol < 40 mg/dL (<1.03 mmol/L), blood pressure $\geq$ 130/85 mmHg, and fasting glucose $\geq$ 100 mg/dL ($\geq$5.6 mmol/L). Among participants aged 16 and older, MetS was defined by the IDF adult criteria as WC $\geq$ 94 cm for men and WC $\geq$ 80 cm for women, triglycerides $\geq$ 150 mg/dL ($\geq$1.7 mmol/L), HDL cholesterol < 40 mg/dL (<1.03 mmol/L) for men and < 50 mg/dL (<1.29 mmol/L) for women, blood pressure $\geq$ 130/85 mmHg, fasting glucose $\geq$ 100 mg/dL ($\geq$5.6 mmol/L) [21]. All the subjects had an abdominal ultrasound performed by a qualified radiologist to assess the occurrence of liver steatosis. Shortening fraction (SF) and ejection fraction (EF) evaluation in echocardiography were measured by a pediatric cardiology specialist.

All the laboratory tests were performed following an eight-hour overnight fast. Blood samples were stored at −80 °C. Serum levels of A-FABP and E-FABP were analyzed using a commercially available ELISA kit (BioVendor Laboratorni Medicina a.s., Brno, Czech Republic). Additionally, concentrations of biochemical parameters such as alanine

aminotransferase (ALT), high-density lipoprotein (HDL), triglycerides (TG), and glucose were measured by using the enzymatic colorimetric methods.

Statistical analysis was performed with STATA v. 12.1 (StatCorp, College Station, Texas, USA). The Shapiro–Wilk test was used to examine normal distribution. The data were expressed as means ± standard deviation (SD), or median (Me) and interquartile range (IQR) when appropriate. The Mann–Whitney U test was applied to compare independent variables without normal distribution. The correlation analysis between parameters was evaluated with Spearman's rank correlation coefficient. The receiver operating characteristic (ROC) curve was used to establish the diagnostic values of the fatty acid-binding proteins and the optimum cut-off points. Multivariate regression models were used to examine the association between FABPs and the independent variables, which potentially might affect their level. The level of statistical significance was defined as $p < 0.05$.

3. Results

The characteristics of the included childhood cancer survivors (CCS) are shown in Table 1. The mean age at the time of diagnosis was 5.03 ± 3.37 years, and the mean time from treatment cessation to follow-up was 6.61 ± 4.59 years. The anthropometric analyses and biochemical parameters measured in the study according to sex are presented in Table 2. The age and sex of the study group did not differ from the control group.

Table 2. Characteristics of acute lymphoblastic leukemia survivors by gender.

	Total	Females	Males	*p* Value
	Median (IQR)	Median (IQR)	Median (IQR)	
	n = 62	*n* = 34	*n* = 28	
Age at diagnosis (years)	4.13 (3.03; 6.45)	5.23 (2.91; 7.04)	3.74 (3.30; 5.59)	0.784
Age at study (years)	12.36 (8.17; 16.08)	13.55 (10.13; 16.40)	10.89 (6.51; 14.49)	0.164
Follow-up (years)	7.05 (2.24; 9.07)	7.69 (3.45; 9.36)	5.71 (1.71; 8.68)	0.178
Weight (kg)	47.50 (31.30; 65.00)	49.25 (38.00; 62.70)	44.05 (24.80; 71.35)	0.598
Height (cm)	151.25 (133.50; 162.00)	152.50 (140.00; 160.00)	145.75 (118.75; 166.75)	0.648
BMI (kg/m^2)	21.17 (17.93; 24.96)	21.41 (18.82; 24.96)	20.99 (17.53; 24.51)	0.817
WC (cm)	72.00 (63.00; 81.00)	72.50 (65.00; 80.00)	71.50 (57.50; 83.00)	1.00
WHtR	0.50 (0.45; 0.54)	0.48 (0.45; 0.53)	0.50 (0.45; 0.55)	0.425
ALT (U/L)	15.00 (12.00; 23.00)	14.00 (12.00; 22.00)	17.00 (13.00; 23.00)	0.513
TG (mg/dL)	91.00 (62.00; 118.00)	84.00 (62.00; 100.00)	98.00 (63.00; 132.00)	0.443
E-FABP (ng/mL)	10.32 (8.26; 14.08)	11.07 (9.43; 15.08)	9.04 (7.12; 12.00)	0.023
A-FABP (ng/mL)	23.69 (14.62; 30.82)	24.71 (16.21; 31.43)	23.09 (11.66; 30.12)	0.349

BMI body mass index, WC waist circumference, WHtR waist-to-height ratio, ALT alanine aminotransferase, TG triglycerides, E-FABP epidermal fatty acid-binding protein, A-FABP adipocyte fatty acid-binding protein, IQR interquartile range.

ALL survivors had statistically higher A-FABP level than the healthy controls (25.57 ± 14.46 vs. 15.13 ± 7.61 ng/mL, $p < 0.001$). In contrast, the E-FABP level showed no differences in the groups concerned (12.07 ± 7.37 vs. 10.12 ± 3.21 ng/mL, $p = 0.325$). Comparisons of A-FABP and E-FABP levels in acute lymphoblastic leukemia patients with normal BMI with controls are shown in Figures 1 and 2. The level of E-FABP was statistically significantly higher in the examined females than in males (13.59 ± 8.80 vs. 10.33 ± 4.87 ng/mL, $p = 0.023$). However, A-FABP did not reveal any differences according to sex (26.69 ± 14.69 vs. 24.05 ± 14.32 ng/mL, $p = 0.349$).

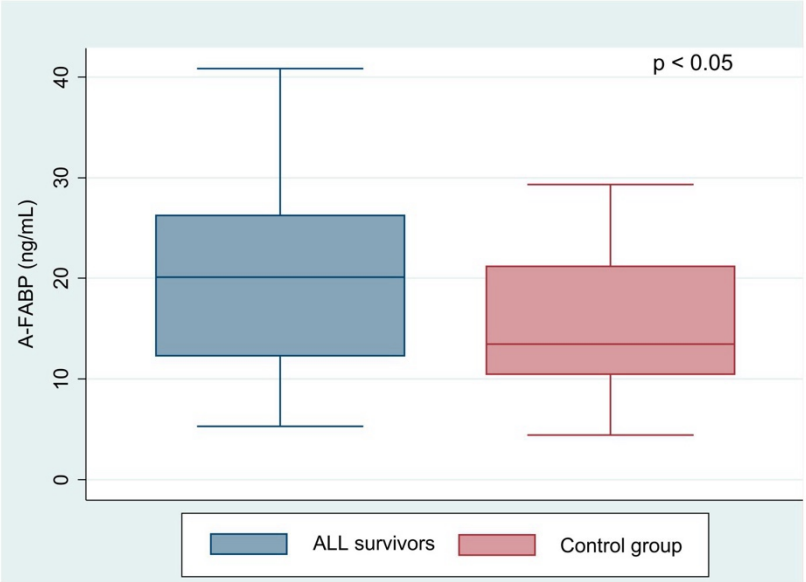

Figure 1. Comparison of the A-FABP level (adipocyte fatty acid-binding protein) between acute lymphoblastic leukemia survivors with normal BMI and the control group.

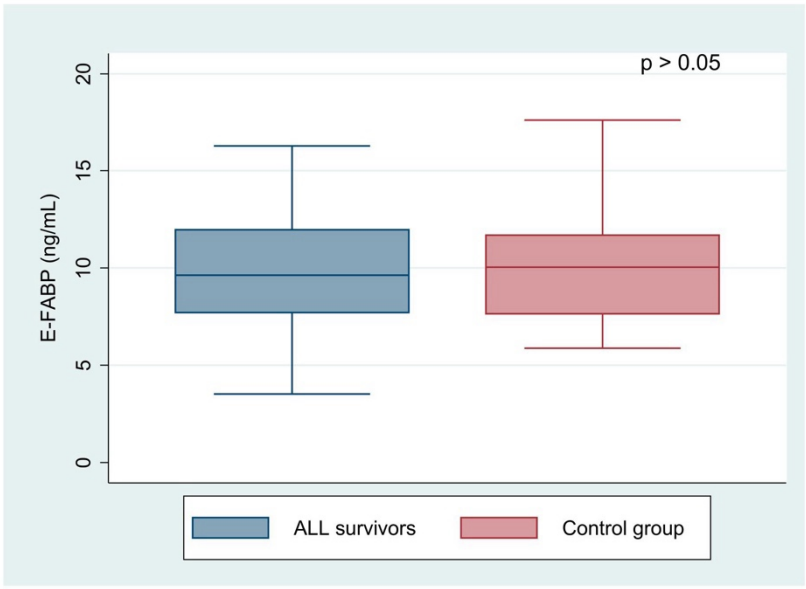

Figure 2. Comparison of the E-FABP level (epidermal fatty acid-binding protein) between acute lymphoblastic leukemia survivors with normal BMI and the control group.

Twenty-eight of the study group participants (45%) were overweight (n = 18) or obese (n = 10). All of them had a significantly higher level of A-FABP compared to the ALL group with normal BMI (32.02 ± 17.10 vs. 20.33 ± 9.24 ng/mL, p = 0.006). No differences in concentrations of E-FABP (13.85 ± 8.97 vs. 10.61 ± 5.46 ng/mL, p = 0.055), TG (107.95 ± 47.88 vs. 87.41 ± 45.62 mg/dL, p = 0.078), and ALT (32.38 ± 36.61 vs. 19.79 ± 16.92 U/L, p = 0.053) were found in these subjects (Table 3).

Table 3. Childhood cancer survivors according to different body mass index.

	Overweight/Obese	Normal Weight	p Value
	n = 28	n = 34	
	Median (IQR)	Median (IQR)	
BMI (kg/m^2)	25.43 (22.36; 28.47)	18.85 (16.18; 21.30)	<0.001
Age at study (years)	12.21 (7.43; 17.34)	12.68 (9.21; 15.42)	0.905
Follow-up (years)	5.58 (2.25; 8.72)	7.58 (2.01; 9.84)	0.427
ALT (U/L)	17.50 (13.50; 33.00)	13.00 (11.00; 19.00)	0.053
TG (mg/dL)	100.00 (66.00; 139.00)	72.00 (56.00; 98.00)	0.078
E-FABP (ng/mL)	10.86 (9.16; 17.52)	9.78 (7.79; 12.04)	0.055
A-FABP (ng/mL)	27.76 (20.84; 38.74)	20.09 (12.32; 26.21)	0.006

BMI body mass index, ALT alanine aminotransferase, TG triglycerides, E-FABP epidermal fatty acid-binding protein, A-FABP adipocyte fatty acid-binding protein, IQR interquartile range.

In the analysis by gender, female subjects with normal BMI showed a higher concentration of A-FABP compared to the control group (22.74 ± 8.28 vs. 11.87 ± 6.77 ng/mL, $p < 0.001$), whereas the male subjects with normal BMI did not reveal significantly statistical differences compared to the control group (A-FABP: 16.80 ± 9.75 vs. 17.30 ± 7.56 ng/mL, p = 0.717). Additionally, the higher levels of both A-FABP and E-FABP were found in females with high BMI in comparison to the control group (E-FABP: 16.07 ± 11.85 vs. 11.87 ± 6.77 ng/mL, p = 0.007; A-FABP: 34.32 ± 20.23 vs. 11.87 ± 6.77 ng/mL, p = 0.001). We observed a similar dependence in overweight male subjects, but it concerned only the A-FABP concentration (30.33 ± 14.93 vs. 17.30 ± 7.56 ng/mL, p = 0.006).

We did not notice any differences in the FABPs levels in patients with and without cranial irradiation (E-FABP: 12.25 ± 7.77 vs. 11.05 ± 4.49 ng/mL, p = 1.000; A-FABP: 25.98 ± 15.00 vs. 23.03 ± 10.92 ng/mL, p = 0.748), respectively. We found a positive correlation between the E-FABP and the A-FABP concentrations in ALL survivors (r = 0.41, p = 0.001) (Figure 3).

Moreover, we observed moderate correlations between FABPs and anthropometric parameters such as BMI (E-FABP: r = 0.42, p = 0.001; A-FABP: r = 0.43, p = 0.001), WHtR (E-FABP: r = 0.35, p = 0.007; A-FABP: r = 0.37, p = 0.004), WC (E-FABP: r = 0.40, p = 0.002; A-FABP: r = 0.40, p = 0.002), and fatty acid-binding proteins. In addition, a positive correlation between high systolic blood pressure and the analyzed biomarkers were found (E-FABP: r = 0.29, p = 0.023; A-FABP: r = 0.35, p = 0.007), while diastolic blood pressure was only positively associated with A-FABP (r = 0.32, p = 0.015); however, the strength of relationships was weak ($0.3 < r < 0.5$) or very weak ($r < 0.3$). We did not find any correlation between FABPs and shortening fraction (SF) or ejection fraction (EF). The multiple regression models were developed regarding each variable that might affect the level of FABPs. The analysis included potential confounding variables listed in Tables 1 and 2. The best models (with the highest coefficient of determination) of the relationship between FABPs and independent variables are presented in Table 4. The first model showed that BMI and SBP significantly affected A-FABP level (coeff. 1.02 and 13.74, respectively; $p < 0.05$), while the second one revealed that only BMI significantly influenced the E-FABP level (coeff. 0.48; p = 0.005).

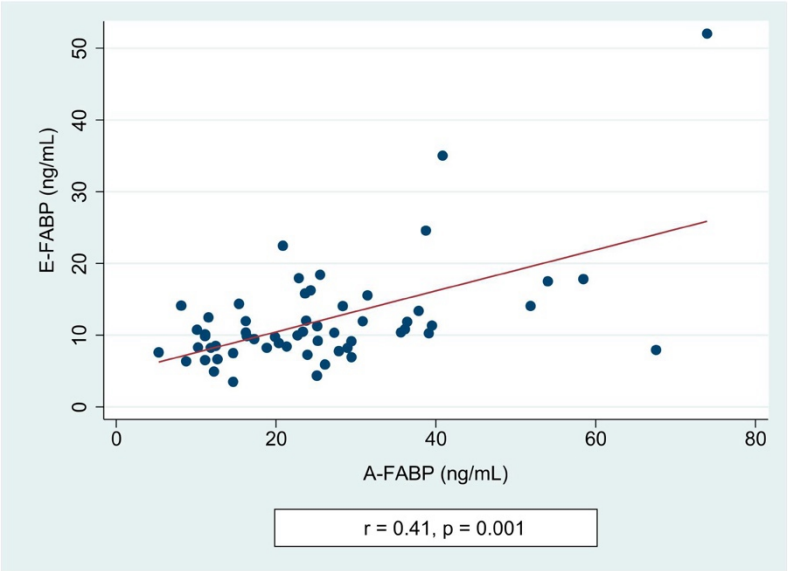

Figure 3. Spearman correlation of A-FABP (adipocyte fatty acid-binding protein) and E-FABP (epidermal fatty acid-binding protein) in childhood acute lymphoblastic leukemia survivors.

Table 4. Multivariate analysis of correlates of the fatty acid-binding proteins (FABPs) in acute lymphoblastic leukemia survivors.

	Independent Variable	Coeff.	t	*p*	95% Conf. Interval	
A-FABP (ng/mL)	BMI (kg/m^2)	1.02	2.87	0.006	0.31	1.73
	SBP (normal vs. high)	13.74	2.04	0.046	0.23	27.3
	DBP (normal vs. high)	−0.64	−0.12	0.907	−11.6	10.3
E-FABP (ng/mL)	BMI (kg/m^2)	0.48	3.43	0.005	0.17	0.78
	Cholesterol (mg/dL)	0.04	1.40	0.186	−0.02	0.11

A-FABP adipocyte fatty acid-binding protein, E-FABP epidermal fatty acid-binding protein, BMI body mass index, SBP systolic blood pressure, DBP diastolic blood pressure. Coefficient of determination (R^2) was 0.25 for A-FABP and 0.60 for E-FABP.

The receiver operating characteristic analyses (Figure 4) for predicting the presence of any components of metabolic syndrome based on the serum levels of A-FABP and E-FABP in the study group were conducted. Adipocyte FABP (AUC 0.72) was found to be a better predictor then E-FABP (AUC 0.62), yet no statistical differences were observed between the two areas under the curve ($p > 0.05$). The same analyses for prediction of overweight and obesity (Figure 5) showed similar results (AUC 0.76 for A-FABP and 0.63 for E-FABP, $p < 0.05$). Interestingly, the examined proteins turned out to be predictors of higher blood pressure in CCS. The AUC for A-FABP was 0.85 for SBP and 0.69 for DBP, while E-FABP showed diagnostic profile describing the AUC of 0.70. There was no diagnostic value of FABPs in hepatic steatosis detected by ultrasound.

In further analysis, we checked the relationship between the FABPs and the anthropometric measurements depending on the time elapsed since the end of treatment. The subjects over 5 years after cessation of therapy presented greater A-FABP (27.85 ± 14.22 vs. 22.10 ± 14.44 ng/mL, $p = 0.045$) and BMI (22.76 ± 4.89 vs. 20.09 ± 5.20 kg/m^2, $p = 0.032$) than participants with a shorter time after completion of treatment. We found no differences in E-FABP concentration in these patient subgroups.

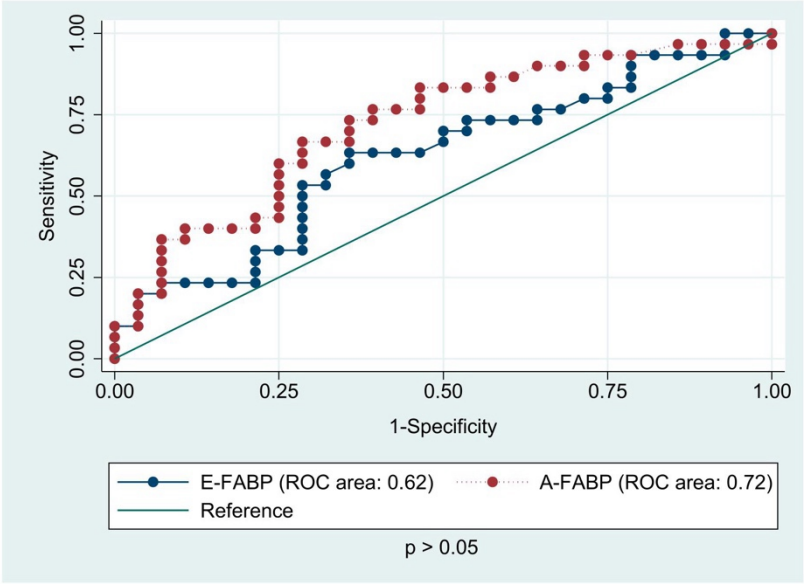

Figure 4. The receiver operating characteristic (ROC) analysis for prediction of the presence of components of metabolic syndrome based on the serum levels of A-FABP (adipocyte fatty acid-binding protein) and E-FABP (epidermal fatty acid-binding protein) in childhood acute lymphoblastic leukemia survivors.

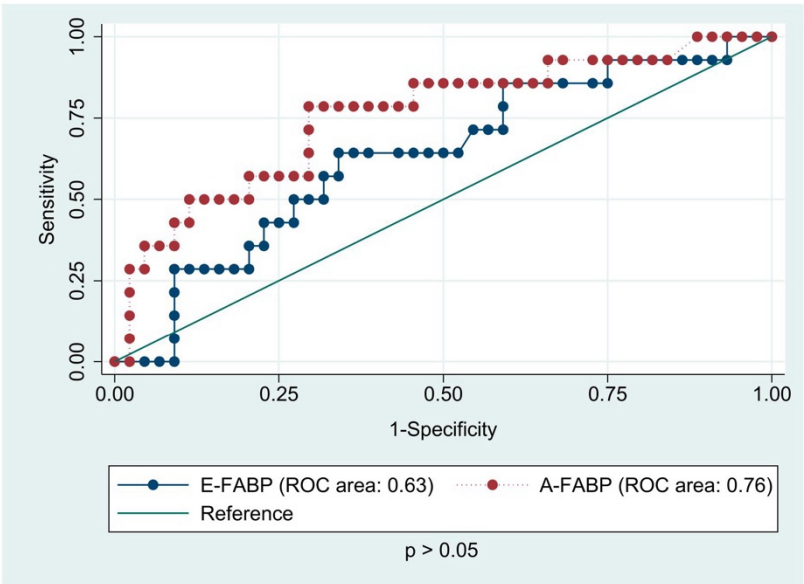

Figure 5. The receiver operating characteristic (ROC) analysis for prediction of overweight/obesity based on the serum levels of A-FABP (adipocyte fatty acid-binding protein) and E-FABP (epidermal fatty acid-binding protein) in childhood acute lymphoblastic leukemia survivors.

Among all participants, at least one risk factor of metabolic syndrome was noticed in 53.23%. In this group, a higher concentration of A-FABP compered to subjects without MetS features was found (30.63 ± 15.91 vs. 20.14 ± 10.52 ng/mL, $p = 0.003$). Interestingly, the subset with two or more metabolic risk factors showed a significant difference in the concentration of both proteins (A-FABP: 33.62 ± 17.16 vs. 20.27 ± 10.35 ng/mL, $p = 0.018$; E-FABP: 13.37 ± 3.62 vs. 10.19 ± 4.02 ng/mL, $p = 0.026$). When compared to the control group, the patients with any metabolic derangements had higher levels of A-FABP (30.63 ± 15.91 vs. 15.13 ± 7.61 ng/mL, $p < 0.001$) but not E-FABP (13.73 ± 9.25 vs. 10.12 ± 3.21 ng/mL, $p = 0.090$). The participants with two or more metabolic risk factors (16.13%) presented significantly higher levels of A-FABP (33.62 ± 17.16 vs. 15.13 ± 7.61 ng/mL, $p = 0.001$) and E-FABP (13.37 ± 3.62 vs. 10.12 ± 3.21 ng/mL, $p = 0.021$) than the control group (Table 5). Due to a small number of patients, we did not analyze CCS with three or four metabolic risk factors separately.

Table 5. Comparison of childhood cancer survivors with the control group in terms of the number of metabolic derangements.

	≥1 Metabolic Risk Factor	Control Group	*p* Value
	n = 33	*n* = 25	
	Median (IQR)	**Median (IQR)**	
E-FABP (ng/mL)	11.28 (8.44; 14.60)	10.04 (7.64; 11.68)	0.090
A-FABP (ng/mL)	25.81 (21.32; 37.84)	13.44 (10.48; 21.15)	<0.001
	≥2 Metabolic Risk Factors		
	n = 10	*n* = 25	
E-FABP (ng/mL)	13.74 (9.88; 16.27)	10.04 (7.64; 11.68)	0.021
A-FABP (ng/mL)	25.51 (24.32; 37.84)	13.44 (10.48; 21.15)	0.001

E-FABP epidermal fatty acid-binding protein, A-FABP adipocyte fatty acid-binding protein, IQR interquartile range.

4. Discussion

The association between obesity-related risk factors and anticancer treatment during childhood has been established [22]. However, metabolic pathways leading to the development of obesity and its complications have not been fully elucidated in this group of patients. In this cross-sectional study, we sought to evaluate whether serum levels of A-FABP and E-FABP are elevated in acute lymphoblastic leukemia survivors and to assess their relationship with overweight and features of metabolic syndrome.

The studies on the importance of fatty acid-binding proteins in various diseases have been conducted [14,15]. The increased FABPs levels have been previously reported among the patients with obesity, hyperglycemia, insulin resistance, type 2 diabetes mellitus, hypertension, left ventricular diastolic dysfunction, atherosclerosis, and heart failure [13,23–30]. Moreover, in a 5-year prospective study, A-FABP has been noted to be a significant predictor of the occurrence of metabolic syndrome regardless of adiposity and insulin resistance [24]. Other studies showed that a higher level of E-FABP positively correlated with the components of MetS, yet it was not as strongly correlated as A-FABP [15,16]. Few studies on A-FABP levels have been conducted in children. Some of them indicate its higher level in overweight children and a possible association with the development of MetS [31,32].

In this study, elevated levels of A-FABP, but not E-FABP, were found among ALL survivors compared to healthy peers. These results support the hypothesis that this particular group of subjects may be susceptible to altered lipid metabolism due to anticancer treatment used in childhood. It was also shown that overweight subjects had higher levels of A-FABP compared to normal-weight subjects, which may indicate the presence of greater metabolic disturbances among overweight ALL survivors. Since A-FABP is expressed

mainly in adipocytes, it may be a better predictor of lipid metabolism changes than E-FABP, which is also secreted by other tissues less involved in fat metabolism.

This study also showed that overweight females had higher concentrations of both A-FABP and E-FABP than the female control group. In contrast, overweight males had only higher levels of A-FABP compared to healthy male controls. The underlying mechanism of the gender difference in serum A-FABP concentration has not been fully elucidated. However, Hu et al. [33] showed a negative correlation between the androgen and A-FABP levels in men, but a positive correlation in women. An additional factor contributing to this difference may be the natural variation in body fat distribution according to gender [15].

Many large epidemiologic studies have demonstrated that pediatric ALL survivors treated with cranial radiotherapy are particularly vulnerable to metabolic disturbances and unhealthy weight gain regardless of sex or weight status before the treatment [22,34–36]. In the present study, we found no differences according to the radiotherapy used. However, the number of individuals with a cranial radiotherapy (CRT) history was too small to conduct a reliable analysis.

The presence of one or more metabolic risk components among ALL survivors accounted for 53.23%. Data from the literature indicate that the population of CCS is more likely to develop metabolic syndrome, yet not all risk factors have been identified [35]. Smith et al. [37] reported that over 30% of adult CCS with the mean follow-up time after treatment discontinuation 25.6 years suffered from MetS, and the most common component in men was high blood pressure, while women had more often low HDL. According to other studies, the incidence of MetS was associated with male sex, age, and BMI at diagnosis. However, there are still inconsistent data in this field, with some papers suggesting that it is the female gender that increases the risk of developing MetS [38,39]. In our study, only one patient met all criteria of MetS, but roughly half of the survivors (children and young adults) met at least one criterion, which may indicate the early onset of the MetS. Xu et al. [13] have reported that A-FABP concentration is associated with obesity and the higher number of metabolic syndrome components in the general population in both genders, which is in accordance with our results. Only an increased A-FABP level in a subset of subjects who met at least one MetS criterion was identified in the current study. On the other hand, those who met at least two criteria had higher concentrations of both A-FABP and E-FABP than the control group or participants who did not meet any criteria. Our study supports the findings of previous research studies that CCS are more likely to develop the components of MetS. However, further studies on a larger group of patients are needed to determine the suitability of fatty acid-binding proteins for distinguishing a subset of CCS at high risk of developing MetS.

Furthermore, Levy et al. [40] described that ALL survivors were significantly at a higher risk of having pre-hypertension and hypertension than healthy counterparts. In our study, we observed a positive correlation between A-FABP and E-FABP and systolic blood pressure, although they were within the normal range. This may be a cause of hypertension in the future, but further studies are needed. All of these individuals should have their blood pressure checked regularly.

Interestingly, Oeffinger et al. [41] reported that ALL survivors were significantly more predisposed to develop insulin resistance even when compared to a control group 10 years older. Meachan et al. [42] showed that CCS were more likely to take medications for diabetes than controls. Our previous study examining obesity and insulin resistance among CCS supports these findings [43].

Some studies showed that obese patients diagnosed with cancer had higher levels of A-FABP than normal weight patients. In addition, these patients exhibit greater cancer progression [44,45]. Thus, the potential association between A-FABP and obesity in ALL survivors may already be due to the primary alteration in lipid metabolism. It is difficult to unequivocally answer the question of whether it is the FABPs that promote the development of ALL-associated MetS or vice versa. A long-term prospective study should be conducted to find the underlying process.

There are several limitations to this study. It was a single-center analysis, with a relatively small number of participants. No analysis of total body fat mass was performed; thus, the relationship between the visceral fat percentage and the levels of analyzed FABPs could not be determined. Furthermore, due to the age of the study population, we did not analyze the insulin resistance; however, this is an important component linked to MetS and should be considered in longitudinal studies. The study could have the added value of comparing the results, with a control group of obese children, which was not considered in this cross-sectional study. This would allow for a better understanding of whether differences in FABPs levels are due to cancer treatment or obesity itself. To conclusively confirm that elevated FABPs in normal weight ALL survivors after treatment promotes obesity later in life, long-term prospective studies would have to be conducted in these patients.

The strengths of our research include the homogenous group of acute lymphoblastic leukemia, relatively long follow-up time, and no ethnic diversity. Moreover, to our best knowledge, this is the first study assessing the role of fatty acid-binding proteins in the development of obesity and metabolic syndrome in acute lymphoblastic leukemia survivors.

In conclusion, this is the first study that demonstrated that ALL survivors present higher A-FABP levels than the control group. Moreover, the elevated levels of A-FABP and E-FABP were observed in overweight subjects and those with metabolic syndrome features. These findings suggest that the increased levels of fatty acid-binding proteins may be involved in the pathogenesis of overweight and the onset of metabolic syndrome in acute lymphoblastic leukemia survivors. Further longitudinal, prospective analyses of the A-FABP and E-FABP levels in ALL survivors and their potential role as the biomarkers in the pathogenesis of overweight, insulin resistance, and cardiovascular complications remain to be performed.

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References

1. Ma, H.; Sun, H.; Sun, X. Survival improvement by decade of patients aged 0–14 years with acute lymphoblastic Leukemia: A SEER analysis. *Sci. Rep.* **2014**, *4*, 1–7. [CrossRef]
2. Ward, E.; DeSantis, C.; Robbins, A.; Kohler, B.; Jemal, A. Childhood and adolescent cancer statistics, 2014: Cancer in children and adolescents. *CA A Cancer J. Clin.* **2014**, *64*, 83–103. [CrossRef] [PubMed]
3. Tai, E.W.; Ward, K.C.; Bonaventure, A.; Siegel, D.A.; Coleman, M.P. Survival among children diagnosed with acute lymphoblastic Leukemia in the United States, by race and age, 2001 to 2009: Findings from the CONCORD-2 study. *Cancer* **2017**, *123* (Suppl. 24), 5178–5189. [CrossRef] [PubMed]
4. Oeffinger, K.C.; Mertens, A.C.; Sklar, C.A.; Kawashima, T.; Hudson, M.M.; Meadows, A.T.; Friedman, D.L.; Marina, N.; Hobbie, W.; Kadan-Lottick, N.S.; et al. Childhood cancer survivor study. Chronic health conditions in adult survivors of childhood cancer. *N. Engl. J. Med.* **2006**, *355*, 1572–1582. [CrossRef] [PubMed]
5. Krawczuk-Rybak, M.; Panasiuk, A.; Stachowicz-Stencel, T.; Zubowska, M.; Skalska-Sadowska, J.; Sęga-Pondel, D.; Czajńska-Deptuła, A.; Sławińska, D.; Badowska, W.; Kamieńska, E.; et al. Health status of polish children and adolescents after cancer treatment. *Eur. J. Pediatr.* **2018**, *177*, 437–447. [CrossRef]

6. Suh, E.; Stratton, K.L.; Leisenring, W.M.; Nathan, P.C.; Ford, J.S.; Freyer, D.R.; McNeer, J.L.; Stock, W.; Stovall, M.; Krull, K.R.; et al. Late mortality and chronic health conditions in long-term survivors of early-adolescent and young adult cancers: A retrospective cohort analysis from the childhood cancer survivor study. *Lancet Oncol.* **2020**, *21*, 421–435. [CrossRef]
7. Ness, K.K.; Kirkland, J.L.; Gramatges, M.M.; Wang, Z.; Kundu, M.; McCastlain, K.; Li-Harms, X.; Zhang, J.; Tchkonia, T.; Pluijm, S.M.F.; et al. Premature physiologic aging as a paradigm for understanding increased risk of adverse health across the lifespan of survivors of childhood cancer. *J. Clin. Oncol.* **2018**, *36*, 2206–2215. [CrossRef]
8. Fulbright, J.M.; Raman, S.; McClellan, W.S.; August, K.J. Late effects of childhood Leukemia therapy. *Curr. Hematol. Malig. Rep.* **2011**, *6*, 195–205. [CrossRef]
9. Essig, S.; Li, Q.; Chen, Y.; Hitzler, J.; Leisenring, W.; Greenberg, M.; Sklar, C.; Hudson, M.M.; Armstrong, G.T.; Krull, K.R.; et al. Estimating the risk for late effects of therapy in children newly diagnosed with standard risk acute lymphoblastic leukemia using an historical cohort: A report from the childhood cancer survivor study. *Lancet Oncol.* **2014**, *15*, 841–851. [CrossRef]
10. Bizzarri, C.; Bottaro, G.; Pinto, R.M.; Cappa, M. Metabolic syndrome and diabetes mellitus in childhood cancer survivors. *Pediatr. Endocrinol. Rev.* **2014**, *11*, 365–373.
11. Nam, G.E.; Kaul, S.; Wu, Y.P.; Nelson, R.E.; Wright, J.; Fluchel, M.N.; Hacking, C.C.; Kirchhoff, A.C. A meta-analysis of body mass index of adolescent and adult survivors of pediatric acute lymphoblastic Leukemia. *J. Cancer Surviv.* **2015**, *9*, 412–421. [CrossRef]
12. Morel, S.; Leahy, J.; Fournier, M.; Lamarche, B.; Garofalo, C.; Grimard, G.; Poulain, F.; Delvin, E.; Laverdière, C.; Krajinovic, M.; et al. Lipid and lipoprotein abnormalities in acute lymphoblastic Leukemia survivors. *J. Lipid Res.* **2017**, *58*, 982–993. [CrossRef]
13. Xu, A.; Wang, Y.; Xu, J.Y.; Stejskal, D.; Tam, S.; Zhang, J.; Wat, N.M.; Wong, W.K.; Lam, K.S. Adipocyte fatty acid–binding protein is a plasma biomarker closely associated with obesity and metabolic syndrome. *Clin. Chem.* **2006**, *52*, 405–413. [CrossRef]
14. Furuhashi, M.; Ishimura, S.; Ota, H.; Miura, T. Lipid chaperones and metabolic inflammation. *Int. J. Inflam.* **2011**, *2011*. [CrossRef]
15. Yeung, D.C.Y.; Wang, Y.; Xu, A.; Cheung, S.C.W.; Wat, N.M.S.; Fong, D.Y.T.; Fong, C.H.Y.; Chau, M.T.; Sham, P.C.; Lam, K.S.L. Epidermal fatty-acid-binding protein: A new circulating biomarker associated with cardio-metabolic risk factors and carotid atherosclerosis. *Eur. Heart J.* **2008**, *29*, 2156–2163. [CrossRef] [PubMed]
16. Ishimura, S.; Furuhashi, M.; Watanabe, Y.; Hoshina, K.; Fuseya, T.; Mita, T.; Okazaki, Y.; Koyama, M.; Tanaka, M.; Akasaka, H.; et al. Circulating levels of fatty acid-binding protein family and metabolic phenotype in the general population. *PLoS ONE* **2013**, *8*, e81318. [CrossRef] [PubMed]
17. Kułaga, Z.; Litwin, M.; Tkaczyk, M.; Palczewska, I.; Zajączkowska, M.; Zwolińska, D.; Krynicki, T.; Wasilewska, A.; Moczulska, A.; Morawiec-Knysak, A.; et al. Polish 2010 growth references for school-aged children and adolescents. *Eur. J. Pediatr.* **2011**, *170*, 599–609. [CrossRef]
18. Kułaga, Z.; Grajda, A.; Gurzkowska, B.; Góźdź, M.; Wojtyło, M.; Świąder, A.; Różdżyńska-Świątkowska, A.; Litwin, M. Polish 2012 growth references for preschool children. *Eur. J. Pediatr.* **2013**, *172*, 753–761. [CrossRef]
19. McCarthy, H.D.; Ashwell, M. A study of central fatness using waist-to-height ratios in UK children and adolescents over two decades supports the simple message—'keep your waist circumference to less than half your height'. *Int. J. Obes.* **2006**, *30*, 988–992. [CrossRef]
20. Kułaga, Z.; Litwin, M.; Grajda, A.; Gurzkowska, B.; Napieralska, E.; Kułaga, K.; Grupa Badaczy, O.L.A.F. Distribution of blood pressure in school-aged children and adolescents reference population. *Stand. Med.* **2010**, *7*, 853–864.
21. Zimmet, P.; Alberti, K.G.M.; Kaufman, F.; Tajima, N.; Silink, M.; Arslanian, S.; Wong, G.; Bennett, P.; Shaw, J.; Caprio, S. The metabolic syndrome in children and adolescents—An IDF consensus report. *Pediatric Diabetes* **2007**, *8*, 299–306. [CrossRef] [PubMed]
22. Zhang, F.F.; Liu, S.; Chung, M.; Kelly, M.J. Growth patterns during and after treatment in patients with pediatric ALL: A meta-analysis. *Pediatr. Blood Cancer* **2015**, *62*, 1452–1460. [CrossRef]
23. Möhlig, M.; Weickert, M.O.; Ghadamgadai, E.; Machlitt, A.; Pfüller, B.; Arafat, A.M.; Pfeiffer, A.F.H.; Schöfl, C. Adipocyte fatty acid-binding protein is associated with markers of obesity, but is an unlikely link between obesity, insulin resistance, and hyperandrogenism in polycystic ovary syndrome women. *Eur. J. Endocrinol.* **2007**, *157*, 195–200. [CrossRef] [PubMed]
24. Xu, A.; Tso, A.W.K.; Cheung, B.M.Y.; Wang, Y.; Wat, N.M.S.; Fong, C.H.Y.; Yeung, D.C.Y.; Janus, E.D.; Sham, P.C.; Lam, K.S.L. Circulating adipocyte–fatty acid binding protein levels predict the development of the metabolic syndrome. *Circulation* **2007**, *115*, 1537–1543. [CrossRef]
25. Yeung, D.C.Y.; Xu, A.; Cheung, C.W.S.; Wat, N.M.S.; Yau, M.H.; Fong, C.H.Y.; Chau, M.T.; Lam, K.S.L. Serum adipocyte fatty acid-binding protein levels were independently associated with carotid atherosclerosis. *Arter. Thromb. Vasc. Biol.* **2007**, *27*, 1796–1802. [CrossRef]
26. Hsu, B.G.; Chen, Y.C.; Lee, R.P.; Lee, C.C.; Lee, C.J.; Wang, J.H. Fasting serum level of fatty-acid-binding protein 4 positively correlates with metabolic syndrome in patients with coronary artery disease. *Circ. J.* **2010**, *74*, 327–331. [CrossRef] [PubMed]
27. Ota, H.; Furuhashi, M.; Ishimura, S.; Koyama, M.; Okazaki, Y.; Mita, T.; Fuseya, T.; Yamashita, T.; Tanaka, M.; Yoshida, H.; et al. Elevation of fatty acid-binding protein 4 is predisposed by family history of hypertension and contributes to blood pressure elevation. *Am. J. Hypertens.* **2012**, *25*, 1124–1130. [CrossRef] [PubMed]
28. Fuseya, T.; Furuhashi, M.; Yuda, S.; Muranaka, A.; Kawamukai, M.; Mita, T.; Ishimura, S.; Watanabe, Y.; Hoshina, K.; Tanaka, M.; et al. Elevation of circulating fatty acid-binding protein 4 is independently associated with left ventricular diastolic dysfunction in a general population. *Cardiovasc. Diabetol.* **2014**, *13*, 126. [CrossRef] [PubMed]

29. Nakamura, R.; Okura, T.; Fujioka, Y.; Sumi, K.; Matsuzawa, K.; Izawa, S.; Ueta, E.; Kato, M.; Taniguchi, S.; Yamamoto, K. Serum fatty acid-binding protein 4 (FABP4) concentration is associated with insulin resistance in peripheral tissues, a clinical study. *PLoS ONE* **2017**, *12*, e0179737. [CrossRef] [PubMed]
30. Rodríguez-Calvo, R.; Girona, J.; Alegret, J.M.; Bosquet, A.; Ibarretxe, D.; Masana, L. Role of the fatty acid-binding protein 4 in heart failure and cardiovascular disease. *J. Endocrinol.* **2017**, *233*, R173–R184. [CrossRef]
31. Choi, K.M.; Yannakoulia, M.; Park, M.S.; Cho, G.J.; Kim, J.H.; Lee, S.H.; Hwang, T.G.; Yang, S.J.; Kim, T.N.; Yoo, H.J.; et al. Serum adipocyte fatty acid–binding protein, retinol-binding protein 4, and adiponectin concentrations in relation to the development of the metabolic syndrome in Korean boys: A 3-y prospective cohort study12345. *Am. J. Clin. Nutr* **2011**, *93*, 19–26. [CrossRef]
32. Krzystek-Korpacka, M.; Patryn, E.; Bednarz-Misa, I.; Mierzchala, M.; Hotowy, K.; Czapinska, E.; Kustrzeba-Wojcicka, I.; Gamian, A.; Noczynska, A. Circulating adipocyte fatty acid-binding protein, juvenile obesity, and metabolic syndrome. *J. Pediatr. Endocrinol. Metab.* **2011**, *24*, 921–928. [CrossRef]
33. Hu, X.; Ma, X.; Pan, X.; Luo, Y.; Xu, Y.; Xiong, Q.; Bao, Y.; Jia, W. Association of androgen with gender difference in serum adipocyte fatty acid binding protein levels. *Sci. Rep.* **2016**, *6*. [CrossRef]
34. Gurney, J.G.; Ness, K.K.; Sibley, S.D.; O'Leary, M.; Dengel, D.R.; Lee, J.M.; Youngren, N.M.; Glasser, S.P.; Baker, K.S. Metabolic syndrome and growth hormone deficiency in adult survivors of childhood acute lymphoblastic Leukemia. *Cancer* **2006**, *107*, 1303–1312. [CrossRef]
35. Nottage, K.A.; Ness, K.K.; Li, C.; Srivastava, D.; Robison, L.L.; Hudson, M.M. Metabolic syndrome and cardiovascular risk among long-term survivors of acute lymphoblastic Leukaemia—From the St. Jude lifetime cohort. *Br. J. Haematol.* **2014**, *165*, 364–374. [CrossRef] [PubMed]
36. Zhang, F.F.; Rodday, A.M.; Kelly, M.J.; Must, A.; MacPherson, C.; Roberts, S.B.; Saltzman, E.; Parsons, S.K. Predictors of being overweight or obese in survivors of pediatric acute lymphoblastic Leukemia (ALL). *Pediatr. Blood Cancer* **2014**, *61*, 1263–1269. [CrossRef]
37. Smith, W.A.; Li, C.; Nottage, K.; Mulrooney, D.A.; Armstrong, G.T.; Lanctot, J.Q.; Chemaitilly, W.; Laver, J.H.; Srivastava, D.K.; Robison, L.L.; et al. Lifestyle and metabolic syndrome in adult survivors of childhood cancer: A report from the St. Jude lifetime cohort study. *Cancer* **2014**, *120*, 2742–2750. [CrossRef]
38. Saultier, P.; Auquier, P.; Bertrand, Y.; Vercasson, C.; Oudin, C.; Contet, A.; Plantaz, D.; Poirée, M.; Ducassou, S.; Kanold, J.; et al. Metabolic syndrome in long-term survivors of childhood acute Leukemia treated without hematopoietic stem cell transplantation: An, L.E.A. study. *Haematologica* **2016**, *101*, 1603–1610. [CrossRef]
39. Özdemir, Z.C.; Düzenli Kar, Y.; Demiral, M.; Sırmagül, B.; Bör, Ö.; Kırel, B. The Frequency of metabolic syndrome and serum osteopontin levels in survivors of childhood acute lymphoblastic Leukemia. *J. Adolesc. Young Adult Oncol.* **2018**, *7*, 480–487. [CrossRef]
40. Levy, E.; Samoilenko, M.; Morel, S.; England, J.; Amre, D.; Bertout, L.; Drouin, S.; Laverdière, C.; Krajinovic, M.; Sinnett, D.; et al. Cardiometabolic risk factors in childhood, adolescent and young adult survivors of acute lymphoblastic Leukemia—A petale cohort. *Sci. Rep.* **2017**, *7*. [CrossRef] [PubMed]
41. Oeffinger, K.C.; Adams-Huet, B.; Victor, R.G.; Church, T.S.; Snell, P.G.; Dunn, A.L.; Eshelman-Kent, D.A.; Ross, R.; Janiszewski, P.M.; Turoff, A.J.; et al. Insulin resistance and risk factors for cardiovascular disease in young adult survivors of childhood acute lymphoblastic Leukemia. *J. Clin. Oncol.* **2009**, *27*, 3698–3704. [CrossRef] [PubMed]
42. Meacham, L.R.; Chow, E.J.; Ness, K.K.; Kamdar, K.Y.; Chen, Y.; Yasui, Y.; Oeffinger, K.C.; Sklar, C.A.; Robison, L.L.; Mertens, A.C. Cardiovascular risk factors in adult survivors of pediatric cancer—A report from the childhood cancer survivor study. *Cancer Epidemiol. Biomark. Prev.* **2010**, *19*, 170–181. [CrossRef] [PubMed]
43. Latoch, E.; Muszynska-Roslan, K.; Panas, A.; Panasiuk, A.; Sawicka-Zukowska, M.; Zelazowska-Rutkowska, B.; Zabrocka, E.; Krawczuk-Rybak, M. Adipokines and insulin resistance in young adult survivors of childhood cancer. *Int. J. Endocrinol.* **2016**, *2016*, 1–7. [CrossRef]
44. Hao, J.; Zhang, Y.; Yan, X.; Yan, F.; Sun, Y.; Zeng, J.; Waigel, S.; Yin, Y.; Fraig, M.M.; Egilmez, N.K.; et al. Circulating adipose fatty acid binding protein is a new link underlying obesity-associated breast/mammary tumor development. *Cell Metab.* **2018**, *28*, 689–705.e5. [CrossRef] [PubMed]
45. Yan, F.; Shen, N.; Pang, J.; Zhang, Y.; Rao, E.; Bode, A.; Al-Kali, A.; Zhang, D.; Litzow, M.; Li, B.; et al. Fatty acid binding protein FABP4 mechanistically links obesity with aggressive AML by enhancing aberrant DNA methylation in AML cells. *Leukemia* **2017**, *31*, 1434–1442. [CrossRef]

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Biomarkers of Glucose Metabolism Alterations and the Onset of Metabolic Syndrome in Survivors of Childhood Acute Lymphoblastic Leukemia

Katarzyna Konończuk [1,*], Katarzyna Muszyńska-Rosłan [1], Karolina Konstantynowicz-Nowicka [2], Maryna Krawczuk-Rybak [1], Adrian Chabowski [2] and Eryk Latoch [1,*]

[1] Department of Pediatric Oncology and Hematology, Medical University of Bialystok, 15-274 Białystok, Poland; kmroslan@post.pl (K.M.-R.); maryna.krawczuk-rybak@umb.edu.pl (M.K.-R.)
[2] Department of Physiology, Medical University of Bialystok, 15-222 Białystok, Poland; karolina.konstantynowicz@umb.edu.pl (K.K.-N.); adrian@umb.edu.pl (A.C.)
* Correspondence: kononczukk@gmail.com (K.K.); eryklatoch@gmail.com (E.L.); Tel.: +48-85-745-0846 (K.K.)

Abstract: Owing to advances in treatment modalities and supportive care, overall survival rates have reached up to 90% among children with acute lymphoblastic leukemia (ALL). However, due to the underlying illness and therapy, they are at a greater risk of developing lifestyle diseases. Hence, special attention is paid to early detection of the components of metabolic syndrome (MetS). This study aimed at investigating the association of plasma levels of nine diabetes markers with being overweight and components of MetS in ALL survivors. The study included 56 subjects with mean age of 12.36 ± 5.15 years. The commercially available Bio-Plex Pro Human Diabetes 10-Plex Panel kit was used to evaluate levels of diabetes biomarkers. ALL survivors presented statistically higher concentrations of GIP ($p = 0.026$), glucagon ($p = 0.001$), leptin ($p = 0.022$), and PAI-1 ($p = 0.047$), whereas the concentration of ghrelin was lower ($p < 0.001$) compared to the control group. Moreover, subjects within normal BMI range showed higher GIP ($p = 0.005$) and lower ghrelin concentration ($p < 0.001$) compared to healthy peers. At least one risk factor of MetS was present in 58.9% of participants, who showed significantly higher levels of C-peptide ($p = 0.028$), leptin ($p = 0.003$), and PAI-1 ($p = 0.034$) than survivors who did not meet any MetS criteria. In conclusion, ALL survivors are at greater risk of disturbances in carbohydrate metabolism. Understanding the pathogenesis and applicability of diabetes markers is crucial for developing strategies to prevent metabolic syndrome in ALL survivors.

Keywords: ALL; childhood cancer survivors; CCS; children; diabetes biomarkers; obesity; overweight; C-peptide; ghrelin; gastric inhibitory peptide (GIP); glucagon; insulin; plasminogen activator inhibitor-1 (PAI-1); resistin; leptin; visfatin

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1. Introduction

The most common malignancy diagnosed in childhood is acute lymphoblastic leukemia (ALL). Due to remarkable advances in treatment modalities and excellent supportive care, overall survival rates have reached up to 90% or more depending on the risk group [1]. As a result, the population of adolescents and young adults who have experienced childhood cancer therapy is growing significantly. Nowadays, the world is witnessing an increase in lifestyle diseases leading to cardiovascular incidents that can result in premature death. Recent studies also indicate an enhanced risk of earlier onset of age-related chronic diseases in childhood cancer survivors (CCS). The main causes of increased morbidity and mortality among CCS include obesity, insulin resistance (IR), hypertension (HT), dyslipidemia, diabetes mellitus (DM), and consequently metabolic syndrome (MetS) and cardiovascular disease [2,3].

The best-studied detrimental factors in the development of metabolic diseases in ALL survivors at a younger age include the use of glucocorticosteroids and cranial radiotherapy (CRT) during treatment, yet not all agents have been identified so far. The etiopathogenesis

of the earlier occurrence of the many diseases among CCS has not been clearly elucidated. Some studies suggest that triggers may include chemotherapeutics used in childhood that alter carbohydrate and lipid metabolism (anthracycline and asparaginase, among others) [4,5]. In addition, poor eating habits and reduced activity persisting beyond the completion of treatment enhance the risk of obesity and lifestyle diseases in this population [6,7].

The metabolic pathways are regulated by the number of factors whose changed secretion may be associated with the onset of insulin resistance and obesity [8]. The major markers include C-peptide, ghrelin, gastric inhibitory peptide (GIP), glucagon, insulin, plasminogen activator inhibitor-1 (PAI-1), resistin, leptin, and visfatin. The detailed characteristics and roles in metabolism are shown in Table 1. As the number of survivors increases substantially, there is a growing interest in the early detection of the predisposition to develop late effects in this particular population.

Table 1. Descriptive characteristics of diabetes markers [9–16].

Biomarker	Function
C-peptide	• A reliable marker of β-cell function. • High levels are associated with macrovascular complications. • Low levels are associated with microvascular and nerve function complications.
Ghrelin	• Secreted by pancreatic islets where it stimulates glucagon release and inhibits insulin secretion. • Negatively correlates with the occurrence of insulin resistance and obesity in a population with normal fasting glucose. • Lower levels are observed in obese people.
Gastric inhibitory peptide (GIP)	• Secreted by K cells in the duodenum and upper jejunum. • High levels are observed in obesity. • Normal to increased levels in subjects with type 2 diabetes mellitus.
Glucagon	• Secreted by pancreatic α-cells. • High levels are observed in obesity. • Higher fasting glucagon levels are associated with more severe insulin resistance.
Insulin	• produced by pancreatic β-cells. • Hyperinsulinemia affects the development of obesity by inhibiting lipolysis and promoting lipogenesis. • Hyperinsulinemia and insulin resistance promote hypertension and atherosclerosis.
Plasminogen activator inhibitor-1 (PAI-1)	• a pivotal regulator of fibrinolysis. • Higher levels have been reported in subjects with obesity, metabolic syndrome, and type 2 diabetes mellitus.
Resistin	• Secreted mainly by peripheral blood mononuclear cells and macrophages. • High levels are associated with promoting inflammation and may be involved in the pathogenesis of insulin resistance and metabolic syndrome.
Leptin	• Produced by adipocytes. • Involved in the low-grade inflammatory state. • Contributes to the secretion of proinflammatory cytokines, which results in the development of insulin resistance and type 2 diabetes mellitus.
Visfatin	• Secreted mainly by adipocytes and macrophages. • Higher levels are observed in obese patients and type 2 diabetes mellitus. • Possibly involved in the release of proinflammatory cytokines, contributing to insulin resistance.

This study aimed at investigating the association of plasma level of nine diabetes markers with being overweight, insulin resistance, and components of metabolic syndrome in childhood survivors of acute lymphoblastic leukemia.

2. Results

The clinical characteristic of ALL childhood survivors is presented in Table 2. The mean age at diagnosis was 5.01 ± 3.46 years, and follow-up after cessation of the treatment was 6.58 ± 4.64 years. The age and sex did not differ between the study and control groups.

Table 2. Descriptive characteristics of acute lymphoblastic leukemia survivors.

	Study Group	
	Number (%) [a] n = 56	**Mean ± SD [b]**
Male	26 (46.4)	
Female	30 (53.6)	
Age at diagnosis (years)		5.01 ± 3.46
Age at study (years)		12.36 ± 5.15
Follow-up after treatment (years)		6.58 ± 4.64
Chemotherapy		
Methotrexate (cumulative dose (mg/m^2))		10,321.43 ± 6644.50
Cumulative corticosteroid dose (mg/m^2) [c]		3538.05 ± 901.85
Prednisone (cumulative dose (mg/m^2))		1680.00 ± 0.00
Dexamethasone (cumulative dose (mg/m^2))		277.32 ± 134.60
Cyclophosphamide (cumulative dose (mg/m^2))		3957.14 ± 2632.93
Anthracycline (cumulative dose (mg/m^2))		225.00 ± 45.41
Radiotherapy	9 (16.1)	
Cranial radiotherapy (CRT) (cumulative dose (Gy))	8 (14.3)	12.75 ± 2.12
Total body irradiation (TBI)	2 (3.6)	12 ± 0.00
No	47 (83.9)	
HSCT	6 (10.7)	
Metabolic derangements		
1 Metabolic risk factor	21 (37.5)	
2 Metabolic risk factors	7 (12.5)	
3 Metabolic risk factors	4 (7.1)	
4 Metabolic risk factors	1 (1.8)	

[a] percent of the total; [b] standard deviation (SD); [c] calculated as prednisone equivalents; *9 the Gray.

Compared to the study group, the control group presented statistically higher concentrations of GIP ($p = 0.026$), glucagon ($p = 0.001$), leptin ($p = 0.022$), and PAI-1 ($p = 0.047$), whereas the concentration of ghrelin was lower ($p < 0.001$). We did not find any differences in the C-peptide ($p = 0.386$), insulin ($p = 0.158$), resistin ($p = 0.429$), and visfatin ($p = 0.066$) levels (Table 3).

Table 3. Comparison of diabetes marker concentrations between the ALL survivors and the control group.

	ALL Survivors n = 56	**Control Group n = 22**	***p* Value**
C-peptide (pg/mL)	611.08 (332.59; 962.57)	479.47 (268.64; 799.61)	0.386
Ghrelin (pg/mL)	224.07 (161.76; 356.32)	634.33 (377.65; 1070.13)	<0.001
GIP (pg/mL)	1050.12 (592.44; 1479.55)	417.67 (280.34; 741.02)	0.026
Glucagon (pg/mL)	394.94 (234.92; 612.39)	237.62 (140.22; 324.11)	0.001
Insulin (pg/mL)	530.18 (296.77; 964.83)	377.87 (140.28; 631.33)	0.158
Leptin (pg/dL)	5219.36 (1329.38; 12551.94)	1846.23 (765.72; 3361.22)	0.022
PAI-1 (pg/mL)	4914.04 (3638.52; 6040.11)	3936.78 (3091.16; 4900.93)	0.047
Resistin (pg/mL)	8448.39 (4983.02; 14698.13)	7420.48 (4239.87; 12889.74)	0.429
Visfatin (pg/mL)	1032.53 (689.50; 2632.74)	133.00 (55.58; 1296.86)	0.066

GIP insulin-dependent insulinotropic polypeptide; PAI-1 plasminogen activator inhibitor-1; IQR interquartile range. Data are given as Median and Interquartile range (IQR).

In the analysis stratified by body mass index (BMI) value, ALL survivors with normal BMI range demonstrated greater levels of GIP (4993.09 ± 10750.06 pg/mL vs. 487.86 ± 278.73 pg/mL, $p = 0.005$) and lower levels of ghrelin (296.64 ± 232.40 pg/mL vs. 764.97 ± 557.20 pg/mL, $p < 0.001$) when compared to the control group. In turn, overweight and obese survivors presented higher levels of glucagon (572.58 ± 327.20 pg/mL vs. 363.39 ± 230.83 pg/mL, $p = 0.006$) and leptin (9180.43 ± 6989.29 pg/mL vs. 4952.19 ± 4642.43 pg/mL, $p = 0.034$) than normal weight subjects. Subsequently, we performed an analysis comparing survivors with a division by BMI value and control group (normal BMI survivors vs. overweight and obese survivors vs. control group). As before, the analysis revealed an increased GIP level among survivors with normal BMI ($p = 0.029$), whereas leptin and glucagon levels were higher in the overweight and obese subgroup ($p = 0.009$, $p < 0.001$, respectively). Finally, decreased ghrelin level was found in both normal BMI and high BMI survivors in comparison to the control group ($p < 0.001$).

According to sex, C-peptide ($p = 0.014$), leptin ($p = 0.031$), and resistin ($p = 0.028$) levels were higher in females than males within the study group. Moreover, ALL females showed higher insulin ($p = 0.041$) and leptin ($p = 0.003$) levels, while ALL males had higher levels of GIP ($p = 0.036$) compared to the control group.

We also assessed the diabetes markers by the time of cessation of anticancer treatment. The participants over 5 years after the end of treatment presented higher levels of PAI-1 (5524.84 ± 2338.48 pg/mL vs. 3711.59 ± 2360.43 pg/mL, $p < 0.001$) and resistin (12247.92 ± 7238.51 pg/mL vs. 5182.93 ± 3620.10 pg/mL, $p = 0.002$) than subjects with a shorter time after completion of treatment.

In the further analysis, we checked the relationship between diabetes markers and risk factors of metabolic syndrome. At least one risk factor was present in 58.9% of the participants. Twenty-nine subjects had increased waist circumference (WC), eleven met the criterion related to blood pressure, six had elevated glucose levels, three presented decreased HDL levels, and six had elevated triglycerides (TG) levels. Compared to the control group, survivors with MetS factors showed greater levels of GIP ($p = 0.030$), glucagon ($p < 0.001$), leptin ($p = 0.001$), PAI-1 ($p = 0.009$), and lower level of ghrelin ($p < 0.001$) (Table 4).

Table 4. Characteristic of acute lymphoblastic leukemia survivors according to number of metabolic risk (MetS) factors compared to the control group.

	≥1 MetS Risk Factor n = 33	**Control Group n = 22**	***p* Value**
C-peptide (pg/mL)	792.42 (444.15; 1046.09)	475.47 (268.64; 799.61)	0.094
Ghrelin (pg/mL)	220.49 (183.58; 351.21)	634.33 (377.65; 1070.13)	<0.001
GIP (pg/mL)	1151.39 (592.44; 1731.89)	417.67 (280.34; 741.02)	0.030
Glucagon (pg/mL)	400.89 (269.81; 608.71)	237.62 (140.22; 324.11)	<0.001
Insulin (pg/dL)	588.46 (365.81; 974.98)	377.87 (140.28; 631.33)	0.071
Leptin (pg/mL)	6999.47 (3347.83; 16,562.53)	1846.23 (765.72; 3361.22)	0.001
PAI-1 (pg/mL)	5305.50 (3814.72; 6898.52)	3936.78 (3091.16; 4900.93)	0.009
Resistin (pg/mL)	10,585.10 (5861.14; 15,774.47)	7420.48 (4239.87; 12,889.74)	0.129
Visfatin (pg/mL)	1285.36 (827.91; 3103.90)	133.00 (55.58; 1296.86)	0.068
	≥2 MetS risk factor n = 12	**Control Group n = 22**	***p* Value**
C-peptide (pg/mL)	936.48 (591.03; 1546.72)	475.47 (268.64; 799.61)	0.008
Ghrelin (pg/mL)	216.77 (193.13; 286.35)	634.33 (377.65; 1070.13)	0.001
GIP (pg/mL)	704.05 (438.26; 1403.35)	417.67 (280.34; 741.02)	0.138
Glucagon (pg/mL)	445.20 (355.39; 765.60)	237.62 (140.22; 324.11)	<0.001
Insulin (pg/dL)	967.72 (454.60; 1729.53)	377.87 (140.28; 631.33)	0.020
Leptin (pg/mL)	10,981.25 (5654.11; 16,772.59)	1846.23 (765.72; 3361.22)	<0.001
PAI-1 (pg/mL)	5757.94 (4203.10; 7416.83)	3936.78 (3091.16; 4900.93)	0.001
Resistin (pg/mL)	12,026.93 (5382.83; 16,882.36)	7420.48 (4239.87; 12,889.74)	0.299
Visfatin (pg/mL)	1016.43 (971.42; 1760.47)	133.00 (55.58; 1296.86)	0.240

GIP insulin-dependent insulinotropic polypeptide, PAI-1 plasminogen activator inhibitor-1, IQR interquartile range. Data are given as Median and Interquartile range (IQR).

ALL survivors with two or more MetS risk factors compared to the control group presented higher levels of C-peptide ($p = 0.008$), glucagon ($p < 0.001$), insulin ($p = 0.020$), leptin ($p < 0.001$), PAI-1 ($p = 0.001$), and a lower level of ghrelin ($p = 0.001$) (Table 4).

Compared to survivors who did not meet any MetS criteria, the ALL survivors with at least one MetS risk factor presented significantly higher levels of C-peptide ($p = 0.028$), leptin ($p = 0.003$), and PAI-1 ($p = 0.034$). In addition, we investigated the subset of participants who met two or more MetS risk factors in comparison to those without any risk factors. Beyond higher C-peptide ($p = 0.002$), leptin ($p < 0.001$), and PAI-1 ($p = 0.031$) higher insulin levels ($p = 0.021$) were found. The results of the CCS analysis with and without the presence of metabolic derangements are presented in Table 5.

Table 5. Characteristic of acute lymphoblastic leukemia survivors according to number of metabolic risk (MetS) factors.

	≥1 MetS Risk Factor n = 33	**No MetS Risk Factors n = 23**	***p* Value**
C-peptide (pg/mL)	792.42 (444.15; 1046.09)	419.15 (258.64; 727.40)	0.028
Ghrelin (pg/mL)	220.49 (183.58; 351.21)	225.45 (118.91; 360.40)	0.817
GIP (pg/mL)	1151.39 (592.44; 1731.89)	1031.18 (605.88; 1093.86)	0.409
Glucagon (pg/mL)	400.89 (269.81; 608.71)	319.44 (174.91; 616.06)	0.220
Insulin (pg/dL)	588.46 (365.81; 974.98)	386.98 (201.06; 814.23)	0.136
Leptin (pg/mL)	6999.47 (3347.83; 16562.53)	3613.77 (664.69; 6269.79)	0.003
PAI-1 (pg/mL)	5305.50 (3814.72; 6898.52)	4478.74 (3409.32; 5383.39)	0.034
Resistin (pg/mL)	10,585.10 (5861.14; 15774.47)	6608.98 (3902.95; 12059.42)	0.059
Visfatin (pg/mL)	1285.36 (827.91; 3103.90)	729.03 (326.13; 2250.56)	0.253
	≥2 MetS risk factor n = 12	**No MetS risk factors n = 23**	***p* value**
C-peptide (pg/mL)	936.48 (591.03; 1546.72)	419.15 (258.64; 727.40)	0.002
Ghrelin (pg/mL)	216.77 (193.13; 286.35)	225.45 (118.91; 360.40)	0.959
GIP (pg/mL)	704.05 (438.26; 1403.35)	1031.18 (605.88; 1093.86)	0.788
Glucagon (pg/mL)	445.20 (355.39; 765.60)	319.44 (174.91; 616.06)	0.263
Insulin (pg/dL)	967.72 (454.60; 1729.53)	386.98 (201.06; 814.23)	0.021
Leptin (pg/mL)	10,981.25 (5654.11; 16772.59)	3613.77 (664.69; 6269.79)	<0.001
PAI-1 (pg/mL)	5757.94 (4203.10; 7416.83)	4478.74 (3409.32; 5383.39)	0.031
Resistin (pg/mL)	12,026.93 (5382.83; 16882.36)	6608.98 (3902.95; 12059.42)	0.176
Visfatin (pg/mL)	1016.43 (971.42; 1760.47)	729.03 (326.13; 2250.56)	0.408

GIP insulin-dependent insulinotropic polypeptide, PAI-1 plasminogen activator inhibitor-1, IQR interquartile range. Data are given as Median and Interquartile range (IQR).

The subgroup of ALL survivors meeting the metabolic criterion for a diagnosis of insulin resistance (10.7%) showed higher levels of C-peptide (1150.13 ± 626.88 pg/mL vs. 425.84 ± 528.95 pg/mL, $p = 0.005$), glucagon (625.22 ± 355.41 pg/mL vs. 284.69 ± 177.93 pg/mL, $p = 0.042$), and leptin (9930.65 ± 6458.18 pg/mL vs. 2906.16 ± 4843.62 pg/mL, $p = 0.016$) in comparison to subjects with normal HOMA-IR.

Furthermore, a strong positive correlation was found between BMI and C-peptide ($r = 0.56$, $p < 0.001$), glucagon ($r = 0.52$, $p = 0.001$), and leptin ($r = 0.56$, $p < 0.001$). A positive correlation between PAI-1 and cholesterol levels ($r = 0.53$, $p = 0.041$) was observed. Leptin was strongly associated with HOMA-IR ($r = 0.72$, $p = 0.002$). In addition, a strong correlation between PAI-1 and resistin ($r = 0.83$, $p < 0.001$) was found.

There were no differences in the levels of any analyzed biomarkers in subjects treated with both radiotherapy (RT) and hematopoietic stem cell transplantation (HSCT).

The receiver operating curve (ROC) analyses were conducted to assess the diagnostic profile of diabetes markers with the presence of any metabolic syndrome features (Table 6) and overweight/obesity in the study group. Glucagon (AUC 0.71, $p = 0.003$) and leptin (AUC 0.67, $p = 0.026$) were found to be predictors for being overweight or obese in ALL survivors; however, we did not observe statistical differences between the two areas under the curve ($p > 0.05$) Figure 1.

Univariate analysis showed a significant association between the presence of at least one MetS risk factor among ALL survivors and levels of leptin (coef. 0.001, $p = 0.001$),

PAI-1 (coef. 0.001, $p = 0.049$), and resistin (coef. 0.001, $p = 0.049$); however, the coefficient values were very small. Multivariable analysis confirmed only the effect on leptin level (coef. 0.001, $p = 0.011$).

Table 6. The receiver operating characteristic (ROC) analysis for prediction of at least one metabolic risk factor based on the diabetes markers levels in childhood acute lymphoblastic leukemia survivors.

	AUC	95% CI
C-peptide (pg/mL)	0.733	(0.596–0.871)
Ghrelin (pg/mL)	0.473	(0.317–0.630)
GIP (pg/mL)	0.638	(0.377–0.899)
Glucagon (pg/mL)	0.642	(0.490–0.795)
Insulin (pg/dL)	0.675	(0.527–0.823)
Leptin (pg/mL)	0.797	(0.684–0.911)
PAI-1 (pg/mL)	0.685	(0.542–0.828)
Resistin (pg/mL)	0.676	(0.531–0.822)
Visfatin (pg/mL)	0.649	(0.429–0.868)

GIP insulin-dependent insulinotropic polypeptide; PAI-1 plasminogen activator inhibitor-1; IQR interquartile range.

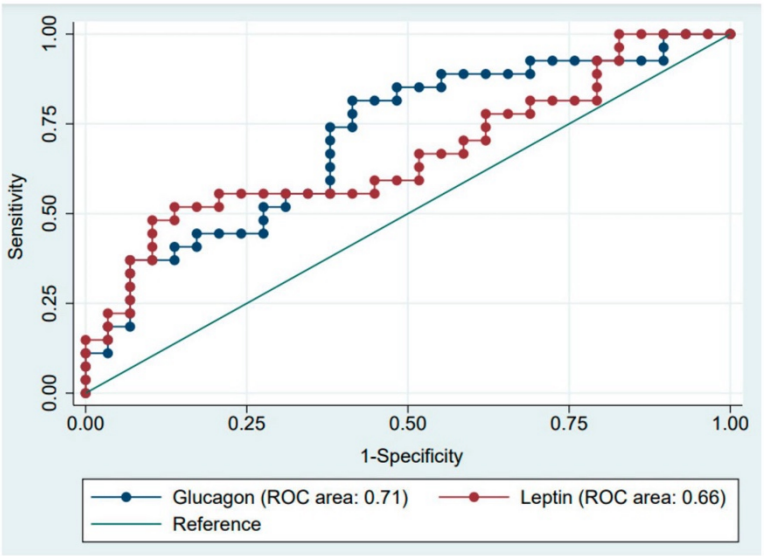

Figure 1. The receiver operating characteristic (ROC) analysis for prediction of overweight/obesity based on the serum levels of glucagon and leptin in childhood acute lymphoblastic leukemia survivors.

3. Discussion

In this study, we focused on evaluating glucose metabolism biomarkers and their applicability in assessing the late effects associated with the development of metabolic syndrome after childhood cancer treatment.

Of the nine markers tested, we showed that levels of GIP, glucagon, leptin, and PAI-1 were significantly higher in ALL survivors, whilst ghrelin was lower compared to healthy controls. In addition, our study confirms alterations in carbohydrate metabolism among patients with normal BMI, which may contribute to the onset of metabolic disease in later life.

Due to the greater risk of developing lifestyle diseases in adolescents and young adults after childhood cancer treatment, diagnostic markers are being sought to detect the

onset of late complications. There has been an increasing amount of literature focusing on being overweight or obese in childhood as well as more generally in adulthood. In CCS, metabolic syndrome is among the most frequently reported medical conditions that may confer essential public health issues. In this regard, special attention is paid to early detection of the components of metabolic syndrome that promote the development of lifestyle diseases. To successfully prevent possible complications, it is, however, necessary to understand the underlying mechanisms and their etiopathogenesis [8,17].

This is the first study to concurrently and comprehensively evaluate multiple markers of impaired carbohydrate metabolism in individuals after childhood ALL treatment.

Some of the analyzed hormones are well known and widely useful in clinical practice, including diagnosis and treatment of type 2 DM, which is characterized by insulin resistance, resulting in hyperinsulinemia, and, over time, β-cell failure. Elevated C-peptide levels are observed in IR and early type 2 DM. Moreover, in the study by Cabrera et al., C-peptide is a risk factor for coronary heart disease in a population with normal blood glucose levels, and thus according to the authors may be potentially more valuable as an early predictor of coronary events than impaired fasting glucose levels [18]. In our study, C-peptide was higher in patients who met at least one criterion of metabolic syndrome compared to those who did not meet any of the criteria. Similarly, survivors with abnormal HOMA-IR also presented increased level of C-peptide. We did not find any cardiovascular diseases among participants enrolled in the study; however, the mean age of the study was low. We may only speculate that this group of CCS might have a stronger predisposition to developing metabolic syndrome and perhaps cardiac events later in life. Therefore, regular cardiovascular evaluation in these patients is needed [19].

Although the main alteration in type 2 diabetes is β-cell dysfunction and insulin resistance, there is also increased α-cell activity. The result of these abnormalities is hyperglucagonemia, which has long been acknowledged to contribute to hyperglycemia in patients with diabetes by promoting hepatic glucose production. Overweight and obese subjects presented higher glucagon levels compared with both the normal weight and the control group. Furthermore, individuals with any risk factors for metabolic syndrome also demonstrated statistically significant differences compared to the control group. A similar observation was made by Mannell et al., who reported more elevated glucagon levels in obese adolescents with normal glucose tolerance than in lean adolescents. [20]. All these data suggest that subjects with a BMI above the normal range are more predisposed to carbohydrate dysregulation.

A meta-analysis of 23 studies on the role of GIP in patients with type 2 DM revealed increased GIP secretion in subjects with high BMI after glucose or meal stimulation compared to normal weight patients [21]. In our study, we did not perform fasting oral glucose challenge (except for single subjects with impaired fasting glucose); however, we showed higher GIP levels in the whole study group and in subgroups with at least one risk factor for MetS compared to the control group. Interestingly, ALL survivors with a normal weight revealed greater levels of GIP than the control group. In contrast, we did not confirm this trend in participants with a high BMI. The results do not clearly establish the role of GIP in the development of obesity in survivors; nevertheless, they may indicate an inadequate tissue response in children and young adults with a preserved normal body weight. This may be due to the fact that GIP influences the secretion of adipokines and proinflammatory cytokines, which leads to low-grade inflammation and IR, which in turn may promote obesity and MetS [22,23]. However, the relationship between obesity and increased GIP secretion is not well established and further studies are needed.

Ghrelin has been reported to be decreased in obese children and negatively correlated with BMI [24,25]. Our results are consistent with previous findings, and demonstrated that not only the subset of subjects with abnormal BMI had significantly lower ghrelin levels, but also the subgroup with normal BMI, which may indicate a potential detrimental effect of anticancer treatment administered in childhood on ghrelin secretion later in life. This finding may also support the hypothesis that being overweight increases tissue sensitivity

to ghrelin. Moreover, the decreased levels of ghrelin have been also observed in patients reaching criteria for metabolic syndrome when compared to controls.

Leptin is a well-known peptide hormone which is mainly secreted by adipocytes and plays an important role in both energy balance and energy expenditure. Leptin level increases in obesity due to enhanced adipose tissue secretion of adipokine and decreases when body weight is reduced. Our study confirmed positive correlation with BMI and HOMA-IR in pediatric population. Furthermore, females presented with higher leptin concentrations compared to males in the study group, and female survivors had greater leptin levels than females in the control group. This finding confirmed leptin as a good predictor of obesity and MetS features [26,27].

In this study, it has been showed that ALL survivors had higher PAI-1 levels than the control group and was a good predictor of features of MetS in univariate analysis. Previous studies in humans also provide evidence that elevated PAI-1 has a direct effect on the development of insulin resistance and type 2 DM, as well as metabolic syndrome. It is possible that the cause leading to the dysfunction may be considered to be endothelial damage and its consequences [19,28–30].

Resistin is a poorly established adipokine in ALL survivors. It is known to promote insulin resistance and may be associated with abdominal fat deposition in the general population. Siviero-Miachion et al. found no association between resistin levels and CRT exposure or BMI in young survivors of ALL [31]. Our study showed no difference in resistin levels between the study and control groups. However, women had higher resistin concentrations than men. Additionally, children more than 5 years from the end of treatment demonstrated higher levels of this adipokine than those with a shorter time from the completion of treatment. As studies have shown a decrease in resistin with age, this may indicate lipid abnormalities in this group of patients.

Some studies, but not all, suggested that visfatin may be a potential predictor of insulin resistance in adults. Furthermore, recent studies have revealed that obese and type 2 DM patients presented higher visfatin levels [32,33]. This protein may play a role in contributing to insulin resistance by indicating the secretion of proinflammatory cytokines [34]. Nevertheless, we did not observe any relationship of visfatin with insulin resistance and features of MetS in ALL survivors.

Our study indicates disturbances in carbohydrate metabolism in ALL survivors, which may affect not only the quality and lifespan of this group of individuals, but also the possibility of earlier diagnosis of treatment-related late sequelae that may lead to the development of metabolic syndromes in the future. Dealing with the late effects of cancer therapy raises significant public health issues and substantial medical costs. From an ethical as well as an economic point of view, a preventive strategy is much more justified. Therefore, it is essential to determine the usefulness of new markers in the early diagnosis of complications in this group of patients, perhaps resulting in earlier implementation of preventive interventions among CCS.

In light of the results obtained in our study and the current knowledge about the increased risk of developing metabolic syndrome in ALL survivors, special attention should be paid to educating parents and children about healthy diet and physical activity after treatment. On the other hand, physicians should provide ongoing health care in the form of regular follow-up visits, including assessment of health status, and carbohydrate and lipid parameters, among others, in order to identify early abnormalities and initiate treatment as early as possible. There is still a need for uniform guidelines to identify the group at highest risk of developing MetS and specialized care for these patients. This challenge has been taken up by an international team of experts who are currently developing harmonized guidelines for diabetes and metabolic syndrome screening in childhood cancer survivors. More information is available at www.ighg.org (accessed on 15 February 2022) [35].

The results obtained should be considered in the context of limitations. The study included a small number of patients in whom total body fat mass was not assessed. Therefore, the analysis between the visceral fat percentage and the diabetes markers was not

possible. In addition, we did not include a control group of obese children, which might have increased the value of the findings.

To the best of our knowledge, this is the first study to evaluate a broad panel of diabetes markers in individuals who experienced anticancer treatment in childhood. In addition, our research includes a homogeneous group of ALL survivors, relatively long time after cessation of treatment, and no ethnic diversity.

In conclusion, the current study indicates that ALL survivors present abnormalities in carbohydrate metabolism. Subjects with features of metabolic syndrome had higher levels of C-peptide, GIP, insulin, leptin, PAI-1, and lower levels of ghrelin, whereas overweight and obese individuals had increased levels of GIP, leptin, glucagon, and decreased levels of ghrelin. This study may suggest a role of diabetes biomarkers in the pathogenesis and risk assessment of overweight and metabolic syndrome in acute lymphoblastic leukemia survivors. However, further studies are still required to establish the precise significance in pathogenesis and applicability of diabetes markers in the early diagnosis of metabolic syndrome in acute lymphoblastic leukemia survivors.

4. Materials and Methods

4.1. Study Population

Survivors of childhood acute lymphoblastic leukemia (56 patients, 30 female), who were treated at the Department of Pediatric Oncology and Hematology of the Medical University of Bialystok, were recruited for the study. All the subjects were in continuous clinical remission and participated in a follow-up visit at the clinic. The treatment was administered according to the applicable protocols for each patient using international protocols (The International Berlin-Frankfurt-Münster Group-I-BFM) approved by Polish Pediatrics Leukemia and Lymphoma Group. The written informed consent was obtained from all the participants or their parents. The study was affirmed by the Ethics Committee of the Medical University of Bialystok in accordance with the Declaration of Helsinki (permission number: APK.002.319.2021).

The mean age at the time of the study was 12.36 $\pm$ 5.15 years. The control group consisted of 22 healthy peers (8 females) at the mean age of 11.39 $\pm$ 4.25 years, with normal body weight, BMI, and fasting blood glucose.

The medical records were performed to obtain data, including age, sex, and anticancer treatment. During the follow-up visit every patient underwent a clinical examination. Anthropometric traits were collected using standard procedure and rigorously recorded. Body Mass Index was calculated as weight in kilograms divided by height in square meters (kg/m^2). The study group was separated into overweight and obese groups and a subset with BMI in a normal range based on the OLA/OLAF growth charts BMI for age and sex, in which overweight was defined as BMI values +1 standard deviation (SD), while obesity -as +2 SD [36,37]. The waist-to-height ratio (WHtR) was calculated by dividing waist circumference by height, assuming the norm to be <0.5. Blood pressure was measured using a standardized sphygmomanometer (performed three times at 1–2 min intervals); before the measurement, the participants rested peacefully for 5 min. Hypertension was defined as a mean systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) level $\geq$ 95th percentile adjusted for age, sex, and height [38]. HOMA-IR was evaluated according to the following formula: serum insulin (uIU/mL) x plasma glucose (mmol/L)/22.5. An abnormal HOMA-IR was considered to be more than 2.86 [39]. Echocardiography was performed to assess shortening fraction (SF) and ejection fraction (EF) by a pediatric cardiology specialist.

The metabolic syndrome and its components in children under 16 were defined by the International Diabetes Federation (IDF) recommendations as WC $\geq$90th centile, triglycerides $\geq$150 mg/dL, HDL-cholesterol <40 mg/dL, blood pressure $\geq$130/85 mmHg, fasting glucose $\geq$100 mg/dL. Among the participants aged 16 and older, MetS was defined by the IDF adult criteria as WC $\geq$94 cm for men and WC $\geq$80 cm for women, triglycerides

≥150 mg/dL, HDL-cholesterol <40 mg/dL for men and <50 mg/dL for women, blood pressure ≥130/85 mmHg, and fasting glucose ≥100 mg/dL [40].

4.2. Biochemical Analysis

All the laboratory tests were performed following an eight-hour overnight fast. Venous blood samples were stored at −80 °C. The enzymatic colorimetric method was used to measure biochemical parameters.

The commercially available Bio-Plex Pro Human Diabetes 10-Plex Panel kit (catalogue number 171A7001M, Bio-Rad Laboratories, Hercules, CA, USA) was performed on the serum specimens according to the manufacturers' instructions. The Bio-Plex system allows the simultaneous determination of 10 diabetes-related biomarkers in each well of a 96-well plate. The assay principle was based on the reaction with an antibody against a specific biomarker which was covalently bound to fluorescently dyed magnetic beads, each with a distinct wavelength specific for the target biomarker. The bead-conjugated antibodies reacted with the biomarker sample of interest. After the series of washes, biotinylated antibodies, optional for different epitopes of the target biomarkers, were added to the reaction. The final complex was made by adding a streptavidin-phycoerythrin (SA-PE) conjugate. A dual-laser, flow-based microplate reader -Bio-Plex 200 Reader (Bio-Rad Laboratories, Hercules, CA, USA)—detected the internal fluorescence of the individually dyed beads and the intensity of the signal on the bead surface. The obtained signal was expressed as median fluorescence intensity (MFI), analyzed, and presented as concentration (pg/mL) by the Bio-Plex Manager Software (Bio-Rad Laboratories, Hercules, CA, USA). The concentration of the biomarker attached to the individual beads was proportional to the MFI of the phycoerythrin signal.

4.3. Statistical Analysis

Statistical analysis was performed with STATA v. 12.1 (StatCorp, College Station, TX, USA). Normal distribution was examined using the Shapiro-Wilk test. The data were presented as mean ± SD, or median (Me) and interquartile range (IQR) when appropriate. The Mann-Whitney U test was used to assess the difference between two groups, while the Kruskal-Wallis test was applied to compare more than two groups without normal distribution. Analysis of the correlation between parameters was calculated by the Spearman's rank correlation coefficient. We used the receiver operating characteristic curve to establish the diagnostic values of the diabetes markers. Multivariate analysis was used to examine the association between diabetes markers and the independent variables which potentially might affect their level. A p-value less than 0.05 was defined to be statistically significant.

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References

1. Tai, E.W.; Ward, K.C.; Bonaventure, A.; Siegel, D.A.; Coleman, M.P. Survival Among Children Diagnosed With Acute Lymphoblastic Leukemia in the United States, by Race and Age, 2001 to 2009: Findings From the CONCORD-2 Study. *Cancer* **2017**, *123* (Suppl. 24), 5178–5189. [CrossRef] [PubMed]
2. Oeffinger, K.C.; Mertens, A.C.; Sklar, C.A.; Kawashima, T.; Hudson, M.M.; Meadows, A.T.; Friedman, D.L.; Marina, N.; Hobbie, W.; Kadan-Lottick, N.S.; et al. Childhood Cancer Survivor Study. Chronic Health Conditions in Adult Survivors of Childhood Cancer. *N. Engl. J. Med.* **2006**, *355*, 1572–1582. [CrossRef] [PubMed]
3. Krawczuk-Rybak, M.; Panasiuk, A.; Stachowicz-Stencel, T.; Zubowska, M.; Skalska-Sadowska, J.; Sęga-Pondel, D.; Czajńska-Deptuła, A.; Sławińska, D.; Badowska, W.; Kamieńska, E.; et al. Health status of polish children and adolescents after cancer treatment. *Eur. J. Pediatr.* **2018**, *177*, 437–447. [CrossRef] [PubMed]
4. de Lima Junior, E.A.; Yamashita, A.S.; Pimentel, G.D.; De Sousa, L.G.O.; Santos, R.V.T.; Gonçalves, C.L.; Streck, E.L.; de Lira, F.S.; Rosa Neto, J.C. Doxorubicin Caused Severe Hyperglycaemia and Insulin Resistance, Mediated by Inhibition in AMPk Signalling in Skeletal Muscle. *J. Cachexia Sarcopenia Muscle* **2016**, *7*, 615–625. [CrossRef] [PubMed]
5. Lowas, S.R.; Marks, D.; Malempati, S. Prevalence of Transient Hyperglycemia during Induction Chemotherapy for Pediatric Acute Lymphoblastic Leukemia. *Pediatr. Blood Cancer* **2009**, *52*, 814–818. [CrossRef] [PubMed]
6. Zhang, F.F.; Kelly, M.J.; Saltzman, E.; Must, A.; Roberts, S.B.; Parsons, S.K. Obesity in Pediatric ALL Survivors: A Meta-Analysis. *Pediatrics* **2014**, *133*, e704–e715. [CrossRef]
7. Zhang, F.F.; Saltzman, E.; Kelly, M.J.; Liu, S.; Must, A.; Parsons, S.K.; Roberts, S.B. Comparison of Childhood Cancer Survivors' Nutritional Intake with US Dietary Guidelines. *Pediatr. Blood Cancer* **2015**, *62*, 1461–1467. [CrossRef]
8. Pluimakers, V.G.; van Santen, S.S.; Fiocco, M.; Bakker, M.E.; van der Lelij, A.J.; van den Heuvel-Eibrink, M.M.; Neggers, S.J.C.M.M. Can Biomarkers Be Used to Improve Diagnosis and Prediction of Metabolic Syndrome in Childhood Cancer Survivors? A Systematic Review. *Obes. Rev.* **2021**, *22*, e13312. [CrossRef]
9. Vejrazkova, D.; Vankova, M.; Lukasova, P.; Vcelak, J.; Bendlova, B. Insights Into the Physiology of C-Peptide. *Physiol. Res.* **2020**, *69* (Suppl. 2), S237–S243. [CrossRef]
10. Alamri, B.N.; Shin, K.; Chappe, V.; Anini, Y. The Role of Ghrelin in the Regulation of Glucose Homeostasis. *Horm. Mol. Biol. Clin. Investig.* **2016**, *26*, 3–11. [CrossRef]
11. Kolb, H.; Kempf, K.; Röhling, M.; Martin, S. Insulin: Too Much of a Good Thing Is Bad. *BMC Med.* **2020**, *18*, 224. [CrossRef] [PubMed]
12. Kulina, G.R.; Rayfield, E.J. The role of glucagon in the pathophysiology and management of diabetes. *Endocr. Pract.* **2016**, *22*, 612–621. [CrossRef] [PubMed]
13. Nauck, M.A.; Quast, D.R.; Wefers, J.; Pfeiffer, A.F.H. The Evolving Story of Incretins (GIP and GLP-1) in Metabolic and Cardiovascular Disease: A Pathophysiological Update. *Diabetes Obes. Metab.* **2021**, *23* (Suppl. 3), 5–29. [CrossRef] [PubMed]
14. Recinella, L.; Orlando, G.; Ferrante, C.; Chiavaroli, A.; Brunetti, L.; Leone, S. Adipokines: New Potential Therapeutic Target for Obesity and Metabolic, Rheumatic, and Cardiovascular Diseases. *Front. Physiol.* **2020**, *11*, 578966. [CrossRef] [PubMed]
15. Atawia, R.T.; Bunch, K.L.; Toque, H.A.; Caldwell, R.B.; Caldwell, R.W. Mechanisms of Obesity-Induced Metabolic and Vascular Dysfunctions. *Front. Biosci. (Landmark Ed.)* **2019**, *24*, 890–934.
16. Park, H.K.; Kwak, M.K.; Kim, H.J.; Ahima, R.S. Linking Resistin, Inflammation, and Cardiometabolic Diseases. *Korean J. Intern. Med.* **2017**, *32*, 239–247. [CrossRef] [PubMed]
17. Williams, H.E.; Howell, C.R.; Chemaitilly, W.; Wilson, C.L.; Karol, S.E.; Nolan, V.; Smeltzer, M.P.; Green, D.M.; Ehrhardt, M.J.; Mulrooney, D.A.; et al. Diabetes Mellitus among Adult Survivors of Childhood Acute Lymphoblastic Leukemia: A Report from the St. Jude Lifetime Cohort Study. *Cancer* **2020**, *126*, 870–878. [CrossRef] [PubMed]
18. Cabrera de León, A.; Oliva García, J.G.; Marcelino Rodríguez, I.; Almeida González, D.; Alemán Sánchez, J.J.; Brito Díaz, B.; Domínguez Coello, S.; Bertomeu Martínez, V.; Aguirre Jaime, A.; Rodríguez Pérez, M. C-Peptide as a Risk Factor of Coronary Artery Disease in the General Population. *Diabetes Vasc. Dis. Res.* **2015**, *12*, 199–207. [CrossRef]
19. Morel, S.; Léveillé, P.; Samoilenko, M.; Franco, A.; England, J.; Malaquin, N.; Tu, V.; Cardin, G.B.; Drouin, S.; Rodier, F.; et al. Biomarkers of Cardiometabolic Complications in Survivors of Childhood Acute Lymphoblastic Leukemia. *Sci. Rep.* **2020**, *10*, 21507. [CrossRef]
20. Manell, H.; Staaf, J.; Manukyan, L.; Kristinsson, H.; Cen, J.; Stenlid, R.; Ciba, I.; Forslund, A.; Bergsten, P. Altered Plasma Levels of Glucagon, GLP-1 and Glicentin During OGTT in Adolescents With Obesity and Type 2 Diabetes. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 1181–1189. [CrossRef]
21. Calanna, S.; Christensen, M.; Holst, J.J.; Laferrère, B.; Gluud, L.L.; Vilsbøll, T.; Knop, F.K. Secretion of Glucose-Dependent Insulinotropic Polypeptide in Patients with Type 2 Diabetes: Systematic Review and Meta-Analysis of Clinical Studies. *Diabetes Care* **2013**, *36*, 3346–3352. [CrossRef] [PubMed]
22. Timper, K.; Grisouard, J.; Sauter, N.S.; Herzog-Radimerski, T.; Dembinski, K.; Peterli, R.; Frey, D.M.; Zulewski, H.; Keller, U.; Müller, B.; et al. Glucose-Dependent Insulinotropic Polypeptide Induces Cytokine Expression, Lipolysis, and Insulin Resistance in Human Adipocytes. *Am. J. Physiol. Endocrinol. Metab.* **2013**, *304*, E1–E13. [CrossRef] [PubMed]
23. Chen, S.; Okahara, F.; Osaki, N.; Shimotoyodome, A. Increased GIP Signaling Induces Adipose Inflammation via a HIF-1α-Dependent Pathway and Impairs Insulin Sensitivity in Mice. *Am. J. Physiol. Endocrinol. Metab.* **2015**, *308*, E414–E425. [CrossRef] [PubMed]

24. Reinehr, T.; Sousa, G.D.; Roth, C.L. Obestatin and Ghrelin Levels in Obese Children and Adolescents before and after Reduction of Overweight. *Clin. Endocrinol.* **2008**, *68*, 304–310. [CrossRef] [PubMed]
25. Fittipaldi, A.S.; Hernández, J.; Castrogiovanni, D.; Lufrano, D.; Francesco, P.N.D.; Garrido, V.; Vitaux, P.; Fasano, M.V.; Fehrentz, J.-A.; Fernández, A.; et al. Plasma Levels of Ghrelin, Des-Acyl Ghrelin and LEAP2 in Children with Obesity: Correlation with Age and Insulin Resistance. *Eur. J. Endocrinol.* **2020**, *182*, 165–175. [CrossRef]
26. Sawicka-Żukowska, M.; Krawczuk-Rybak, M.; Muszynska-Roslan, K.; Panasiuk, A.; Latoch, E.; Konstantynowicz, J. Does Q223R Polymorphism of Leptin Receptor Influence on Anthropometric Parameters and Bone Density in Childhood Cancer Survivors? *Int. J. Endocrinol.* **2013**, *2013*, 805312. [CrossRef]
27. Latoch, E.; Muszynska-Roslan, K.; Panas, A.; Panasiuk, A.; Sawicka-Zukowska, M.; Zelazowska-Rutkowska, B.; Zabrocka, E.; Krawczuk-Rybak, M. Adipokines and Insulin Resistance in Young Adult Survivors of Childhood Cancer. *Int. J. Endocrinol.* **2016**, *2016*, 6349134. [CrossRef]
28. Brouwer, C.A.J.; Postma, A.; Hooimeijer, H.L.H.; Smit, A.J.; Vonk, J.M.; van Roon, A.M.; van den Berg, M.P.; Dolsma, W.V.; Lefrandt, J.D.; Bink-Boelkens, M.T.E.; et al. Endothelial Damage in Long-Term Survivors of Childhood Cancer. *JCO* **2013**, *31*, 3906–3913. [CrossRef]
29. Altalhi, R.; Pechlivani, N.; Ajjan, R.A. PAI-1 in Diabetes: Pathophysiology and Role as a Therapeutic Target. *Int. J. Mol. Sci.* **2021**, *22*, 3170. [CrossRef]
30. Vecchiola, A.; García, K.; González-Gómez, L.M.; Tapia-Castillo, A.; Artigas, R.; Baudrand, R.; Kalergis, A.M.; Carvajal, C.A.; Fardella, C.E. Plasminogen Activator Inhibitor-1 and Adiponectin Are Associated With Metabolic Syndrome Components. *Am. J. Hypertens.* **2021**, No. hpab138. [CrossRef]
31. Siviero-Miachon, A.A.; Spinola-Castro, A.M.; Andreoni, S.; de Martino Lee, M.L.; Calixto, A.R.; Geloneze, B.; Guerra-Junior, G. Adipokines in Young Survivors of Childhood Acute Lymphocytic Leukemia Revisited: Beyond Fat Mass. *Ann. Pediatr. Endocrinol. Metab.* **2020**, *25*, 174–181. [CrossRef]
32. Sandeep, S.; Velmurugan, K.; Deepa, R.; Mohan, V. Serum Visfatin in Relation to Visceral Fat, Obesity, and Type 2 Diabetes Mellitus in Asian Indians. *Metabolism* **2007**, *56*, 565–570. [CrossRef]
33. Yin, C.; Hu, W.; Wang, M.; Xiao, Y. The Role of the Adipocytokines Vaspin and Visfatin in Vascular Endothelial Function and Insulin Resistance in Obese Children. *BMC Endocr. Disord.* **2019**, *19*, 127. [CrossRef]
34. Moschen, A.R.; Kaser, A.; Enrich, B.; Mosheimer, B.; Theurl, M.; Niederegger, H.; Tilg, H. Visfatin, an Adipocytokine with Proinflammatory and Immunomodulating Properties. *J. Immunol.* **2007**, *178*, 1748–1758. [CrossRef]
35. Friedman, D.N.; Tonorezos, E.S.; Cohen, P. Diabetes and Metabolic Syndrome in Survivors of Childhood Cancer. *Horm. Res. Paediatr.* **2019**, *91*, 118–127. [CrossRef]
36. Kułaga, Z.; Litwin, M.; Tkaczyk, M.; Palczewska, I.; Zajączkowska, M.; Zwolińska, D.; Krynicki, T.; Wasilewska, A.; Moczulska, A.; Morawiec-Knysak, A.; et al. Polish 2010 growth references for school-aged children and adolescents. *Eur. J. Pediatr.* **2011**, *170*, 599–609. [CrossRef]
37. Kułaga, Z.; Grajda, A.; Gurzkowska, B.; Góźdź, M.; Wojtyło, M.; Świąder, A.; Różdżyńska-Świątkowska, A.; Litwin, M. Polish 2012 growth references for preschool children. *Eur. J. Pediatr.* **2013**, *172*, 753–761. [CrossRef]
38. Kułaga, Z.; Litwin, M.; Grajda, A.; Gurzkowska, B.; Napieralska, E.; Kułaga, K.; Grupa Badaczy, O.L.A.F. Distribution of blood pressure in school-aged children and adolescents reference population. *Stand. Med.* **2010**, *7*, 853–864.
39. Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F.; Turner, R.C. Homeostasis Model Assessment: Insulin Resistance and Beta-Cell Function from Fasting Plasma Glucose and Insulin Concentrations in Man. *Diabetologia* **1985**, *28*, 412–419. [CrossRef]
40. Zimmet, P.; Alberti, K.G.M.; Kaufman, F.; Tajima, N.; Silink, M.; Arslanian, S.; Wong, G.; Bennett, P.; Shaw, J.; Caprio, S. The metabolic syndrome in children and adolescents—an IDF consensus report. *Pediatric Diabetes* **2007**, *8*, 299–306. [CrossRef]

6. STRESZCZENIE

W ostatnich latach nastąpił znaczący rozwój diagnostyki i terapii nowotworów wieku dziecięcego, zwiększając 5-letnie przeżycie do około 80% w krajach rozwiniętych. Skutki uboczne intensywnego leczenia, obejmującego chemioterapię, radioterapię i zabiegi chirurgiczne, prowadzą do licznych powikłań zdrowotnych. U około 60% pacjentów występuje przynajmniej jedno przewlekłe schorzenie, a u 27,5% zagrażające życiu. Wczesne wykrywanie powikłań, szczególnie zaburzeń metabolicznych, jest kluczowe dla poprawy jakości i długości życia ozdrowieńców. Coraz większą uwagę poświęca się poszukiwaniu markerów wczesnych zaburzeń metabolizmu lipidów i węglowodanów, które pozwolą na diagnostykę chorób sercowo-naczyniowych i zmniejszenie śmiertelności w tej grupie pacjentów.

Celem pracy była analiza zależności wybranych markerów gospodarki lipidowej i węglowodanowej z występowaniem nadwagi, otyłości i czynników zespołu metabolicznego u pacjentów po zakończonym leczeniu przeciwnowotworowym w dzieciństwie. W pierwszej publikacji oceniono stężenia adipocytarnego i epidermalngo białka wiążącego kwasy tłuszczowe (A-FABP, E-FABP) po zakończonym leczeniu ostrej białaczki limfoblastycznej (ALL) w dzieciństwie. Natomiast w drugiej publikacji zbadano zależność pomiędzy wybranymi markerami gospodarki węglowodanowej (C-peptyd, grelina, GIP, glukagon, insulina, PAI-1, rezystyna, leptyna i wisfatyna) a wstępowaniem insulinooporności i czynników zespołu metabolicznego u ozdrowieńców ALL.

Badania przeprowadzono w dwóch grupach pacjentów: grupa 1: 62 pacjentów (śr. wiek w dniu badania 12.41 ± 4.98 lat); grupa 2: 56 pacjentów (śr. wiek w dniu badania 12.36 ± 5.15 lat) leczonych z powodu ALL w dzieciństwie. Nadwaga i otyłość w grupie badanej została określona na podstawie wartość BMI zgodnie z siatkami centylowymi OLA/OLAF dla wieku i płci. Wskaźnik HOMA-IR został wyliczony zgodnie ze wzorem: stężenie insuliny (μIU/mL) x stężenie glukozy (mmol/L) / 22.5. Czynniki zespołu metabolicznego dla dzieci poniżej 16 roku życia zostały zdefiniowane na podstawie wytycznych IDF: obwód talii (WC) ≥ 90c, trójglicerydy (TG) ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL. Pacjenci w wieku 16 lat lub starsi zostali ocenieni według wytycznych IDF dla dorosłych: WC ≥ 94 cm dla mężczyzn and WC ≥ 80 cm dla kobiet, TG ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL dla mężczyzn i <

50 mg/dL dla kobiet, ciśnienie krwi ≥ 130/85 mmHg, stężenie glukozy na czczo ≥ 100 mg/dL. Stężenie A-FABP i E-FABP w surowicy oceniono za pomocą dostępnego zestawu ELISA (BioVendor Laboratorni Medicina a.s., Brno, Czech Republic), natomiast stężenie wybranych markerów gospodarki węglowodanowej oceniono za pomocą dostępnego pakietu Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA, USA). Oba badania uzyskały zgodę Komisji Bioetycznej Uniwersytetu Medycznego w Białymstoku.

Grupa badana prezentowała wyższe stężenia A-FABP ($p < 0.001$) w porównaniu do grupy kontrolnej, natomiast stężenie E-FABP ($p = 0.325$) nie różniło się istotnie statystycznie w powyższych grupach. Pacjenci z nadwagą i otyłością wykazywali wyższe stężenia A-FABP ($p = 0.006$) w porównaniu do grupy z prawidłowym BMI. W grupie badanej 53.23% pacjentów spełniało przynajmniej jeden czynnik zespołu metabolicznego. Dzieci po zakończonym leczeniu ze spełnionymi przynajmniej dwoma czynnikami MetS prezentowały wyższe stężenia A-FABP ($p = 0.018$) i E-FABP ($p = 0.026$) w porównaniu do grupy bez spełnionych kryteriów MetS, jak i w porównaniu do grupy kontrolnej (A-FABP - $p = 0.001$; E-FABP - $p = 0.021$). W drugim badaniu grupa badana wykazywała wyższe stężenie GIP ($p = 0.026$), glukagonu ($p = 0.001$), leptyny ($p = 0.022$) i PAI-1 ($p = 0.047$) oraz niższe stężenie greliny ($p < 0.001$) w porównaniu do grupy kontrolnej. Dzieci leczone z powodu ALL z nadwagą i otyłością prezentowały wyższe stężenia glukagonu ($p = 0.006$) i leptyny ($p = 0.034$) niż pacjenci z prawidłowym BMI. Pacjenci powyżej pięciu lat od zakończonego leczenia wykazywali wyższe stężenia PAI-1 ($p < 0.001$) i rezystyny ($p = 0.002$) w porównaniu do krótszego czasu obserwacji. W analizie porównującej pacjentów ze spełnionym przynajmniej jednym kryterium MetS z grupą bez spełnionych kryteriów MetS, zaobserwowano wyższe stężenia C-peptydu ($p = 0.028$), leptyny ($p = 0.003$) i PAI-1 ($p = 0.034$) w grupie z czynnikami MetS. Nieprawidłową wartość HOMA-IR wykazało 10.7% pacjentów, prezentowali oni wyższe stężenia C-peptydu ($p = 0.005$), glukagonu ($p = 0.042$) i leptyny ($p = 0.016$) w porównaniu do podgrupy z prawidłowym HOMA-IR. Glukagon (AUC 0.71; $p = 0.003$) i leptyna (AUC 0.67; $p = 0.026$) okazały się najlepszymi predyktorami nadwagi i otyłości w grupie badanej.

Pacjenci po zakończonym leczeniu ALL w dzieciństwie wykazują zaburzenia w gospodarce lipidowej i węglowodanowej, co może zwiększać ryzyko rozwoju zespołu metabolicznego i chorób sercowo-naczyniowych w późniejszym życiu.

Nadwaga i otyłość przyczyniają się do wzrostu ryzyka zaburzeń metabolicznych w tej grupie pacjentów. W związku z tym, niezbędne jest regularne monitorowanie ozdrowieńców pod kątem zaburzeń metabolicznych, aby umożliwić wczesną interwencję i zapobieganie długoterminowym powikłaniom zdrowotnym.

7. SUMMARY

In recent years, there has been significant improvement in the diagnosis and treatment of childhood cancers, increasing 5-year survival rates to around 80% in developed countries. However, the side effects of intensive therapies, including chemotherapy, radiotherapy, and surgical interventions, have resulted in numerous health complications. About 60% of survivors experience at least one chronic condition, and 27.5% face life-threatening complications. Early detection of disturbances, particularly metabolic disorders, is crucial for improving survivors' quality of life and lifespan. Increasing attention is being given to identifying early biomarkers for lipid and carbohydrate metabolism disorders, which could enable the diagnosis of cardiovascular diseases and reduce mortality in this patient group.

The aim of this study was to analyze the relationship between selected markers of lipid and carbohydrate metabolism with the occurrence of overweight, obesity, and metabolic syndrome factors in patients who have completed childhood cancer treatment. The first publication evaluated the concentrations of adipocyte and epidermal fatty acid binding proteins (A-FABP, E-FABP) after the completion of treatment for acute lymphoblastic leukemia (ALL) in childhood. The second publication investigated the relationship between selected carbohydrate metabolism markers (C-peptide, ghrelin, GIP, glucagon, insulin, PAI-1, resistin, leptin, and visfatin) and the presence of insulin resistance and metabolic syndrome factors in ALL survivors.

The study was conducted in two groups of patients: Group 1 consisted of 62 patients (mean age: 12.41 ± 4.98 years), and Group 2 included 56 patients (mean age: 12.36 ± 5.15 years) who had been treated for ALL in childhood. Overweight and obesity in the study group were determined based on BMI values according to the OLA/OLAF percentile charts for age and sex. HOMA-IR was calculated using the formula: insulin concentration (μIU/mL) × glucose concentration (mmol/L) / 22.5. Metabolic syndrome factors for children under 16 years old were defined according to the IDF guidelines: waist circumference (WC) ≥ 90th percentile, triglycerides (TG) ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL, blood pressure ≥ 130/85 mmHg, fasting glucose concentration ≥ 100 mg/dL. Patients aged 16 years or older were assessed according to the IDF guidelines for adults: WC ≥ 94 cm for men and WC ≥ 80 cm for women, TG ≥ 150 mg/dL, HDL-cholesterol < 40 mg/dL for men and < 50 mg/dL for women, blood

pressure ≥ 130/85 mmHg, fasting glucose concentration ≥ 100 mg/dL. Serum concentrations of A-FABP and E-FABP were assessed using an ELISA kit (BioVendor Laboratorni Medicina a.s., Brno, Czech Republic). In contrast, concentrations of selected carbohydrate metabolism markers were evaluated using the Bio-Plex Pro Human Diabetes 10-Plex Panel (Bio-Rad Laboratories, Hercules, CA, USA). Ethical approval for both studies was obtained from the Bioethics Committee of the Medical University of Białystok.

The study group showed higher concentrations of A-FABP ($p < 0.001$) compared to the control group, while the concentration of E-FABP ($p = 0.325$) did not differ between the two groups. Overweight and obese patients had higher A-FABP concentrations ($p = 0.006$) compared to those with normal BMI. In the study group, 53.23% of patients met at least one criterion for metabolic syndrome. Children with at least two metabolic syndrome factors presented higher concentrations of A-FABP ($p = 0.018$) and E-FABP ($p = 0.026$) compared to those without metabolic syndrome criteria, as well as compared to the control group (A-FABP: $p = 0.001$; E-FABP: $p = 0.021$). In the second study, the study group exhibited higher concentrations of GIP ($p = 0.026$), glucagon ($p = 0.001$), leptin ($p = 0.022$), and PAI-1 ($p = 0.047$), and lower concentrations of ghrelin ($p < 0.001$) compared to the control group. Overweight and obese children treated for ALL showed higher concentrations of glucagon ($p = 0.006$) and leptin ($p = 0.034$) than patients with normal BMI. Patients more than five years after anticancer treatment exhibited higher concentrations of PAI-1 ($p < 0.001$) and resistin ($p = 0.002$) compared to those with shorter follow-up times. In the comparison of patients with at least one metabolic syndrome criterion to those without, higher concentrations of C-peptide ($p = 0.028$), leptin ($p = 0.003$), and PAI-1 ($p = 0.034$) were observed in the group with metabolic syndrome factors. Abnormal HOMA-IR was observed in 10.7% of patients, who showed higher concentrations of C-peptide ($p = 0.005$), glucagon ($p = 0.042$), and leptin ($p = 0.016$) compared to the subgroup with normal HOMA-IR. Glucagon (AUC 0.71; $p = 0.003$) and leptin (AUC 0.67; $p = 0.026$) were identified as the best predictors of overweight and obesity in the study group.

Patients who have completed treatment for ALL in childhood exhibit disturbances in lipid and carbohydrate metabolism, which increases the risk of developing metabolic syndrome and cardiovascular diseases later in life. Overweight and obesity contribute to an increased risk of metabolic disorders in this patient group. Therefore, regularly monitoring patients after childhood anticancer treatment for

metabolic disorders is essential to enable early intervention and prevent long-term health complications.

8. PIŚMIENNICTWO

1. Krawczuk-Rybak, Maryna, Anna Panasiuk, Teresa Stachowicz-Stencel, Małgorzata Zubowska, Jolanta Skalska-Sadowska, Dorota Sęga-Pondel, Aneta Czajńska-Deptuła, et al. "Health Status of Polish Children and Adolescents after Cancer Treatment." *European Journal of Pediatrics* 177, no. 3 (March 2018): 437–47. Doi: 10.1007/s00431-017-3066-x.
2. Wang, Xiaoyan, Derek S. Brown, Yin Cao, Christine C. Ekenga, Shenyang Guo, and Kimberly J. Johnson. "Disparities in Survival Improvement for U.S. Childhood and Adolescent Cancer between 1995 and 2019: An Analysis of Population-Based Data." *Cancer Epidemiology* 85 (August 2023): 102380. Doi: 10.1016/j.canep.2023.102380.
3. Ward, Elizabeth, Carol DeSantis, Anthony Robbins, Betsy Kohler, and Ahmedin Jemal. "Childhood and Adolescent Cancer Statistics, 2014." *CA: A Cancer Journal for Clinicians* 64, no. 2 (2014): 83–103. Doi: 10.3322/caac.21219.
4. Oeffinger, Kevin C., Ann C. Mertens, Charles A. Sklar, Toana Kawashima, Melissa M. Hudson, Anna T. Meadows, Debra L. Friedman, et al. "Chronic Health Conditions in Adult Survivors of Childhood Cancer." *The New England Journal of Medicine* 355, no. 15 (October 12, 2006): 1572–82. Doi: 10.1056/NEJMsa060185.
5. Chemaitilly, Wassim, Laurie E. Cohen, Sogol Mostoufi-Moab, Briana C. Patterson, Jill H. Simmons, Lillian R. Meacham, Hanneke M. van Santen, and Charles A. Sklar. "Endocrine Late Effects in Childhood Cancer Survivors." *Journal of Clinical Oncology: Official Journal of the American Society of Clinical Oncology* 36, no. 21 (July 20, 2018): 2153–59. Doi: 10.1200/JCO.2017.76.3268.
6. Lowas, Stefanie R., Daniel Marks, and Suman Malempati. "Prevalence of Transient Hyperglycemia during Induction Chemotherapy for Pediatric Acute Lymphoblastic Leukemia." *Pediatric Blood & Cancer* 52, no. 7 (July 2009): 814–18. Doi: 10.1002/pbc.21980.
7. Lima Junior, Edson Alves de, Alex Shimura Yamashita, Gustavo Duarte Pimentel, Luís G. O. De Sousa, Ronaldo Vagner T. Santos, Cinara Ludvig Gonçalves, Emilio Luiz Streck, Fábio Santos de Lira, and Jose Cesar Rosa Neto.

"Doxorubicin Caused Severe Hyperglycaemia and Insulin Resistance, Mediated by Inhibition in AMPk Signalling in Skeletal Muscle." *Journal of Cachexia, Sarcopenia and Muscle* 7, no. 5 (December 2016): 615–25. Doi: 10.1002/jcsm.12104.

8. Choi, Kyung M., Mary Yannakoulia, Min S. Park, Geum J. Cho, Jung H. Kim, Seung H. Lee, Taik G. Hwang, et al. "Serum Adipocyte Fatty Acid-Binding Protein, Retinol-Binding Protein 4, and Adiponectin Concentrations in Relation to the Development of the Metabolic Syndrome in Korean Boys: A 3-y Prospective Cohort Study." *The American Journal of Clinical Nutrition* 93, no. 1 (January 2011): 19–26. Doi: 10.3945/ajcn.2010.29667.
9. Krzystek-Korpacka, Malgorzata, Eliza Patryn, Iwona Bednarz-Misa, Magdalena Mierzchala, Katarzyna Hotowy, Elzbieta Czapinska, Irena Kustrzeba-Wojcicka, Andrzej Gamian, and Anna Noczynska. "Circulating Adipocyte Fatty Acid-Binding Protein, Juvenile Obesity, and Metabolic Syndrome." *Journal of Pediatric Endocrinology & Metabolism: JPEM* 24, no. 11–12 (2011): 921–28. Doi: 10.1515/jpem.2011.323.
10. Yeung, Dennis C. Y., Yu Wang, Aimin Xu, Stephen C. W. Cheung, Nelson M. S. Wat, Daniel Y. T. Fong, Carol H. Y. Fong, M. T. Chau, Pak C. Sham, and Karen S. L. Lam. "Epidermal Fatty-Acid-Binding Protein: A New Circulating Biomarker Associated with Cardio-Metabolic Risk Factors and Carotid Atherosclerosis." *European Heart Journal* 29, no. 17 (September 2008): 2156–63. Doi: 10.1093/eurheartj/ehn295.
11. Ishimura, Shutaro, Masato Furuhashi, Yuki Watanabe, Kyoko Hoshina, Takahiro Fuseya, Tomohiro Mita, Yusuke Okazaki, et al. "Circulating Levels of Fatty Acid-Binding Protein Family and Metabolic Phenotype in the General Population." *PloS One* 8, no. 11 (2013): e81318. Doi: 10.1371/journal.pone.0081318.
12. Vejrazkova, D., M. Vankova, P. Lukasova, J. Vcelak, and B. Bendlova. "Insights into the Physiology of C-Peptide." *Physiological Research* 69, no. Suppl 2 (September 30, 2020): S237–43. Doi: 10.33549/physiolres.934519.
13. Reinehr, Thomas, Gideon de Sousa, and Christian L. Roth. "Obestatin and Ghrelin Levels in Obese Children and Adolescents before and after Reduction of Overweight." *Clinical Endocrinology* 68, no. 2 (February 2008): 304–10. Doi: 10.1111/j.1365-2265.2007.03042.x.

14. Alamri, Bader N., Kyungsoo Shin, Valerie Chappe, and Younes Anini. “The Role of Ghrelin in the Regulation of Glucose Homeostasis.” *Hormone Molecular Biology and Clinical Investigation* 26, no. 1 (April 1, 2016): 3–11. Doi: 10.1515/hmbci-2016-0018.
15. Fittipaldi, Antonela S., Julieta Hernández, Daniel Castrogiovanni, Daniela Lufrano, Pablo N. De Francesco, Verónica Garrido, Patrick Vitaux, et al. “Plasma Levels of Ghrelin, Des-Acyl Ghrelin and LEAP2 in Children with Obesity: Correlation with Age and Insulin Resistance.” *European Journal of Endocrinology* 182, no. 2 (February 2020): 165–75. Doi: 10.1530/EJE-19-0684.
16. Kulina, Georgia R., and Elliot J. Rayfield. “THE ROLE OF GLUCAGON IN THE PATHOPHYSIOLOGY AND MANAGEMENT OF DIABETES.” *Endocrine Practice: Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists* 22, no. 5 (May 2016): 612–21. Doi: 10.4158/EP15984.RA.
17. Manell, Hannes, Johan Staaf, Levon Manukyan, Hjalti Kristinsson, Jing Cen, Rasmus Stenlid, Iris Ciba, Anders Forslund, and Peter Bergsten. “Altered Plasma Levels of Glucagon, GLP-1 and Glicentin During OGTT in Adolescents With Obesity and Type 2 Diabetes.” *The Journal of Clinical Endocrinology and Metabolism* 101, no. 3 (March 2016): 1181–89. Doi: 10.1210/jc.2015-3885.
18. Kolb, Hubert, Kerstin Kempf, Martin Röhling, and Stephan Martin. “Insulin: Too Much of a Good Thing Is Bad.” *BMC Medicine* 18, no. 1 (August 21, 2020): 224. Doi: 10.1186/s12916-020-01688-6.
19. Timper, Katharina, Jean Grisouard, Nadine S. Sauter, Tanja Herzog-Radimerski, Kaethi Dembinski, Ralph Peterli, Daniel M. Frey, et al. “Glucose-Dependent Insulinotropic Polypeptide Induces Cytokine Expression, Lipolysis, and Insulin Resistance in Human Adipocytes.” *American Journal of Physiology. Endocrinology and Metabolism* 304, no. 1 (January 1, 2013): E1-13. Doi: 10.1152/ajpendo.00100.2012.
20. Nauck, Michael A., Daniel R. Quast, Jakob Wefers, and Andreas F. H. Pfeiffer. “The Evolving Story of Incretins (GIP and GLP-1) in Metabolic and Cardiovascular Disease: A Pathophysiological Update.” *Diabetes, Obesity & Metabolism* 23 Suppl 3 (September 2021): 5–29. Doi: 10.1111/dom.14496.
21. Morel, Sophia, Pauline Léveillé, Mariia Samoilenko, Anita Franco, Jade England, Nicolas Malaquin, Véronique Tu, et al. “Biomarkers of

Cardiometabolic Complications in Survivors of Childhood Acute Lymphoblastic Leukemia." *Scientific Reports* 10, no. 1 (December 9, 2020): 21507. Doi: 10.1038/s41598-020-78493-x.

22. Vecchiola, Andrea, Killén García, Luis M. González-Gómez, Alejandra Tapia-Castillo, Rocío Artigas, René Baudrand, Alexis M. Kalergis, Cristian A. Carvajal, and Carlos E. Fardella. "Plasminogen Activator Inhibitor-1 and Adiponectin Are Associated With Metabolic Syndrome Components." *American Journal of Hypertension* 35, no. 4 (April 2, 2022): 311–18. Doi: 10.1093/ajh/hpab138.
23. Siviero-Miachon, Adriana Aparecida, Angela Maria Spinola-Castro, Solange Andreoni, Maria Lucia de Martino Lee, Antonio Ramos Calixto, Bruno Geloneze, and Gil Guerra-Junior. "Adipokines in Young Survivors of Childhood Acute Lymphocytic Leukemia Revisited: Beyond Fat Mass." *Annals of Pediatric Endocrinology & Metabolism* 25, no. 3 (September 2020): 174–81. Doi: 10.6065/apem.1938174.087.
24. Latoch, Eryk, Katarzyna Muszynska-Roslan, Agata Panas, Anna Panasiuk, Malgorzata Sawicka-Zukowska, Beata Zelazowska-Rutkowska, Ewa Zabrocka, and Maryna Krawczuk-Rybak. "Adipokines and Insulin Resistance in Young Adult Survivors of Childhood Cancer." *International Journal of Endocrinology* 2016 (2016): 6349134. Doi: 10.1155/2016/6349134.
25. Moschen, Alexander R., Arthur Kaser, Barbara Enrich, Birgit Mosheimer, Milan Theurl, Harald Niederegger, and Herbert Tilg. "Visfatin, an Adipocytokine with Proinflammatory and Immunomodulating Properties." *Journal of Immunology (Baltimore, Md.: 1950)* 178, no. 3 (February 1, 2007): 1748–58. Doi: 10.4049/jimmunol.178.3.1748.
26. Yin, Chunyan, Wei Hu, Ming Wang, and Yanfeng Xiao. "The Role of the Adipocytokines Vaspin and Visfatin in Vascular Endothelial Function and Insulin Resistance in Obese Children." *BMC Endocrine Disorders* 19, no. 1 (November 26, 2019): 127. Doi: 10.1186/s12902-019-0452-6.
27. Kułaga, Zbigniew, Mieczysław Litwin, Marcin Tkaczyk, Iwona Palczewska, Małgorzata Zajączkowska, Danuta Zwolińska, Tomasz Krynicki, et al. "Polish 2010 Growth References for School-Aged Children and Adolescents." *European Journal of Pediatrics* 170, no. 5 (May 2011): 599–609. Doi: 10.1007/s00431-010-1329-x.

28. Kułaga, Zbigniew, Aneta Grajda, Beata Gurzkowska, Magdalena Góźdź, Małgorzata Wojtyło, Anna Swiąder, Agnieszka Różdżyńska-Świątkowska, and Mieczysław Litwin. “Polish 2012 Growth References for Preschool Children.” *European Journal of Pediatrics* 172, no. 6 (June 2013): 753–61. Doi: 10.1007/s00431-013-1954-2.
29. Zimmet, Paul, K. George Mm Alberti, Francine Kaufman, Naoko Tajima, Martin Silink, Silva Arslanian, Gary Wong, et al. “The Metabolic Syndrome in Children and Adolescents - an IDF Consensus Report.” *Pediatric Diabetes* 8, no. 5 (October 2007): 299–306. Doi: 10.1111/j.1399-5448.2007.00271.x.

9. INFORMACJE O CHARAKTERZE UDZIAŁU WSPÓŁAUTORÓW W PUBLIKACJACH

Konończuk, Katarzyna, Eryk Latoch, Beata Żelazowska-Rutkowska, Maryna Krawczuk-Rybak, and Katarzyna Muszyńska-Rosłan. "Increased Levels of Adipocyte and Epidermal Fatty Acid-Binding Proteins in Acute Lymphoblastic Leukemia Survivors." *Journal of Clinical Medicine* 10, no. 8 (April 8, 2021): 1567. DOI: 10.3390/jcm10081567

Imię i nazwisko współautora	Charakter udziału
Lek. Katarzyna Konończuk	Planowanie badania Rekrutowanie pacjentów Zebranie i interpretacja danych Przeprowadzenie analizy statystycznej Tworzenie manuskryptu
Dr hab. n. med. Eryk Latoch	Planowanie badania Rekrutowanie pacjentów Współtworzenie manuskryptu Akceptacja ostatecznej wersji do publikacji
Dr hab. n. med. Beata Żelazowska-Rutkowska	Przeprowadzenie oznaczeń
Prof. dr hab. n. med. Maryna Krawczuk-Rybak	Nadzorowanie procesu powstawania publikacji Akceptacja ostatecznej wersji do publikacji
Prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan	Nadzorowanie procesu powstawania publikacji Akceptacja ostatecznej wersji do publikacji

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w pracy doktorskiej lek. Katarzyny Konończuk

Katarzyna Konończuk

Podpis kandydata

Potwierdzam opisany powyżej merytoryczny wkład kandydata w powstanie publikacji wchodzącej w skład rozprawy doktorskiej.

Podpis promotora

K. Muszyńska-Rosłan

Białystok, 20.08.2024 r.

Dr hab. n. med. Eryk Latoch
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji: **„Increased Levels of Adipocyte and Epidermal Fatty Acid-Binding Proteins in Acute Lymphoblastic Leukemia Survivors"** autorów Katarzyna Konończuk, Eryk Latoch, Beata Żelazowska-Rutkowska, Maryna Krawczuk-Rybak, Katarzyna Muszyńska-Rosłan, opublikowanej w *Journal of Clinical Medicine,* wchodzącej w skład rozprawy doktorskiej **„Ocena stężenia wybranych markerów gospodarki węglowodanowej i lipidowej oraz ich związek z wystąpieniem nadwagi i otyłości u dzieci i młodzieży po leczeniu przeciwnowotworowym."** polegał na planowaniu badania, rekrutowaniu pacjentów, współtworzeniu manuskryptu oraz akceptacji ostatecznej wersji manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez Lek. Katarzynę Konończuk publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

dr hab. n. med. Eryk Latoch
specjalista pediatrii, onkologii
i hematologii dziecięcej
3632652

Białystok, 23.08.2024 r.

Dr hab. n. med. Beata Żelazowska-Rutkowska
Zakład Laboratoryjnej Diagnostyki Pediatrycznej
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

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Dr hab. n. med. Beata Żelazowska-Rutkowska
DIAGNOSTA LABORATORYJNY
Specjalista laboratoryjnej diagnostyki pediatrycznej
05893

Białystok, 20.08.2024 r.

Prof. dr hab. n. med. Maryna Krawczuk-Rybak
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

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KIEROWNIK
Kliniki Pediatrii, Onkologii i Hematologii
.....
prof. zw. dr hab. n. med. Maryna Krawczuk-Rybak

Białystok, 16.08.2024 r.

Prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
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15-089 Białystok

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Prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan
specjalista pediatra
i onkologii dziecięcej
1362-48

Konończuk, Katarzyna, Katarzyna Muszyńska-Rosłan, Karolina Konstantynowicz-Nowicka, Maryna Krawczuk-Rybak, Adrian Chabowski, and Eryk Latoch. "Biomarkers of Glucose Metabolism Alterations and the Onset of Metabolic Syndrome in Survivors of Childhood Acute Lymphoblastic Leukemia." *International Journal of Molecular Sciences* 23, no. 7 (March 28, 2022): 3712. DOI: 10.3390/ijms23073712

Imię i nazwisko współautora	Charakter udziału
Lek. Katarzyna Konończuk	Planowanie badania Rekrutowanie pacjentów Zebranie i interpretacja danych Przeprowadzenie analizy statystycznej Tworzenie manuskryptu
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Dr n. med. Karolina Konstantynowicz-Nowicka	Przeprowadzenie oznaczeń
Prof. dr hab. n. med. Maryna Krawczuk-Rybak	Nadzorowanie procesu powstawania publikacji Akceptacja ostatecznej wersji do publikacji
Prof. dr hab. n. med. Adrian Chabowski	Nadzorowanie procesu powstawania publikacji Akceptacja ostatecznej wersji do publikacji
Dr hab. n. med. Eryk Latoch	Planowanie badania Rekrutowanie pacjentów Współtworzenie manuskryptu Akceptacja ostatecznej wersji do publikacji

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Katarzyna Konończuk

Podpis kandydata

Potwierdzam opisany powyżej merytoryczny wkład kandydata w powstanie publikacji wchodzącej w skład rozprawy doktorskiej.

Podpis promotora

K. Muszyńska-Rosłan

Białystok, 16.08.2024 r.

Prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

Oświadczenie

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Prof. dr hab. n. med. Katarzyna Muszyńska-Rosłan
specjalista pediatra
i onkologii i hematologii dziecięcej
1368446

.....

Białystok, 20.08.2024 r.

Dr n. med. Karolina Konstantynowicz-Nowicka
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

Oświadczenie

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Konstantynowicz-Nowicka Karolina

Białystok, 20.08.2024 r.

Prof. dr hab. n. med. Maryna Krawczuk-Rybak
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
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KIEROWNIK
Kliniki Pediatrii, Onkologii i Hematologii
prof. zw. dr hab. n. med. Maryna Krawczuk-Rybak

Białystok, 20.08.2024 r.

Prof. Dr hab. n. med. Adrian Chabowski
Zakład Fizjologii
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji: **"Biomarkers of Glucose Metabolism Alterations and the Onset of Metabolic Syndrome in Survivors of Childhood Acute Lymphoblastic Leukemia."** autorów Katarzyna Konończuk, Katarzyna Muszyńska-Rosłan, Karolina Konstantynowicz-Nowicka, Maryna Krawczuk-Rybak, Adrian Chabowski, Eryk Latoch opublikowanej w *International Journal of Molecular Sciences,* wchodzącej w skład rozprawy doktorskiej **„Ocena stężenia wybranych markerów gospodarki węglowodanowej i lipidowej oraz ich związek z wystąpieniem nadwagi i otyłości u dzieci i młodzieży po leczeniu przeciwnowotworowym."** polegał na nadzorowaniu procesu powstawania publikacji oraz akceptacji ostatecznej wersji manuskryptu.

Jednocześnie wyrażam zgodę na wykorzystanie przez Lek. Katarzynę Konończuk publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

KIEROWNIK
Zakładu Fizjologii
prof. dr hab. Adrian Chabowski

Białystok, 20.08.2024 r.

Dr hab. n. med. Eryk Latoch
Klinika Pediatrii, Onkologii i Hematologii Dziecięcej
Uniwersytet Medyczny w Białymstoku
ul. J. Kilińskiego 1
15-089 Białystok

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji: "**Biomarkers of Glucose Metabolism Alterations and the Onset of Metabolic Syndrome in Survivors of Childhood Acute Lymphoblastic Leukemia.**" autorów Katarzyna Konończuk, Katarzyna Muszyńska-Rosłan, Karolina Konstantynowicz-Nowicka, Maryna Krawczuk-Rybak, Adrian Chabowski, Eryk Latoch opublikowanej w *International Journal of Molecular Sciences,* wchodzącej w skład rozprawy doktorskiej **„Ocena stężenia wybranych markerów gospodarki węglowodanowej i lipidowej oraz ich związek z wystąpieniem nadwagi i otyłości u dzieci i młodzieży po leczeniu przeciwnowotworowym."** polegał na planowaniu badania, rekrutowaniu pacjentów, współtworzeniu manuskryptu i akceptacji ostatecznej wersji do publikacji.

Jednocześnie wyrażam zgodę na wykorzystanie przez Lek. Katarzynę Konończuk publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne.

dr hab. n. med. Eryk Latoch
specjalista pediatrii, onkologii
i hematologii dziecięcej
2632652

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10. ZGODA KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
UNIWERSYTETU MEDYCZNEGO w BIAŁYMSTOKU
ul. Jana Kilińskiego 1
15-089 Białystok
tel. (085) 748 54 07, fax. (085) 748 55 08
prorektorkl@umb.edu.pl

Białystok, 22-12-2016

Uchwała nr: R-I-002/463/2016

Komisja Bioetyczna Uniwersytetu Medycznego w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Znaczenie białek wiążących kwasy tłuszczowe (FABPs) u wieloletnich ozdrowieńców z choroby nowotworowej przebytej w dzieciństwie jako predyspozycja do rozwoju zespołu metabolicznego" przez dr n. med. Annę Panasiuk wraz z zespołem badawczym z UMB.

Przewodnicząca Komisji Bioetycznej UMB

prof. dr hab. Elżbieta Hassman-Poznańska

KOMISJA BIOETYCZNA
PRZY UNIWERSYTECIE MEDYCZNYM W BIAŁYMSTOKU
ul. Jana Kilińskiego 1
15-089 Białystok
tel. 85 748 54 07, fax 85 748 55 08
komisjabioetyczna@umb.edu.pl

Białystok, 24.06.2021 r.

Uchwała nr: APK.002.319.2021

Na podstawie art. 29 ust. 2 i 14 ustawy dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (t.j. Dz. U z 2020, poz. 514 ze zm.), Komisja Bioetyczna przy Uniwersytecie Medycznym w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Analiza wybranych parametrów gospodarki lipidowej oraz węglowodanowej u dzieci i młodzieży przed, w trakcie oraz po leczeniu przeciwnowotworowym" przez dr n. med. Eryka Latocha wraz z zespołem badawczym z UMB.

Planowany okres realizacji od 24.06.2021 r. do czerwca 2023 r.

Przewodnicząca Komisji Bioetycznej przy UMB

prof. dr hab. Otylia Kowal-Bielecka

Pouczenie:
1. Odwołanie od uchwały komisji bioetycznej wyrażającej opinię może wnieść:
1) wnioskodawca;
2) kierownik podmiotu, w którym eksperyment medyczny ma być przeprowadzony;
3) komisja bioetyczna właściwa dla ośrodka, który ma uczestniczyć w wieloośrodkowym eksperymencie medycznym.
2. Odwołanie, o którym mowa w ust. 1, wnosi się za pośrednictwem komisji bioetycznej, która podjęła uchwałę, do Odwoławczej Komisji Bioetycznej w terminie 14 dni od dnia doręczenia uchwały wyrażającej opinię.

11. SPIS SKRÓTÓW

A-FABP – Adipocyte Fatty Acid Binding Protein, Adipocytarne Białka wiążące kwasy tłuszczowe

E-FABP – Epidermal Fatty Acid Binding Protein, Epidermalne Białka wiążące kwasy tłuszczowe

ALL - Acute lymphoblastic leukemia, Ostra białaczka limfoblastyczna

AUC - Area Under the Curve, pole pod krzywą ROC

BMI - Body Mass Index, wskaźnik masy ciała

FABP – Fatty Acid Binding Protein, białka wiążące kwasy tłuszczowe

GIP - Gastric inhibitory peptide, glukozozależny peptyd insulinotropowy

HOMA-IR - Homeostasis Model Assessment of Insulin Resistance, wskaźnik insulinooporności

IDF - International Diabetes Federation

IR – Insulin resistance, insulinooporność

MetS – Metabolic syndrome, zespół metaboliczny

PAI-1 - Plasminogen activator inhibitor-1, inhibitor aktywatorów plazminogenu typu 1

TG – Triglycerides, triglicerydy

WC – Waist circumference, obwód talii