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Związek powikłań metabolicznych w akromegalii z ekspresją H19 RNA

Promotor:

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Spis treści

Wykaz publikacji stanowiących rozprawę doktorską

Niniejszy cykl obejmuje trzy prace, w tym dwie prace oryginalne i jedną przeglądową, o łącznym IF: **13,247** i punktacji MNiSW: **270**.

1. **Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Kolačkov Katarzyna, Bolanowski Marek: H19 in endocrine system tumours, Anticancer Research, 2021, vol. 41, nr 2, s.557-565. DOI:10.21873/anticanres.14808
   IF: 2,435
   Pkt. MNiSW: 70

2. **Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Halupczok-Żyła Jowita, Kałużny Marcin, Konopka Bogumil M., Błoniecka Izabela, Zieliński Grzegorz, Bolanowski Marek: Complications and comorbidities of acromegaly - retrospective study in Polish Center, Frontiers in Endocrinology, 2021, vol. 12, art.642131[10 s.]. DOI:10.3389/fendo.2021.642131
   IF: 6,055
   Pkt. MNiSW: 100

3. **Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Kolačkov Katarzyna, Zembska Agnieszka, Bolanowski Marek: Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study, Biomedicines, 2023, vol. 11, nr 4, art.1211 [10 s.]. DOI:10.3390/biomedicines11041211
   IF: 4,757
   Pkt. MNiSW: 100

Streszczenie

Wstęp: Rozprawa doktorska składa się z cyklu trzech publikacji podejmujących temat powikłań akromegalii oraz zastosowania długołańcuchowego niekodującego H19 RNA w diagnostyce akromegalii i jej powikłań oraz innych nowotworach układu dokrewnego.

Akromegalia to choroba rzadka związana z obecnością gruczolaka przysadki wydzielającego hormon wzrostu. Pacjenci chorujący na akromegalię, oprócz występowania typowych objawów klinicznych choroby, są narażeni na rozwój wielu powikłań, między innymi powikłań sercowo-naczyniowych, metabolicznych i współistniejących zaburzeń endokrynologicznych. Podkreśla się, że aktywna diagnostyka i monitorowanie powikłań akromegalii są istotnymi elementami opieki nad tą grupą chorych.

Długołańcuchowy niekodujący H19 RNA (long noncoding H19 RNA – lnc H19 RNA, H19 RNA) jest nowopoznanym markerem nowotworzenia, którego potencjalne zastosowanie opisywano w diagnostyce, ocenie zaawansowania i ryzyka progresji wielu nowotworów złośliwych. Dodatkowo zmienna ekspresja H19 RNA może być związana z występowaniem zaburzeń metabolicznych, osteoporozy czy chorobami mięśnia sercowego. Jak dotąd nie przeprowadzono badań oceniających związek ekspresji H19 RNA z występowaniem akromegalii oraz towarzyszącymi tej chorobie powikłaniami.

Cel pracy: Głównymi celami rozprawy doktorskiej było scharakteryzowanie powikłań akromegalii oraz ocena związku pomiędzy poziomem ekspresji długołańcuchowego niekodującego H19 RNA a występowaniem akromegalii i jej powikłań.

Materiał i metody: Artykuł pierwszy oparty jest o przegląd piśmiennictwa dotyczącego związku H19 RNA z nowotworami układu dokrewnego. W artykule drugim dokonano analizy retrospektywnej powikłań akromegalii, metod służących ich diagnozowaniu oraz wyników badań biochemicznych, hormonalnych i obrazowych 179 pacjentów z akromegalią. Badanie opisane w artykule trzecim przeprowadzono w grupie 32 chorych na akromegalię oraz 25 pacjentów z wykluczonym guzem przysadki, stanowiących grupę kontrolną. W obu grupach analizowano poziom ekspresji H19 RNA we krwi obwodowej. Otrzymane wyniki korelowano z obecnością powikłań, parametrami radiologicznymi, biochemicznymi i hormonalnymi.

Wyniki: Najczęstszą grupą powikłań stanowiły choroby metaboliczne, a wśród nich najczęściej rozpoznawana była dyslipidemia. Rzadziej obserwowano powikłania sercowo-naczyniowe i endokrynologiczne. Mężczyźni częściej niż kobiety chorowali na niedoczynność przysadki, hipogonadyzm hipogonadotropowy, panhipopituitaryzm i stan przedcukrzycowy. Publikacje dotyczące nowotworów układu dokrewnego, w tym guzów przysadki przedstawiają

H19 jako potencjalny marker diagnostyczny. W przeprowadzonym przez nas badaniu nie stwierdzono istotnej statystycznie różnicy w poziomie ekspresji H19 RNA między grupą chorych na akromegalię a grupą kontrolną. Poziom ekspresji H19 RNA nie zależał od aktywności choroby, wyników badań biochemicznych, hormonalnych, wielkości gruczolaka przysadki czy ekspansji pozasiodłowej. Współwystępowanie kamicy żółciowej wiązało się z wyższą ekspresją H19 RNA.

Wnioski: Ocena ekspresji H19 RNA to metoda, która może znaleźć zastosowanie w diagnostyce i monitorowaniu nowotworów złośliwych. Jak dotąd prace dotyczące zastosowania H19 RNA dla guzów układu endokrynnego nie są liczne i dostarczają niejednoznacznych wyników.

U większości pacjentów z akromegalią rozwijają się powikłania z kręgu chorób metabolicznych, układu krążenia i endokrynologicznych. Chorzy na akromegalię powinni być objęci wielospecjalistyczną opieką celem aktywnego wykrywania, leczenia i monitorowania współistniejących schorzeń.

Wyniki dotychczasowej analizy ekspresji H19 RNA we krwi pełnej obwodowej nie wskazują, by była to metoda przydatna w diagnostyce i monitorowaniu akromegalii i jej powikłań. Ciekawym i po raz pierwszy opisanym odkryciem było uzyskanie znamiennie wyższego poziomu ekspresji H19 RNA u pacjentów z kamicą żółciową.

Słowa kluczowe: akromegalia, powikłania akromegalii, guzy przysadki, markery nowotworzenia, długołańcuchowe niekodujące kwasy nukleinowe, H19 RNA

Summary

Introduction: The doctoral dissertation consists of a series of three publications concerning complications of acromegaly and an application of long non-coding H19 RNA in the diagnostics of acromegaly and other endocrine tumors.

Acromegaly is a rare disease associated with the somatotroph adenoma overproducing growth hormone. Acromegaly patients, despite presenting typical symptoms of the disease, are at risk of cardiovascular, metabolic, and endocrine complications. It is emphasized that diagnostics and monitoring of acromegaly complications are essential elements of care for this group.

Long noncoding H19 RNA (lnc H19 RNA, H19 RNA) is a novel marker of carcinogenesis, which potential application was presented in diagnostics, staging and prognosis of the risk of progression in many types of malignancies. Additionally, there may be an association between a variance expression of H19 RNA and metabolic disorders, osteoporosis and diseases of the myocardium. So far, there was no other study investigating the association between the expression of H19 RNA and acromegaly and its complications.

Aim of the study: The main objectives of the dissertation have been to evaluate acromegaly comorbidities and assessment of the association between the expression of long noncoding H19 RNA and the occurrence of acromegaly and its complications.

Materials and methods: First publication is a review concerning associations between H19 RNA and tumors of the endocrine system. In the second publication, a retrospective analysis of acromegaly complications, diagnostic procedures and biochemical, hormonal and radiological results was performed on the group of 179 acromegaly patients. The research described in the third publication was performed in the group of 32 acromegaly patients and 25 patients, after the exclusion of pituitary tumor diagnosis. Obtained outcomes were correlated with the occurrence of acromegaly comorbidities, and imaging, biochemical and hormonal results.

Results: Metabolic disorders were the main group of diagnosed complications. Among them, dyslipidemia occurred with the highest frequency. Cardiovascular and endocrine diseases were observed less frequently. Pituitary insufficiency, secondary hypogonadism, panhypopituitarism and prediabetes occurred more frequently in males than in females. Publications concerning endocrine tumors, including pituitary tumors, present H19 RNA as a useful diagnostic tool. In our study, we did not observe a significant variation in H19 expression between patients with acromegaly and the controls. We did not find any association between H19 RNA and disease

activity, biochemical and hormonal parameters as well as tumor dimension and its extrasellar expansion. Co-occurrence of cholelithiasis was associated with higher H19 expression.

Conclusions: H19 expression is a method, which may be involved in the diagnostics and monitoring of malignancies. So far, research concerning the application of H19 RNA in the endocrine system tumors are limited and they present ambiguous results.

Most of acromegaly patients develop complications of metabolic, cardiovascular and endocrine systems. Acromegaly patients require complex healthcare to diagnose, treat and monitor comorbidities.

According to performed analysis, whole blood H19 expression is not a relevant method in diagnostics or monitoring acromegaly and its complications. An interesting and for the first time depicted finding was the significantly higher H19 expression in patients suffering from cholelithiasis.

Key words: acromegaly, acromegaly complications, pituitary tumors, markers of carcinogenesis, long noncoding nucleic acids, H19 RNA

Wprowadzenie

Akromegalia jest chorobą charakteryzującą się nadmiernym wydzielaniem hormonu wzrostu (GH – growth hormone) oraz insulinopodobnego czynnika wzrostu 1 (IGF-1 – insulin-like growth factor 1). Najczęstszą przyczyną akromegalii jest gruczolak przysadki produkujący w sposób autonomiczny hormon wzrostu [1]. W odpowiedzi na jego wysokie stężenie, wątroba wydziela IGF-1. Somatotropowe gruczolaki przysadki to najczęściej makrogruczolaki, czyli guzy łagodne o średnicy co najmniej 10 mm. Z uwagi na rozrost guza często dochodzi do ekspansji pozasiodłowej i penetracji guza do zatok jamistych oraz ucisku skrzyżowania nerwów wzrokowych. Objawy akromegalii wynikają z nadmiernej produkcji powyższych hormonów oraz z ucisku guza przysadki na okoliczne struktury. U pacjentów obserwowane są zmiana rysów twarzy, powiększenie rąk, stóp, nadmierna potliwość, a także bóle głowy i odskroniowe ograniczenie pola widzenia.

Poza obecnością typowych objawów akromegalii, u większości chorych dochodzi do rozwoju powikłań choroby wynikających z działania GH i IGF-1 na tkanki i narządy [2,3]. Obserwowane są choroby układu krążenia (nadciśnienie tętnicze, przerost mięśnia sercowego, wady zastawowe, makroangiopatie), układu oddechowego (obturacyjny bezdech senny), pokarmowego (polipy jelita grubego), endokrynnego (wole, niedoczynność przysadki), kostnego (osteoporoza, choroba zwyrodnieniowa stawów) czy powikłania metaboliczne (cukrzyca, dyslipidemie). Choroby współistniejące z akromegalią przyczyniają się do pogorszenia jakości życia oraz przedwczesnych zgonów [4]. W ostatnich latach zalecenia dotyczące opieki nad pacjentami z akromegalią podkreślają istotność aktywnego wykrywania oraz leczenia powikłań akromegalii [5–7]. Nierzadki brak możliwości przeprowadzenia leczenia radykalnego powoduje, że akromegalia często ma charakter przewlekły, stąd zaleca się by diagnostyka i monitorowanie powikłań odbywały się cyklicznie, nie tylko w chwili wykrycia choroby. Z uwagi, iż obserwowane schorzenia dotyczą niemal wszystkich układów i narządów, pacjenci z akromegalią wymagają często opieki wielospecjalistycznej.

Akromegalia oraz towarzyszące jej powikłania są bardzo często rozpoznawane z opóźnieniem, co utrudnia osiągnięcie celów terapeutycznych oraz powoduje nieodwracalność niektórych patologii [8]. Zastosowanie nowych narzędzi pomocnych w diagnostyce i monitorowaniu akromegalii i jej powikłań mogłoby ułatwić rozpoznanie choroby na wczesnym etapie i zwiększyć szanse na jej skuteczne leczenie.

Długołańcuchowe niekodujące kwasy nukleinowe (long noncoding RNA - lncRNA) to cząsteczki nie podlegające procesowi translacji, a zatem nie posiadające produktów białkowych [9]. W ostatnich latach odkryto, że zaledwie 2% ludzkiego genomu podlega procesom transkrypcji i translacji [10]. Geny kodujące są matrycą dla tworzenia białek organizmu. Z pozostałej części powstają niekodujące RNA, które nie posiadają otwartej ramki odczytu i nie biorą udziału w procesie translacji [11]. Spośród nich wyodrębniono długołańcuchowe RNA, których nić zbudowana jest z ponad 200 nukleotydów. Dotychczasowe badania nad funkcją lncRNA pokazują, iż ich znaczenie jest znacznie szersze niż początkowo uważano. Prawdopodobnie cząsteczki te są regulatorami ekspresji genów w procesach transkrypcji i translacji oraz przy użyciu mechanizmów epigenetycznych [12].

H19 RNA to pierwszy odkryty długołańcuchowy niekodujący RNA, opisany w 1984 r. w pracy Pachnisa i wsp. [13]. Początkowo udowodniono, że wysoki poziom ekspresji H19 występuje w życiu płodowym, głównie w tkankach pochodzenia mezo- i endodermalnego [14,15]. Po narodzinach poziom ekspresji H19 gwałtownie spada w większości tkanek, a jej wyższy poziom obserwowany jest w mięśniu sercowym i mięśniach szkieletowych. W ostatnich latach odkryto, że długołańcuchowe niekodujące RNA, a wśród nich również H19 RNA, mają istotne znaczenie w patogenezie chorób, w tym przede wszystkim w procesach nowotworzenia [15]. Przypuszcza się, iż związki te odgrywają rolę w powstawaniu nowotworów, ich progresji, inwazji i tworzeniu przerzutów. Udowodniono związek H19 RNA z takimi procesami jak przyspieszanie proliferacji, inwazyjności i migracji komórek nowotworowych, a także stymulowanie angiogenezy i hamowanie apoptozy [16]. Dotychczasowe badania nad rolą H19 RNA w procesie inicjacji i progresji nowotworów dostarczają niejednoznacznych doniesień. W większości typów nowotworów wykazano wysoką ekspresję omawianego RNA, co wskazuje na jego proonkogenny charakter. Tego typu relacje wykazano m.in. dla nowotworów złośliwych piersi, płuc, układu pokarmowego, pęcherza moczowego i jajnika [17–22]. Z kolei supresyjny wpływ H19 oraz jego niską ekspresję wykazano w guzie Wilmsa oraz rakach kory nadnerczy [23,24]. Dotychczasowe wyniki badań wskazują, że informacja o poziomie ekspresji H19 może być potencjalnym biomarkerem mającym zastosowanie w diagnostyce oraz ocenie zaawansowania nowotworów. Ponadto wyższa niż w zdrowych tkankach ekspresja H19 może być punktem uchwytu w terapii celowanej, co dotychczas było tematem badań dla nowotworów złośliwych pęcherza moczowego i trzustki [25,26].

Dotychczasowe badania oceniające poziom ekspresji H19 RNA w nowotworach układu endokrynnego dostarczają niejednoznacznych wniosków. Do tej pory ukazało się tylko kilka publikacji skupiających się na roli H19 RNA w gruczolakach przysadki [27–30]. Również nieliczne badania podejmują temat związku H19 RNA z zaburzeniami metabolizmu węglowodanów, lipidów czy tkanki kostnej [31–33]. Jak dotąd brak jest badań oceniających związek ekspresji H19 RNA z występowaniem akromegalii oraz towarzyszących jej powikłań.

Cele rozprawy doktorskiej

Głównymi celami niniejszej rozprawy doktorskiej było scharakteryzowanie powikłań akromegalii oraz ocena związku pomiędzy poziomem ekspresji długołańcuchowego niekodującego H19 RNA a występowaniem akromegalii i jej powikłań.

Cele szczegółowe:

1. Przedstawienie aktualnego stanu wiedzy na temat znaczenia H19 RNA jako potencjalnego biomarkera w nowotworach układu dokrewnego.
2. Charakterystyka powikłań akromegalii oraz metod stosowanych w ich diagnostyce.
3. Ocena przydatności zastosowania analizy ekspresji H19 RNA we krwi pełnej obwodowej w diagnostyce akromegalii.
4. Ocena korelacji między ekspresją H19 RNA a wielkością i ekspansją gruczolaków somatotropowych.
5. Analiza związku między poziomem ekspresji H19 RNA a występowaniem powikłań akromegalii.

Materiał i metody

Materiał i metody w artykule pierwszym:

Przegląd aktualnego (do grudnia 2020 r.) piśmiennictwa dotyczącego zagadnień nowotworów układu endokrynnego, w tym guzów przysadki, tarczycy, przytarczyc, nadnerczy i neuroendokrynnych przy użyciu bazy danych PubMed.

Materiał i metody w artykule drugim:

Retrospektywnie przeanalizowano dane 179 pacjentów (119 kobiet i 60 mężczyzn) hospitalizowanych w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami we Wrocławiu w latach 1976-2018. Na ich podstawie stworzono bazę danych dla przeprowadzenia analizy statystycznej. Zgromadzone dane zawierały wiek pacjentów, wyniki pomiarów antropometrycznych, badań biochemicznych, hormonalnych i obrazowych. Ponadto na podstawie dokumentacji medycznej oceniono rodzaj występujących powikłań towarzyszących akromegalii, a także rodzaj badań zlecanych w kierunku ich diagnostyki i monitorowania. Na realizację badania otrzymano zgodę Komisji Bioetycznej.

Materiał i metody w artykule trzecim:

Badanie przeprowadzono w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami w latach 2020-2022 r. Finansowanie badania pochodziło z grantu dla Młodych Naukowców (STM. C120.20.096). Do grupy badanej zakwalifikowano 32 pacjentów chorujących na akromegalię (24 kobiety i 8 mężczyzn), a do grupy kontrolnej – 25 osoby zdrowe (16 kobiet i 9 mężczyzn), z wykluczonym na podstawie badania rezonansu magnetycznego (MR) guzem przysadki. Spośród grupy badanej 5 pacjentów miało świeżo rozpoznaną akromegalię, 15 chorych było w trakcie leczenia farmakologicznego, a 12 chorych było skutecznie wyleczonych. Od wszystkich uczestników badania zebrano szczegółowy wywiad lekarski, przeprowadzono pomiary antropometryczne oraz wykonano badania biochemiczne i hormonalne oraz MR przysadki. Ponadto od wszystkich pacjentów zabezpieczono 5 ml krwi pełnej obwodowej. Zebrany materiał posłużył do ekstrakcji RNA. Następnie przeprowadzono ilościową reakcję łańcuchową polimerazy (real-time PCR). Poziom ekspresji H19 RNA był oceniony względem dwóch genów referencyjnych – beta-aktyny (BACT) i dehydrogenazy aldehydu 3-fosfoglicerynowego (GAPDH) metodą 2-ΔΔCt. Zebrane dane zostały poddane analizie statystycznej. Badanie zostało pozytywnie zaopiniowane przez Komisję Bioetyczną.

Omówienie wyników

Omówienie wyników artykułu pierwszego:

Dotychczasowe badania nad rolą H19 RNA w nowotworach układu endokrynnego były prowadzone przede wszystkim na liniach komórkowych oraz modelach zwierzęcych. Nieliczne prace omawiają funkcję H19 na podstawie badań przeprowadzonych z udziałem ludzi.

Publikacje dotyczące guzów przysadki przedstawiają H19 jako potencjalny marker diagnostyczny. W badaniach Wu i wsp. oraz Zhanga i wsp. obserwowano istotnie niższą ekspresję H19 w tkankach gruczolaków przysadki [27,30]. Ponadto poziom ekspresji H19 ujemnie korelował z objętością guza. Zaobserwowano supresyjne działanie H19 na rozwój gruczolaków, które było silniejsze w porównaniu do działania kabergoliny, co wskazuje na potencjał terapeutyczny omawianego związku [28,30]. Dodatkowo Lu i wsp. w swojej pracy przedstawili H19 jako marker pozwalający na różnicowanie inwazyjnych gruczolaków somatotropowych z gruczolakami nieinwazyjnymi [29].

Dość szeroko badany był związek H19 z rakiem tarczycy, jednak publikacje dostarczają niejednoznacznych wniosków. Część autorów wykazała zwiększoną ekspresję H19 w tkankach raków tarczycy oraz promujący wpływ H19 na rozwój tego nowotworu [34,35]. Wysoka ekspresja H19 wiązała się z większym rozmiarem guza, jego wyższym stopniem zaawansowania oraz mniejszym odsetkiem pięcioletnich przeżyć. Z kolei inne prace donoszą o supresyjnym wpływie H19 na rozwój raka tarczycy [36,37]. Badacze wykazali niższą ekspresję H19 w tkankach nowotworowych oraz gorsze rokowaniu pacjentów z niską ekspresją H19.

Dla guzów nadnerczy istotnym wnioskiem płynącym z badań było wykazanie obniżonej ekspresji H19 RNA w rakach kory nadnerczy [38,39]. Obniżona ekspresja w guzach złośliwych pozwalała na ich różnicowanie ze zmianami łagodnymi.

Do przeciwnych wniosków doszli badacze oceniający związek H19 z guzami neuroendokrynnymi (neuroendocrine tumors - NET) – wyższa ekspresja H19 była czynnikiem różnicującym zmiany złośliwe od łagodnych [40]. Wysoki poziom ekspresji H19 był czynnikiem negatywnym rokowniczo dla pacjentów z NET tj. dodatnio korelował z wielkością guza, jego stopniem zaawansowania czy obecnością przerzutów odległych.

Omówienie wyników artykułu drugiego:

W przebadanej grupie pacjentów z akromegalią dominowały kobiety (66%). Podczas ostatniej hospitalizacji największą grupę (39,7%) stanowili chorzy wyleczeni, następnie chorzy kontrolowani za pomocą farmakoterapii (30,5%) oraz pacjenci z chorobą aktywną (29,9%). Na przestrzeni lat 2000-2018 odsetek pacjentów z chorobą aktywną malał na korzyść pacjentów kontrolowanych farmakologicznie. Potwierdza to korzystny wpływ zwiększenia dostępności do terapii analogami somatostatyny. W badanej populacji pacjentów z chorobą aktywną zaobserwowano większy odsetek guzów o charakterze makrogruczolaka (największy wymiar ≥ 10 mm) w stosunku do mikrogruczolaków. Wielkość guza istotnie korelowała ze stężeniami hormonu wzrostu ($p<0,001$) i IGF-1 ($p<0,001$). Zastosowane leczenie opierało się na leczeniu chirurgicznym z dostępu przezklinowego i przezczaszkowego, radioterapii oraz farmakoterapii z udziałem analogów somatostatyny i agonistów dopaminy. Większość pacjentów (54,7%) wymagała leczenia skojarzonego. Najczęściej łączoną opcją (45,3%) było leczenie chirurgiczne oraz farmakoterapia. U 139 chorych zastosowano leczenie chirurgiczne. Spośród tych chorych 20,9% wymagało więcej niż jednej operacji. W farmakoterapii na przestrzeni lat zwiększało się zastosowanie analogów somatostatyny.

Najczęstszą grupą powikłań obserwowanych w badanej populacji były powikłania metaboliczne, następnie sercowo-naczyniowe i endokrynologiczne. Hiperlipidemia była najczęściej diagnozowanym powikłaniem, stwierdzanym u 74% chorych. W grupie kobiet najczęstszymi chorobami towarzyszącymi były hiperlipidemia, nadciśnienie tętnicze i wole. U mężczyzn dominowały hiperlipidemia, nadciśnienie tętnicze i niedoczynność przysadki. W grupie mężczyzn istotnie częściej występowały niedoczynność przysadki, hipogonadyzm hipogonadotropowy, panhipopituitaryzm i stan przedcukrzycowy. Stwierdzono, że w populacji mężczyzn hipogonadyzm i niedoczynność przysadki istotnie wiązały się z przebyciem radioterapii ($p=0,008$ i $p=0,04$). Niedoczynność przysadki, hipogonadyzm i panhipopituitaryzm częściej występowały u chorych wymagających więcej niż jednej operacji, zarówno w grupie kobiet, jak i mężczyzn.

Spośród powikłań sercowo-naczyniowych najczęściej obserwowanym schorzeniem było nadciśnienie tętnicze obecne u 58% badanych. Na patogenezę nadciśnienia tętniczego u pacjentów z akromegalią składają się zwiększona objętość osocza, przerost mięśniówki naczyń oraz ich zwiększona sztywność, a także dysfunkcja śródbłonka. Innymi powikłaniami powiązanymi z wpływem GH i IGF-1 na układ krążenia były zmiany strukturalne serca

obserwowane w badaniu ECHO (34%), arytmie (20%), choroba niedokrwienna serca (7%) i niewydolność serca (6%).

W badanej grupie chorych na akromegalię obserwowano częstsze niż w populacji ogólnej występowanie cukrzycy i stanu przedcukrzycowego. Najpewniej przyczyniło się do tego antagonistyczne do insuliny działanie hormonu wzrostu, predysponujące do zwiększenia lipolizy i glukoneogenezy oraz insulinooporności. Ponadto GH jest czynnikiem negatywnie wpływającym na gospodarkę lipidową. Dyslipidemie wiążące się ze zwiększonymi stężeniami LDL, cholesterolu i trójglicerydów były najczęściej notowanymi zaburzeniami w badanej grupie.

Wśród powikłań endokrynnych wole było najliczniej diagnozowanym problemem (52%). Dotychczasowe wyniki badań wskazują na dodatnią korelację pomiędzy stężeniami GH i IGF-1 oraz objętością tarczycy. Kolejne obserwowane powikłania z tej grupy to niedoczynność przysadki (37%) i osteoporoza (12%).

Współwystępowanie powikłań ze strony różnych układów i narządów w badanej grupie wskazują na niezbędny udział specjalistów różnych dziedzin w opiece nad chorymi na akromegalię. Ponadto badania służące diagnostyce i monitorowaniu powikłań akromegalii powinny być wykonywane regularnie oraz stanowić nieodłączny element planowanego postępowania. Badaniami najczęściej zlecanymi podczas zarejestrowanych hospitalizacji były: pomiar ciśnienia tętniczego, elektrokardiogram, lipidogram, ocena stężenia glukozy na czczo i w teście obciążenia glukozą. Elementy diagnostyki, których częstość była najniższa, obejmowały polisomnografię, kolonoskopię i echokardiografię.

Omówienie wyników artykułu trzeciego:

Grupa pacjentów z akromegalią charakteryzowała się większą masą ciała oraz wartościami BMI oraz niższym wzrostem w stosunku do grupy badanej (wartość p odpowiednio 0,018; 0,001; 0,045). Makrogruczolaki przysadki dominowały w grupie chorych ze świeżo rozpoznaną akromegalią (60%) jak i w grupie wszystkich chorych, którzy nie byli radykalnie zoperowani (55%). U 40% chorych stwierdzono ekspansję pozasiodłową.

Nie stwierdzono istotnej statystycznie różnicy w poziomie ekspresji H19 RNA pomiędzy grupą kobiet i mężczyzn. W grupie badanej zaobserwowano dodatnią korelację pomiędzy wiekiem chorych a poziomem ekspresji H19 RNA.

Nie stwierdzono istotnej różnicy w poziomie ekspresji H19 RNA pomiędzy grupą chorych na akromegalią a grupą kontrolną. Ponadto nie stwierdzono, by poziom ekspresji H19 RNA zależał od aktywności choroby, wyników badań hormonalnych czy biochemicznych. Nie stwierdzono również związku pomiędzy poziomem ekspresji H19 RNA a wielkością guza przysadki ani jego ekspansją pozasiodłową. Nie wykazano, by ekspresja H19 RNA zależała od zastosowania leczenia chirurgicznego, farmakologicznego czy radioterapii ani od skuteczności powyższych terapii.

Najczęstszymi powikłaniami obserwowanymi w grupie chorych z akromegalią były wole, dyslipidemia i nadciśnienie tętnicze. Pacjenci z akromegalią istotnie częściej chorowali na nadciśnienie tętnicze, wole i kamicę żółciową. Ponadto stwierdzono, że obecność akromegalii jest niezależnym od wieku czynnikiem predysponującym do występowania dyslipidemii i wola. Chorych z kamicą żółciową cechowała wyższa ekspresja H19 RNA. Nie stwierdzono, by jej poziom był powiązany z innymi powikłaniami akromegalii.

Wnioski

- Ocena ekspresji H19 RNA to nowatorska metoda, która można znaleźć zastosowanie w diagnostyce i monitorowaniu nowotworów złośliwych. Jak dotąd prace dotyczące zastosowanie H19 RNA w guzach układu endokrynnego nie są liczne, dotyczą głównie modeli komórkowych lub badań na zwierzętach, dostarczają niejednoznacznych wyników.
- U większości pacjentów z akromegalią rozwijają się powikłania z kręgu chorób metabolicznych, układu krążenia i endokrynologicznych. Chorzy na akromegalię powinni być objęci wielospecjalistyczną opieką celem aktywnego wykrywania, leczenia i monitorowania współistniejących schorzeń.
- Dotychczasowo uzyskane wyniki we wstępnej analizie ekspresji H19 RNA we krwi pełnej obwodowej pacjentów z akromegalią nie wskazują, by była to metoda przydatna w diagnostyce i monitorowaniu tej grupy chorych. Ciekawym i po raz pierwszy opisanym odkryciem było uzyskanie znamiennie wyższego poziomu ekspresji H19 RNA u pacjentów z kamicą żółciową. Nie stwierdzono zależności pomiędzy poziomem ekspresji H19 RNA a obecnością innych powikłań typowych dla akromegalii.

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Publikacja 1

H19 in endocrine system tumours

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Review

H19 in Endocrine System Tumours

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Abstract. *Long non-coding RNAs (lncRNAs) are over 200 nucleotides long recently discovered RNA molecules that are not involved in the translation process. Accumulating evidence shows that H19 lncRNA is an important regulator of gene expression and its altered expression contributes to carcinogenesis. The aim of this review was to reveal current knowledge about H19 lncRNA and its impact on tumours of the endocrine system. We present findings about H19 altered regulation and its association with tumorigenesis, cancer progression and differentiation, and its potential use in diagnostics, prognostics and therapy. The mechanism and molecular pathways involved in these processes are discussed.*

Non-coding RNAs (ncRNAs) are recently discovered molecules, which do not participate in the translation process and do not have their own protein product (1-3). Approximately 80% of human genome is transcribed into functional RNA, but less than 2% is involved in translation and has protein-coding capacity (4). Therefore, ncRNAs are an abundant group of transcripts that can be divided according to their length or function. According to their length, we can distinguish them into small ncRNAs (less than 200 nucleotides long) and long non-coding RNAs (lncRNAs) (1-3). ncRNAs are divided according to their function into housekeeping ncRNAs and regulatory ncRNAs (2, 5). Ribosomal (r-), transfer (t-), small nuclear (sn-) and small nucleolar (sno-) ncRNAs are housekeeping, whereas micro (mi-), small interfering (si-), piwi-interacting (pi-) and long non-coding (lnc-) ncRNAs are regulatory (2, 5). Up to November 2020, over 260,000 types of human lncRNAs had been identified (6). LncRNAs can be located in the nucleus or cytoplasm (5). Their function is still poorly understood, but their biological roles seem to be more crucial than it was initially hypothesized (1, 3, 5). Accumulating evidence shows that lncRNAs are important regulators of gene expression (3). They play roles in regulation and modification of transcription, post-transcription and epigenetic processes (2, 3). Evidence has revealed that they are involved in the development of diabetes (7, 8) and neurological diseases (9-11). Recent studies have shown that aberrant expression of lncRNAs may also contribute to carcinogenesis (2, 3, 12, 13).

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Key Words: H19, lncRNA, pituitary adenoma, thyroid cancer, adrenal tumour, neuroendocrine tumour, review.

H19 RNA

H19 lncRNA was the first discovered lncRNA; it was initially classified as an mRNA with unknown protein product and was extracted from a mouse liver (14). A few years later, Brannan *et al.* isolated *H19* gene from human tissues and stated that the only final product of *H19* gene may be an mRNA transcript, located in the cytoplasm (15). The full length of *H19* RNA chain is 2.3 kb (16). In human, the gene is mapped on chromosome 11p15.5 (17). The expression of *H19* is high during embryonic development (14, 18), mainly in the endoderm and mesoderm (19), and maximum expression has been observed in the liver, muscles and adrenals (19, 20). After birth, it is down-regulated in most tissues, but its expression is still detectable in inter alia, skeletal muscle, myocardium and mammary gland tissues (19, 21).

H19 – Contribution to Carcinogenesis

The linkage between *H19* and cancer development was the subject of many studies since the 1990s (18, 22, 23). Bartolomei *et al.* first discovered that *H19* is expressed exclusively from the maternal allele, due to the imprinting process (24). Knowledge about the influence of imprinting alterations on carcinogenesis led to search for an association between *H19* gene and its

potential role in cancer development (18). Probable mechanisms are loss of imprinting (LOI) – an epigenetic event resulting in biallelic gene expression, and changes in the methylation pattern of promoters sequences, which regulate the levels of gene expression (25). It has been proposed that these two mechanisms are strongly related, due to the involvement of methylation in the inactivation of the paternal allele (26). It is important to note that *H19* and *IGF2* genes are commonly imprinted interdependently due to their close location on 11p15.5 (27). Association between LOI of *H19* gene and tumorigenesis was described inter alia, for oesophageal (28), colorectal (28) and lung cancers (29). Additionally, in Wilms' tumour, LOI of *IGF2* gene contributes to methylation of *H19* promoter, resulting in the down-regulation of *H19* expression (30). Nevertheless, LOI does not always directly correspond to a methylation pattern and level of gene expression, as it has been shown by Byun *et al.* in a study on bladder cancer (25). In contrast to the above studies, Yballe *et al.* have shown no connection between LOI of *H19* gene and the occurrence of breast cancer (31). Similar results were obtained for neuroblastoma by Wada *et al.* (32). Further studies have been performed on the mechanisms of *H19* contribution in carcinogenesis. *H19* has been proposed as an oncogene (33, 34), tumour suppressor (35, 36) or as an oncofoetal RNA, associated with germ cell tumours (18, 19, 37).

The oncogenic properties of *H19* may be due to its increased expression in neoplasm tissues. Over-expression of *H19* RNA has been shown to contribute to the carcinogenesis and progression of tumours of the breast (34, 38), lung (39, 40), oesophagus (28, 41), stomach (42-44), colon (28, 45), liver (46), pancreas (47, 48), kidney (49), bladder (46, 50), cervix (51), ovary (52, 53), as well as in glioma (54, 55), leukaemia (56), oral squamous cell carcinoma (57), cholangiocarcinoma (58), osteosarcoma (59, 60) and melanoma (61). On the other hand, in some tumours down-regulation of *H19* expression was observed [inter alia in Wilms' tumour (30, 62)], which means that *H19* may be classified also as a tumour suppressor.

The mechanisms through which *H19* is involved in the process of cancer development include promotion of gene mutations, cell proliferation, invasion, migration and angiogenesis, immune and pro-apoptotic factors modulation and growth suppressor expression regulation (63, 64). Additionally, some studies have shown that *H19* RNA functions through sponging mi-RNAs including miR-675 (45, 48), miR-107 (39, 40), miR-370-3p (65), miR-106a-5p (61), miR-29a (54), miR-29a-3p (49) and miR-138-5p (51).

In recent meta-analyses (64, 66) the prognostic and clinicopathological values of *H19* in different types of cancers were explored. Both studies demonstrated that high levels of *H19* RNA contribute to shorter overall survival and associate with more advanced clinical stage of tumours and lymph node metastasis. Additionally, *H19* RNA positively correlates with poor tumour differentiation, earlier distant metastasis (64), as well as with poorer histological tumour grade and disease-free survival (66). Summarizing, *H19* RNA has been demonstrated as a potential marker for tumour progression and patient's prognosis.

So far, *H19* has been introduced as an intriguing figure in neoplasms' origin and development. But what is its impact on tumours of the endocrine system?

Pituitary Adenomas

In the study by Lu *et al.*, significantly higher expression of *H19* was observed in aggressive growth hormone-secreting pituitary adenomas compared to non-invasive growth hormone-secreting tumours (67). A similar observation was made for oesophageal cancer (*H19* correlated positively with tumours' depth, stage and metastasis) (41), lung cancer (*H19* enhanced cell proliferation, migration, and invasion of a cell line) (68), glioblastoma (in cell line and xenograft mouse model, *H19* promoted invasion, angiogenesis and tumour growth) (55), cholangiocarcinoma (*H19* positively correlated with tumour size, cell migration and invasiveness in tissues and cell lines) (58). Thus, we could hypothesize that in invasive pituitary adenomas *H19* might be a potential marker of malignancy and patients' prognosis.

On the other hand, Wu *et al.* (69) and Zhang *et al.* (70) observed down-regulation of *H19* expression in pituitary tumour tissues and in the plasma obtained from patients with pituitary adenomas in comparison to normal pituitary glands and healthy controls. In *in vitro* and *in vivo* models, an increase in cell proliferation after knockdown of *H19* gene was observed (69). Furthermore, in mouse models injection of *H19* lentivirus led to shrinkage of tumour volumes. *H19* expression levels negatively correlated with tumour volumes. Antitumor effects were induced by inhibiting 4E-BP1 phosphorylation in the mTORC1/4E-BP1 pathway. Moreover, in xenograft experiments *H19* overexpression was more effective than cabergoline in suppressing tumour growth (69). Additionally, the investigators revealed that cabergoline stimulated *H19* expression and *H19* and dopamine agonists exerted a synergistic therapeutic effect. These results indicate that increasing *H19* expression can be a potential therapy for pituitary adenomas. The mechanism of the synergistic action of *H19* and dopamine agonists in prolactinomas was investigated in a recent study by Wu *et al.* (71). It was revealed that *H19* promotes the effects of dopamine agonists by inhibiting miRNA-93a and stimulating ATG7 expression, and this is another example of *H19* action by sponging mi-RNA. *H19*/miRNA-93a/ATG7 axis was elucidated as a potential target of therapy, especially in drug-resistant prolactinomas.

Opposite results regarding the influence of *H19* on drug resistance, but also describing *H19* impact on ATG7, were demonstrated by Pan *et al.* (72). In non-small cell lung

cancer cell lines and xenograft models, they observed that *H19* sponges miRNA-615-3p and regulates ATG7 expression, and that this mechanism is probably involved in erlotinib resistance.

Thyroid Cancer

Ambiguous associations between *H19* expression and tumour development have also been illustrated for thyroid cancer. In thyroid cancer samples and cell lines, Liu *et al*. (73) observed over-expression of *H19*. *H19* enhanced tumour growth by inhibiting apoptosis and promoting progression, migration and invasion. Moreover, the researchers found that *H19* affects miR-17-5p and antagonizes its effect on YES1 expression. The association between *H19* and miR-17-5p has also been illustrated in gastric cancer cells (74), whereas a positive correlation between the levels of these two RNA was determined. In that study, *H19* was associated with larger tumour size, more advanced TNM stage and lymph node metastases. Corresponding outcomes, but for thyroid cancer, were exemplified in the study by Liu *et al*. (75). Moreover, higher *H19* expression was related with lower 5-year survival rate.

The mechanism through which *H19* contributes to thyroid cancer development was the subject of the studies of Li *et al*. (76) and Wang *et al*. (77). In the Li *et al*. study (76), *H19* was found to function through the PI3K/AKT signalling pathway, which plays an important role in carcinogenesis. Similarly, the association of *H19* with PI3K/AKT was illustrated in colorectal cancer cell lines (78) and melanoma (79). An additional finding of Li *et al*. was over-expression of *H19* in thyroid cancer tissues compared to adjacent healthy thyroid tissues (76). Moreover, *H19* expression was higher in poorly differentiated thyroid cancer tissues. In an *in vitro* model, knockdown of *H19* resulted in cancer cell viability inhibition and induction of apoptosis (76).

In contrary, Wang *et al*. (77) showed that *H19* overexpression inhibits viability, migration and invasion and induces tumour cells apoptosis and these effects might be mediated *via* down-regulating the expression of IRS-I (insulin receptor substrate I). Moreover, IRS-I expression might be induced also by PI3/AKT signalling pathway. The results of Wang *et al*. suggest that *H19* could be potentially used in thyroid cancer treatment.

The *H19* effect on the development of specific types of thyroid cancer was the subject of several studies presented below. For papillary thyroid cancer (PTC), higher tissue expression of *H19* was observed in the studies of Liang *et al*. (80) and Li *et al*. (81). Different mechanisms were proposed for expounding *H19* involvement in PTC development. In the first study, higher expression was positively correlated with mesenchymal phenotype biomarkers (vimentin, ZEB2, Twist, Snail2), which indicates that *H19* RNA induces epithelial–mesenchymal transition (EMT) process. EMT has been described to play a critical role in cancer invasiveness and metastasis (82). A similar effect of *H19* on EMT was depicted for ovarian (65), oesophageal (41) cancers and cholangiocarcinoma (58). Moreover, in the ovarian cell line, *H19* was shown to promote EMT-related activity and contribute to cisplatin resistance (83). Li *et al*. (81) proposed a mechanism that was related to ERβ (oestrogen receptor beta). Oestradiol enhanced *H19* expression by ERβ whereas high expression of *H19* promoted expression of ERβ (as a positive feedback). Additionally, *H19* acted through miR-3126-5p and this is another example of sponging mi-RNA by *H19*.

In the study by Liang *et al*., *H19* expression was positively correlated with tumour size and grade, as well as with lymph node metastases (80). The opposite results were obtained by Lan *et al*. (84). Jiao *et al*. (85) observed down-regulation of *H19* in papillary thyroid cancer tissues compared to paracancerous or benign nodes. Additionally lower expression of *H19* coincided with the presence of lymph node metastasis (84, 85), as well as with other features of poorer prognosis, such as higher tumour size, more aggressive histological type and poorer diseases-free survival (85).

For minimally invasive follicular thyroid cancer, Dai *et al*. examined whether *H19* could be a marker of distant metastasis and patients' prognosis (86). The study revealed low expression of *H19* in cancer tissues and *H19* levels were negatively correlated with tumour size, vascular invasion, distant metastasis and poorer overall survival.

Zhang *et al*. demonstrated that *H19* RNA is over-expressed in anaplastic thyroid carcinoma tissues and cell lines (87). Moreover, they showed that reduction of *H19* expression can be a potential target of molecular therapy – it decreased cell proliferation, migration and invasion *in vitro* as well as inhibited tumorigenesis and metastasis *in vivo*.

Alike divergences of *H19* expression levels in different types of thyroid cancer samples were observed by Wächter *et al*. (88). In anaplastic carcinoma, it was upregulated in six cases, down-regulated in two and was similar to healthy thyroid tissue in four. In follicular thyroid cancer, it was down-regulated in five samples and was the same in three cases. In papillary thyroid cancer it was overexpressed in five samples, down-regulated in two and stable in four. Thus, no association was observed between *H19* levels and type of thyroid cancer. In summary, in thyroid cancer, *H19* was found to act both as an oncogene as well as a suppressor.

Adrenals

During embryonic and foetal life adrenal expression of *H19* is very high (89, 90). In adulthood it remains highly expressed – it shows approximately 50% of the foetal

expression (91). Gao *et al.* (92) and Liu *et al.* (91) showed that in benign adrenal adenomas and hyperplastic adrenals, *H19* is expressed at about the same level as in healthy glands. However, similarly to Glover *et al.* they showed that in adrenocortical carcinomas the expression was reduced and it was significantly lower than in normal adrenals (91-93), whereas in pheochromocytomas the expression was variable, but generally decreased (91). Upon further investigation, Liu *et al.* showed that *H19* expression was also decreased in virilizing adrenal adenomas (94). The proposed mechanism causing the low *H19* expression in adrenocortical carcinomas was methylation of the promoter area (92). The degree of methylation of the promoter CpG regions in patients with adrenocortical cancers and adenomas was the subject of the study of Barreau *et al.* (95). The characterized cancers had a higher degree of methylation compared to adenomas that corresponded to patients' poorer prognosis. *H19* was found to be one of the genes with a hypermethylated promoter region leading to its down-regulation. Moreover, it showed the strongest observed inverse correlation between methylation levels and gene expression in this study, leading to a conclusion that *H19* plays a role as a suppressor. A comparable effect of methylation of the *H19* promoter on carcinogenesis was shown for bladder cancer (25) and Wilms' tumour (30). Additionally, Creemers *et al.* (96) proposed that the methylation status of *IGF2* and *H19* regulatory regions as useful markers in distinguishing malignant adrenocortical carcinomas from benign adenomas. Thus, we could conclude that *H19* expression levels and the methylation pattern of its regulatory regions could be promising tools in the diagnosis of adrenal tumours.

In addition, the various degrees of *H19* promoter methylation in benign ovarian teratomas (97) as well as in different types of germ cell tumours (GCTs) (98, 99), illustrated the diversity in origin and processes involved in the development of these neoplasms. Hence, reduced methylation in adrenocortical carcinomas may reflect their primordial features, however, further investigations are needed to evaluate this hypothesis.

Neuroendocrine Tumours

In the Ji *et al.* study, aberrant expression of *H19* was described as an important element in the development of non-functional pancreatic neuroendocrine neoplasms (pNENs) (100). In primary tumours as well as in metastatic tumours, the levels of *H19* expression were variable. However, after evaluation of the association between *H19* and tumour's malignancy, the researchers revealed that non-malignant tumours were characterized by low expression of *H19*, whereas in malignant pNENs as well as in liver metastases its expression was high. Moreover, high expression correlated positively with tumour size, lymph node and liver metastasis, local invasion, TNM stage, tumour-related death, poorer progression free and overall survival. In the cell line models, the authors showed that silencing *H19* led to inhibition of cell proliferation, growth and colony formation and the opposite effects were observed after *H19* over-expression. Additionally, overexpression of *H19* promoted tumour growth and Ki67 expression in xenograft mouse models. The paper illustrates the possible association between high expression of *H19* and VGF (neuropeptide precursor) in neoplasms origin, progression and poorer patient prognosis. Additionally, similarly to Li *et al.* (76), *H19* was shown to be involved in the activation of PI3K/Akt signalling pathway.

Ramnarine *et al.* showed that *H19* was an epigenetic regulator, which contributed to neuroendocrine transdifferentiation (NEtD) – a transformation from prostate cancer to neuroendocrine prostate cancer (101). In addition, high expression of *H19* was presented as a practical tool in distinguishing neuroendocrine prostate cancers from prostate adenocarcinomas.

Conclusion

The aim of this review was to demonstrate the current knowledge about *H19* lncRNA and its impact on tumours of the endocrine system. The collected data shed light on the mechanisms and molecular pathways involved in tumorigenesis. *H19* was determined to be involved in epigenetic regulation and in miRNA expression control. Moreover, *H19* may be a useful factor in differentiating malignancies from benign lesions, as it was demonstrated in aggressive pituitary adenomas (67), adrenocortical carcinomas (91-93) and pNENs (100). Another promising aspect is the down-regulation of *H19* as a purpose of targeted therapy, which was illustrated in cell line models of thyroid cancer (76, 87) and pNENs (100). On the other hand, upregulation of *H19* has also been proposed as a therapeutic tool (69, 70, 77). In addition, *H19* may improve the effects of treatment, like it was illustrated for dopamine agonists in prolactinomas (69, 71). Furthermore, abnormal expression of *H19* RNA in different types of malignancies makes it a potential biomarker for cancer diagnosis, prognosis and monitoring. In some reports correlation between *H19* expression and clinicopathological features was observed, which highlights the prognostic value of this RNA (75, 80, 84-86, 95).

Nevertheless, there are still many questions without unequivocal answers and are subjects for further investigation. First, studies concerning tumours of the endocrine system are limited. Particularly, there is a lack of studies exemplifying a connection between *H19* and parathyroid tumours. Additionally, only few studies concerned the potential association between *H19* and hormonal function of tumours. Second, there are

contradictory reports regarding *H19* expression in most of the described pathologies. Similarly, to outcomes obtained for other neoplasms, opposite effects of *H19* on tumorigenesis in endocrine gland tumours were demonstrated. *H19* was proposed to act as an oncogene as well as a suppressor. Currently, the possibility to use its levels as a simple tumour marker is limited. In addition, most presented results were obtained using cancer cell lines and xenograft mouse models. Further investigations on human tumour tissues and plasma concentrations are needed. Finally, the samples of the groups were small in some studies, sometimes due to the rare occurrence of the specific pathology. Therefore, studies with larger sample size are necessary.

In conclusion, *H19* is a novel and intriguing factor, which may allow elucidation of processes involved in carcinogenesis and tumour progression. Nevertheless, further investigation of its biological role in endocrine system tumours are still needed.

Conflicts of Interest

The Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' Contributions

MR, AJP and MB contributed to the article's conception and design. MR, AJP and KK collected the literature sources. The first draft of the manuscript was written by MR and corrected by AJP, KK and MB. All Authors contributed to the final version of the manuscript and approved it for publication.

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Complications and comorbidities of acromegaly - retrospective study in Polish Center

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Complications and Comorbidities of Acromegaly—Retrospective Study in Polish Center

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Introduction: In acromegaly, chronic exposure to impaired GH and IGF-I levels leads to the development of typical acromegaly symptoms, and multiple systemic complications as cardiovascular, metabolic, respiratory, endocrine, and bone disorders. Acromegaly comorbidities contribute to decreased life quality and premature mortality. The aim of our study was to assess the frequency of acromegaly complications and to evaluate diagnostic methods performed toward recognition of them.

Materials and Methods: It was a retrospective study and we analyzed data of 179 patients hospitalized in the Department of Endocrinology, Diabetes and Isotope Therapy in Wroclaw Medical University (Poland) in 1976 to 2018 to create a database for statistical analysis.

Results: The study group comprised of 119 women (66%) and 60 men (34%). The median age of acromegaly diagnosis was 50.5 years old for women (age range 20–78) and 46 for men (range 24–76). Metabolic disorders (hyperlipidemia, diabetes, and prediabetes) were the most frequently diagnosed complications in our study, followed by cardiovascular diseases and endocrine disorders (goiter, pituitary insufficiency, osteoporosis). BP measurement, ECG, lipid profile, fasting glucose or OGTT were performed the most often, while colonoscopy and echocardiogram were the least frequent.

Conclusions: In our population we observed female predominance. We revealed a decrease in the number of patients with active acromegaly and an increase in the number of well-controlled patients. More than 50% of patients demonstrated a coexistence of cardiac, metabolic and endocrine disturbances and only 5% of patients did not suffer from any disease from those main groups.

Keywords: acromegaly, complication, comorbidity, pituitary adenoma, IGF-I, GH

INTRODUCTION

Acromegaly is a rare endocrine disease associated with elevated growth hormone (GH) and insulin-like factor I (IGF-I) levels, mainly due to a pituitary adenoma (1). Hypersecretion of GH and IGF-I leads to increased cellular proliferation and differentiation, followed by remodelling of tissues, organ enlargement (organomegaly), and disturbances to metabolism. Most acromegaly patients experience a long delay between the appearance of the first symptoms of the disease, its diagnosis, and the start of the treatment (2). In consequence, there is a chronic exposure to increased GH and IGF-I levels leading to the development of typical acromegaly symptoms, and multiple systemic complications as cardiovascular, metabolic, respiratory, endocrine, and bone disorders (3, 4). Cardiovascular disorders leading to myocardial infarction and stroke, respiratory diseases, and cancers are the main reasons for premature mortality in this group (5, 6). Early diagnosis of acromegaly and its treatment is mandatory to avoid comorbid diseases and further complications leading to premature death. Radical surgery as well as pharmacological control of disease activity decrease mortality to that observed in the normal population (7, 8). Moreover, a lot of acromegaly patients suffer from decreased life quality despite cured or well-controlled disease activity (9, 10). Complications like diabetes, cardiovascular disease and vertebral fractures contribute to a significant reduction of patients' quality of life (11, 12).

First guidelines about diagnosis and treatment of acromegaly complications were published in 2003 (3). Since then some updates emerged, with the most relevant in 2013 (13) and 2020 (14), recommending a change in approach to the disease supporting holistic view on an acromegaly patient with involvement of different types of specialist. As stated in screening for comorbidities should be performed at the time of diagnosis and repeated regularly.

The aim of our study was to perform an in-depth analysis of the gathered database of hospitalization in terms of acromegaly complication frequencies and their co-occurrence. Also, we investigated the changes in procedures used in diagnostics and treatment of acromegaly patients over an eighteen-year period, from 2000 to 2018.

MATERIALS AND METHODS

In this retrospective study, we analyzed data of 179 patients hospitalized in the Department of Endocrinology, Diabetes and Isotope Therapy in Wroclaw Medical University (Poland) in 1976 to 2018. The inclusion criteria were diagnosis of acromegaly according to Endocrine Society Guidelines (15) – elevated IGF-I levels and unsuppressed GH in OGTT at present or in the past. The study included patients with an established acromegaly diagnosis. The database of the patients was created by a single researcher and the registry included 560 records. Data came from patients' hospitalizations at the Department of Endocrinology, Diabetes and Isotope Therapy in Wroclaw and the Neurosurgery Department in Warsaw. Registry included patients' demographic characteristics, laboratory test results and performed procedures, as well as recommendations for the patients. The number of hospitalizations for each patient was between 1 and 16, with a mean of 3.136 for women and 3.151 for men. The average time of monitoring was 5 years.

Statistical analysis was performed using R for Windows, version 3.5.3.

Occurrence of Micro- and Macroadenomas

For 132 patients adenoma sizes were available. An adenoma was classified as a macroadenoma if at least one of its sizes was greater than 10 mm. The comparison of micro- to macroadenomas between males and females was performed using the Chi-squared test.

Comparison of Patients Who Underwent One *vs.* Several Surgeries

All comparisons were performed separately for male and female patients. The IGF-I concentrations and GH concentrations were compared using the Wilcoxon Rank Sum test. GH concentrations were log-transformed prior to analysis. The comparison was also performed with Student's t-test (data not shown) - with consistent results. The comparison of complications occurrence (pituitary insufficiency, secondary hypogonadism, panhypopituitarism) between the groups was performed using the Chi-squared test.

Comparison of Complication Frequencies Between Male and Female Patients

The comparison for each complication was performed using the Chi-squared test with Benjamin-Hochberg False Discovery Rate correction (BH).

Analyzing Complications Occurrence With Respect to Patient Age

The analysis was performed separately for male and female patients. For each patient, we have found at what age the analyzed complication first occurred. Age values were categorized into 10-year ranges starting (0, 30], (30, 40] and so on till (80, 100]. The bins were then compared using Chi-squared with BH correction to see if the occurrence is related to patient age.

Clustering Analysis of Complication's Co-Occurrences

The heatmap was generated by performing agglomerative clustering first, to group patients that suffered from similar complications (this provided the patient clustering dendrogram), second, to group co-occurring complications (this provided the complications clustering dendrogram). In both cases, the Ward's clustering algorithm was used (16) with binary distance metric.

P-value less than 0.05 was considered statistically significant. The study was approved by the local ethical committee.

RESULTS

Among the group, there were 119 women (66%) and 60 men (34%). The median age of acromegaly diagnosis was 50.5 years old for women (age range, 20–78) and 46 for men (range, 24–76). During the last registered hospitalization, among patients with known disease status, 69 patients (39.7%) had cured acromegaly, 52 (29.9%) had still active disease and 53 (30.5%) were pharmacologically well-controlled. Over the years the proportion between active acromegaly patients and well-controlled patients decreased (**Figure 1**). In 5-year periods, analyzed from 2000 to 2018, there is a statistically significant decreasing trend in the number of active disease patients (80 vs. 29, 2000 to 2005 vs. after 2015, respectively; p=0.01), this is accompanied by an increasing fraction of well-controlled acromegaly patients - the trend of percentage of WCA cases was significantly increasing (7.1% vs. 43.5%, p=0.02).

In 132 patients, there was information about tumor size. Among patients with active acromegaly (102 patients), there was the predominance of macroadenomas (n=78, 76.5%). In this group, we found a higher ratio of incidence of macroadenomas to microadenomas in men (31/7) than in women (47/17), however, this difference was not statistically significant (p=0.49). In the tumor sizes analysis, we have found statistically significant correlations with IGF-I ($p<0.000001$) and GH levels ($p<0.000001$) (GH levels were log-transformed prior analysis - for details see Methods section) in the AA group.

139 patients underwent surgery: 110 were operated on once, 20 twice, 6 patients had three, and 3 had four surgeries. 134 patients had transsphenoidal and 10 transcranial surgeries. In 5 cases both types of operation were performed. 108 patients were operated once transsphenoidal, at 26 cases reoperations by the same method were performed.

GH concentrations and tumor sizes (maximal tumor dimensions) were significantly higher among males with more than one surgery compared to operated only once (p=0.00006; p=0.04, respectively). This difference was not observed among females. Interestingly, IGF-I concentrations were significantly lower among females, but not among males, operated more than once in comparison to females with one surgery (p=0.01). These results are presented in **Figure 2**.

In most cases (98/179 – 54.7%) combination therapy was performed. The most often chosen option of treatment was a combination of surgical and pharmacological therapy (in 45.3%, 81/179). Combination of surgery with radiotherapy was performed in 2.2% (4/179) and pharmacotherapy with radiotherapy in 1.1% (2/179). In 6.1% (11/179) combination of all the three methods were applied. In some cases, only one method of treatment was applied – surgery in 24.6% (44/179) and pharmacotherapy in 13.4% (24/179).

The type of pharmacological therapy used in patients underwent changes throughout the years. Sparsely available information regarding therapy used before 2000 indicates that most of the patients were treated with dopamine agonists (**Figure 3**). After 2000, somatostatin analogues became a dominant type of pharmacotherapy. Among them, up to 2015 octreotide LAR was preferred, and after 2015 – lanreotide Autogel became the most often chosen. Pasireotide LAR is used in few patients in recent years while pegvisomant was introduced in Poland later, in 2019.

Complications of Acromegaly

Metabolic disorders (hyperlipidemia, diabetes, and prediabetes) were the most frequently diagnosed complications in our study, followed by cardiovascular diseases and endocrine disorders (goiter, pituitary insufficiency, osteoporosis). More detailed data about the frequency of occurrence of specific complications and their distribution in males and females are presented in **Table 1**.

Hyperlipidemia was the most frequent comorbid disease and metabolic disorder (74%). We analyzed the lipid profiles from the first hospitalizations of the patients with hyperlipidemia. In 126/133 cases we had complete data about cholesterol and triglycerides levels. 56 patients (44%) had high cholesterol (>200 mg/dL) with normal triglycerides levels (≤150 mg/dL). 20 patients (16%) had isolated hypertriglyceridemia, whereas the coincidence of

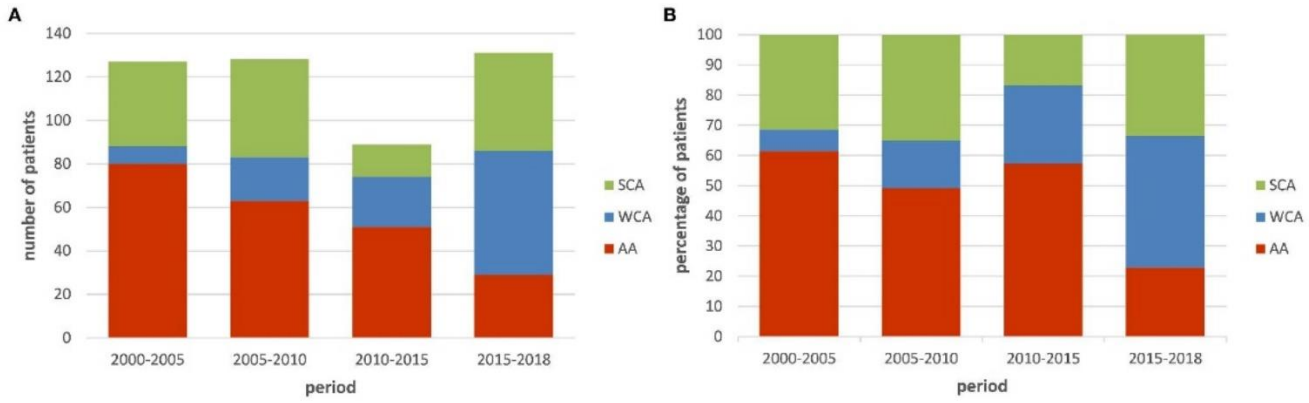

FIGURE 1 | Distribution of patients with active, controlled and cured acromegaly over 5-year time frames. **(A)** – the numerical value; **(B)** – the percentage value AA, active acromegaly; WCA, well-controlled acromegaly; SCA, surgery cured acromegaly.

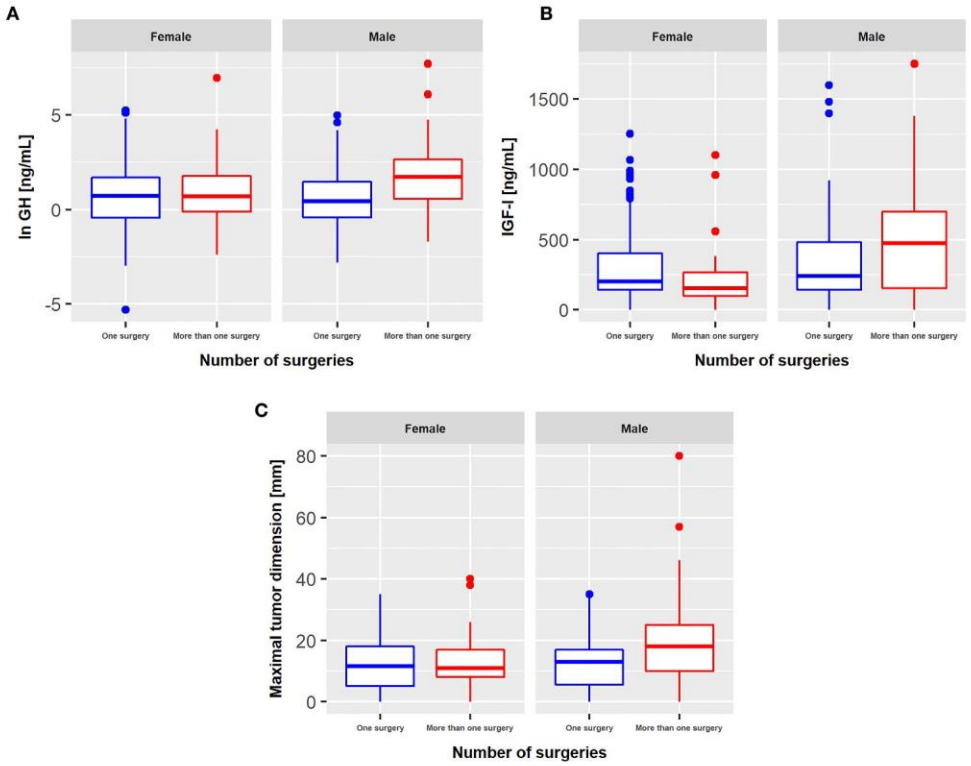

FIGURE 2 | Differences between patients operated once and patients with reoperations. **(A)** GH concentrations; **(B)** IGF-I concentrations; **(C)** maximal tumor dimensions.

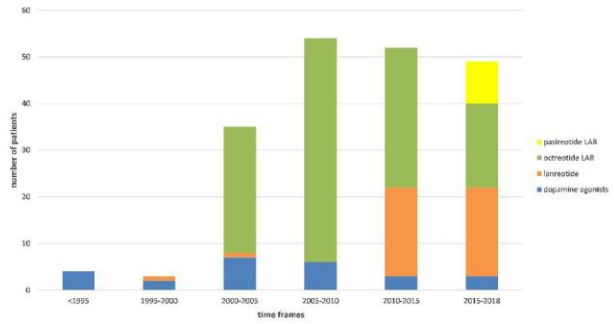

FIGURE 3 | Type of used pharmacotherapy over 5-year time frames.

hypercholesterolemia and hypertriglyceridemia was observed in 40 cases (32%). In 19 patients coexistence of hypertriglyceridemia with low levels of HDL (< 40 mg/dL in men and <45 mg/dL in women) was observed, however in 44 cases there is a lack of information about HDL results. Among cardiovascular diseases, hypertension occurred the most often (58%), and among endocrine – goiter (52%). Hyperlipidemia (90/119), hypertension (72/119), and goiter (65/119) were the most frequent complications in female patients. All the three occurred in more than half of the subgroup. In the male patients hyperlipidemia also dominated (43/60), hypertension was at the second place (31/60), but at third, there was hypopituitarism (30/60). Similarly, all of the three were observed in at least half of the subgroup.

Pituitary insufficiency (insufficiency of one pituitary axis), secondary hypogonadism, panhypopituitarism, and prediabetes occurred more frequently in males than females ($p=0.01$; $p<0.0005$; $p=0.003$; $p=0.01$, respectively). The analysis showed that in males pituitary insufficiency, as well as secondary hypogonadism was more common in patients after radiotherapy ($p=0.04$; $p=0.008$, respectively). Radiotherapy did not have a significant effect on the incidence of panhypopituitarism ($p=0.09$). The size of tumor did not have any impact on the incidence of pituitary insufficiency, secondary hypogonadism, panhypopituitarism ($p=0.23$; $p=0.52$; $p=0.21$, respectively). Among males and females with more than one surgery, pituitary insufficiency, secondary hypogonadism, and panhypopituitarism were observed more frequently compared to males and females with one operation (for males $p=0.001$; $p=0.002$; $p=0.009$, respectively, for females $p=0.0001$; $p=0.04$; $p=0.005$, respectively).

We further explored if specific complications occurred more frequently in specific age ranges within genders. In females, there was a higher occurrence of hypertension, diabetes, and osteoporosis in a range of 60–70 years. In males, hypertension was diagnosed a decade earlier than in females (in range 50–60).

TABLE 1 | Prevalence of complications and their distribution in genders.

Complication	Number of patients	%	F/M	%F	%M
Lipid disorders	133	74	90/43	76	72
Hypertension	103	58	72/31	61	52
Goiter	93	52	65/28	55	47
Joint degeneration	72	40	46/26	39	43
Hypopituitarism	66	37	36/30	30	50
Secondary hypogonadism	33	18	13/20	11	33
Secondary adrenal insufficiency	46	26	25/21	21	35
Secondary hypothyroidism	40	22	21/19	18	32
Panhypopituitarism	18	10	6/12	5	20
Changes in echocardiograms*	61	34	36/25	30	42
Prediabetes**	61	34	33/28	28	47
Diabetes***	59	33	44/15	37	25
Cholelithiasis	51	28	36/15	30	25
Arrhythmias	35	20	20/15	17	25
Osteoporosis	22	12	15/7	13	12
Nephrolithiasis	22	12	13/9	11	15
Colonic polyps	21	12	13/8	11	13
Ischemic heart disease	12	7	11/1	9	2
Heart failure	11	6	6/5	5	8
Carpal tunnel syndrome	8	4	7/1	6	2
Sleep apnea	3	2	1/2	1	3

*Left ventricular and interventricular septum hypertrophy, diastolic dysfunction, valvular defects – mainly mitral valve regurgitation, improper atrial and ventricular dimensions.
**Diagnostic criteria were: fasting plasma glucose between 100 and 125 mg/dL or two-hour plasma glucose value between 140 and 199 mg/dL.
***Diagnostic criteria were: fasting plasma glucose ≥126 mg/dL repeated twice or two-hour plasma glucose value of ≥200 mg/dL during a 75-g oral glucose test (OGTT), or symptomatic hyperglycemia (weight loss, polyuria, polydipsia) and blood glucose ≥200 mg/dL.

TABLE 2 | Prevalence of complications and distribution of the disease activity during the last hospitalization.

Complication	AA (%)	WCA (%)	SCA (%)	Total
Lipid disorders	32 (31)	29 (28)	43 (41)	104
Hypertension	29 (30)	34 (35)	34 (35)	97
Goiter	20 (24)	36 (43)	28 (33)	84
Joint degeneration	20 (33)	24 (39)	17 (28)	61
Hypopituitarism	19 (33)	19 (33)	20 (34)	58
Secondary hypogonadism	9 (33)	8 (30)	10 (37)	27
Secondary adrenal insufficiency	11 (29)	13 (34)	14 (37)	38
Secondary hypothyroidism	12 (32)	12 (32)	13 (35)	37
Panhypopituitarism	5 (33)	3 (20)	7 (47)	15
Changes in echocardiograms	8 (23)	14 (40)	13 (37)	35
Prediabetes	15 (54)	8 (29)	5 (18)	28
Diabetes	18 (34)	27 (51)	8 (15)	53
Cholelithiasis	11 (24)	25 (54)	10 (22)	46
Arrhythmias	10 (50)	7 (35)	3 (15)	20
Osteoporosis	6 (32)	6 (32)	7 (37)	19
Nephrolithiasis	6 (38)	3 (19)	7 (44)	16
Colonic polyps	1 (5)	10 (53)	8 (42)	19
Ischemic heart disease	2 (25)	5 (63)	1 (13)	8
Heart failure	3 (38)	3 (38)	2 (25)	8
Carpal tunnel syndrome	0 (0)	4 (67)	2 (33)	6
Sleep apnea	0 (0)	2 (67)	1 (33)	3

AA, active acromegaly; WCA, well-controlled acromegaly; SCA, surgery cured acromegaly.

Additionally, we analyzed the prevalence of each complication according to a distribution of the acromegaly activity during the last hospitalization (**Table 2**). In order to analyze the coincidence of complications at a more general level, we aggregated complications into the three groups: metabolic, cardiovascular and endocrine (**Table 3**). The coincidence of the groups of complications revealed that 50.8% of patients suffered from at least one complication from each group. Frequency of co-occurrence of metabolic and cardiovascular disorders as well as metabolic and endocrine diseases was 15.5% for both. Only 5.0% of patients did not suffer from any disease belonging to those main groups.

Furthermore, an evaluation of the coexistence of particular complications by using heatmap clustering was performed (**Figure 4**). It revealed that types of pituitary insufficiency co-occurred more frequently. On this map, also a tendency to the coexistence of diabetes, hyperlipidemia, hypertension, and heart remodelling was observed. In addition, a cluster including prediabetes and cholelithiasis was obtained. Groups of diseases distinguished from the clustering are presented in **Table 4**.

Analysis of Diagnostic Procedures

We investigated the frequencies of diagnostic procedures performed or ordered during hospitalizations in the Endocrinology Department for diagnostics and monitoring purposes. They are listed in **Table 5**. The results of colonoscopies are presented in **Table 6**.

We analyzed the changes in numbers of procedures in 5-years periods. We have found that there was an increasing trend in the number of performed thyroid ultrasounds (p=0.01). During this period, the number of performed abdomen ultrasounds correlated positively with the detectability of cholelithiasis (p=0.02). There were no significant trends in other procedures.

DISCUSSION

Acromegaly complications are the main factors contributing to lifespan and its quality in patients, so active diagnostics and treatment are essential. The knowledge about comorbidities of acromegaly has changed in the past decades. For this moment, radical surgical treatment or even pharmacological control of the disease can enable reversibility of some complications, but only if those are recognized and treated in early stages. So, it is very important to shorten the delay in the diagnosis of acromegaly. In addition, it is essential to improve the regularity of diagnostic exams that help us to recognize comorbidities and treat them earlier. Obtaining a strict control of hormone excess is the best strategy to limit the development of complications of acromegaly.

Patients Characteristics

In the population of our patients, we can observe substantial domination of females. Similar outcomes were reported in Polish multicenter study (17), as well as in other European registries (18–23). Not statistically significant, but also a higher ratio of incidence of macroadenomas to microadenomas in men compared to women was detected in the French population (21).

As we expected, positive correlations between maximal tumor dimensions and IGF-I and GH concentrations were obtained in patients with an active phase of the disease. Similarly, such correlations were observed for acromegaly patients before

TABLE 3 | Coincidence of complications belonging to the metabolic, cardiovascular and endocrine categories.

Metabolic	Cardiovascular	Endocrine	N	%
1	1	1	91	50.8
1	1	0	28	15.6
1	0	1	28	15.6
1	0	0	11	6.1
0	1	1	4	2.2
0	0	1	8	4.5
0	0	0	9	5.0

surgery (24). Adenoma measurements correlated positively with GH levels, but not with IGF-I levels in Tirosh et al. (25). and Evran et al. (26). studies, while Schwyzer et al. obtained contrary results with a significant correlation between preoperative tumor volume and IGF-I, but not with GH level (27). In Tirosh et al. study (25) an association between tumor volume and hypogonadism was determined, more pronounced in males, which was not obtained in our study. In that study also an association between size of tumor > 10 mm before the operation and the need for re-operation was observed. In our research, we also observed dependence between the maximal tumor diameter and reoperations in males, but in that analysis, we used data of maximal tumor diameter from all MRI results, also after an operation, not only before.

Acromegaly Treatment

Over the years, we observed a reduction in the number of patients with active acromegaly and an increase of pharmacologically controlled individuals. Consistent outcomes were depicted in the French registry (21). Many of the patients required additional treatment due to non-radical operation or recurrence. Sometimes decisions about reoperation are afflicted by difficult localization of the tumor, risk of damage to important structures (e.g. optic chiasm) or patients' coexisting diseases. Introduction and wide availability of somatostatin analogues allowed to achieve good control of disease activity and reduction in the occurrence of serious complications.

Epidemiology and Pathogenesis of the Main Groups' Complications

Cardiovascular Complications

Cardiovascular complications are the main causes of mortality in acromegaly patients (2, 3, 8, 13). Cure or even control of disease activity decrease the frequency and severity of comorbidities from this group. Some of them can even be totally reversed (28). In our study hypertension was the dominant complication from the cardiovascular group – it occurred in 58% of patients, which is consistent with previous studies. It has been reported that it occurs approximately in one-third of patients, ranging from 18 to 60% (4, 17, 28–30). What is more, the prevalence that we

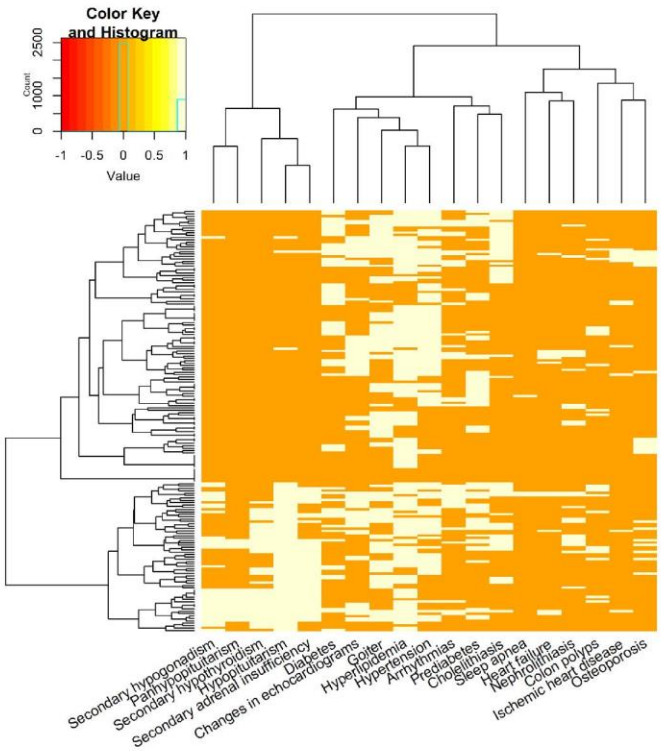

FIGURE 4 | Heatmap of particular complications co-occurrence. Each row in the heatmap denotes a patient. Each column is a complication. Bright fields mark complications that a patient suffered from. In addition, the dendrograms on the left-hand side and the top panel present grouping of patients, and grouping of complications, respectively.

TABLE 4 | Groups of complications obtained from heatmap clustering.

I	hypopituitarism, secondary hypogonadism, secondary hypothyroidism, secondary adrenal insufficiency, panhypopituitarism
II	diabetes, hyperlipidemia, hypertension, changes in echocardiograms, goiter
III	prediabetes, arrhythmias, cholelithiasis
IV	osteoporosis, ischemic heart disease, heart failure, sleep apnea, colonic polyps, nephrolithiasis

TABLE 5 | Number of procedures performed during hospitalizations in the Endocrinology Department.

Type of procedure	Number of procedures/496 hospitalizations	%
BP measurement	481	97.0
Lipid profile	462	93.1
Fasting glucose	453	91.3
OGTT (if no diabetes)	299	79.5
Electrocardiogram	391	78.8
Densitometry	235	47.4
Hormonal profile*	213	42.9
Abdomen ultrasound	224	45.2
Thyroid ultrasound	198	39.5
Echocardiogram	111	22.4
Colonoscopy	43	8.7

FSH, LH, PRL, TSH, fT4, morning cortisol, testosterone (in men), estradiol (in women).

TABLE 6 | The findings of colonoscopy in analyzed acromegaly patients.

Results	Number of patients
Normal endoscopic appearance	7
Colonic polyps	19
Proximal colon to splenic flexure	5
Distal colon to splenic flexure	9
Proximal and distal colon	3
Rectum	2
Diverticulosis of the sigmoid colon	5
Grades Internal hemorrhoids	14
I	6
II	5
III	1
IV	2
External hemorrhoids	2
Colitis	5
Ulcerative colitis	1
Spastic colon	1
Irritable bowel syndrome (IBS) suspicion	2

I, II, III, IV: grades of internal hemorrhoids.

obtained is higher than in the general Polish population, which is 42.7% (31). The etiology of hypertension in acromegaly is multifactorial. It includes, inter alia: increased plasma volume, hypertrophy of vascular smooth muscles, vascular stiffness, and endothelial dysfunction (28, 29). Management of hypertension in acromegaly patients does not differ from that applied in the general population (14).

In other databases, left ventricle hypertrophy has been observed in 70–90% of reported cases [but only in 15% of patients in Liege Acromegaly Survey Database (19)] while valve diseases in 20 to 75% (4, 28). In our study changes in echocardiograms (left ventricular and interventricular septum hypertrophy, diastolic dysfunction, valvular defects – mainly mitral valve regurgitation, improper atrial and ventricular dimensions) were found in 34% of the cases. The excess of GH and IGF-I is known to contribute to myocardial concentric hypertrophy, which leads to a decrease in the cardiac output and heart failure (29). We observed heart failure only in 6% of the cases, whereas the prevalence of this complication was previously described in approximately 10% of the cases (4). These differences may be a consequence of performing fewer examinations, including echocardiograms in the past.

There are conflicting data regarding acromegaly's contribution to a higher occurrence of ischemic heart disease (32). The probable origin of this complication is not associated with GH and IGF-I excess levels, but with other comorbidities like dyslipidemia, insulin resistance, hypertension, and sleep apnea (28). Ischemic heart disease was diagnosed in 7% of our patients. The incidence in previous registries was between 2.5 and 12% (17, 19, 28).

The most common arrhythmias observed in acromegaly are atrial fibrillation, sinus or ventricular tachycardia, sick sinus syndrome, and ventricular extrasystoles (28). Arrhythmias were reported to affect 7% to 48% of patients (4, 28). In our study, we observed them in 20% of the cases. The number of performed electrocardiograms was high (78.8%), but it is a short time examination and it is possible that some disturbances might be detected only in Holter ECG.

Metabolic Complications

The prevalence of diabetes in acromegaly patients is 12% to 53% (17, 19, 30, 33, 34). We observed diabetes in 33% of the cases and prediabetes in 34%, which is much higher than in the general Polish population (6.97%) (25). In Warncke's study, it has been reported that in acromegaly diabetes affects younger patients (50.1 years) than in the general population (59 years) (35). Comparable results were observed for the Italian population (20). In our research, the dominant age of diabetes diagnosis was 60 to 70 and 50 to 60 years for prediabetes, respectively. Factors that contribute to disturbances in glucose metabolism in acromegaly are complex (33). GH is an antagonist hormone to insulin, which increases lipolysis and gluconeogenesis and predisposes to insulin resistance, which is the main factor in diabetes origin (33, 36). Normalization of GH concentration decreases glycemia and improves insulin sensitivity (37). It is important to highlight that treatment with somatostatin analogues, especially pasireotide, may impede control of diabetes and increase insulin resistance (13, 38). Diabetes therapy in acromegaly does not differ from generally used in type 2 diabetes (2, 19).

Dyslipidemia, depending on the source, affects 13-51% or even up to 71% of acromegaly patients (8, 35). GH induces lipolysis and increases levels of plasma free fatty acids (8). Hypertriglyceridemia and decreased HDL level are the main lipid abnormalities in acromegaly (8, 36). In our study dyslipidemia was the most frequent complication in the whole group and it was observed in 74% cases, so it was even higher incidence compared to the previous studies (8, 35). Moreover, we observed elevated triglycerides in 48% cases, which is also higher in comparison to the other reports (4).

Endocrine Complications

There is a proven correlation between thyroid gland volume and GH and IGF-I levels (39, 40). The prevalence of goiter in acromegaly patients reaches between 25 and 92% of the cases (17, 19, 39, 41, 42). We observed this complication in 52% of our patients. Furthermore, a predisposition for the autonomous function of some nodules in nodular goiter contributes to hyperthyroidism (39). Some studies suggest a higher incidence of thyroid cancer in acromegaly (39, 43). In the presented series, we diagnosed one follicular thyroid cancer and one papillary thyroid cancer.

Hypopituitarism is caused by compression or damage of the pituitary gland by an expansion of adenoma or it is an iatrogenic complication that appears after surgery or radiotherapy. The prevalence of hypopituitarism in our study was 37% and it dominated in the male population. Similar prevalence of hormonal deficiency was reported in the Belgian registry (44). On the other hand, for example in the USA database, this complication was observed only in 16.6% of the cases (30). Secondary adrenal insufficiency was the most frequent alternation in our research. What is more, we observed hypopituitarism in 64.7% of patients after radiotherapy. It is known that radiotherapy increases the risk of hypopituitarism – in this group it is diagnosed in more than half of the patients (36, 44). In contrary to the Italian registry (20), we observed the significant impact of radiotherapy only for hypogonadism in males. Hypopituitarism, secondary hypogonadism and panhypopituitarism were revealed more often in the male population. In the Italian registry, hypogonadism also predominated in males (20).

Acromegaly is also associated with secondary osteoporosis and fractures. Our research revealed osteoporosis in 12% of the cases. We did not have complete information about fractures in the analyzed group of patients. In previous studies, osteoporosis was observed in 12-32% of patients (19, 45). It is known that a higher risk of fractures may occur despite normal bone mineral density (BMD) (46). So, we still need new tools to estimate the risk of fractures in acromegaly. Assessment of bone quality can improve this evaluation. Recently introduced trabecular bone score (TBS) method is a promising tool, which enables to assess bone tissue microarchitecture (47, 48).

Coexistence of Complications

In more than half of patients, we observed co-occurrence of cardiovascular, metabolic and endocrine complications. This finding illustrates that they are common problems in acromegaly and acromegaly patients often require complex healthcare and multi-component therapy. Cardiovascular and metabolic, as well as metabolic and endocrine complications co-occurred more frequent than cardiovascular and endocrine disorders. The coincidence of hypertension, heart structural changes, hyperlipidemia and diabetes was also exemplified in heatmap clustering evaluation. Besides the impact of GH and IGF-I excess on cardiovascular and metabolic diseases, similar environmental factors contribute to the origin of morbidities classified in those groups. We speculate that obtained cluster of prediabetes and cholelithiasis coexistence may indicate on the group of patients treated with somatostatin analogues. Moreover, in the analysis of disease activity (**Table 2**), diabetes and cholelithiasis were observed in more than 50% in the well-controlled acromegaly patients treated with somatostatin analogues.

Diagnostics of Complications

We decided to determine if patients from our department underwent procedures that are recommended in the guidelines. According to current worldwide and Polish guidelines (14, 49) BP measurement, fasting glucose or OGTT should be performed every six months; ECG, echocardiography, Epworth scale, spine X-ray, lipid and hormonal profile (towards hypopituitarism diagnostics) and Acromegaly Quality of Life Questionnaire (AcroQoL) annually; thyroid ultrasound every 1 to 2 years; DXA every 2 years and colonoscopy every 10 years. In order to do that we calculated the general number of each procedure during 496 hospitalizations (**Table 5**). According to obtained results BP measurement, ECG, lipid profile, fasting glucose, or OGTT were performed the most often. The least frequent examinations were colonoscopy and echocardiogram. None of our patients had polysomnography. This might be a result of the clinic profile. Polysomnography, colonoscopy and echocardiogram require the presence of other specialists to be performed. Unrecognized sleep apnea, colon cancer or heart failure can lead to serious consequences. It is important to highlight that in care of acromegaly patients, the involvement of different types of specialists is needed and that each patient should obtain referrals for additional consultations when needed. In 21 patients (12%) (in 19 cases colonoscopy was recommended in the Endocrinology Department, in 2 an information about diagnosis was included in the medical histories) colonic polyps were observed. Colonic polyps are the most frequent types of tumors in acromegaly (39), so obtained rate indicates that this diagnosis could be underestimated in the group of our patients, but it is similar to observed in the Liege Acromegaly Survey Database (19). Hemorrhoids and diverticulosis, which also are reported to be associated with GH and IGF-I excess (4), were detected in 37 and 12% of examinations, respectively. The number of performed abdomen ultrasounds correlated positively with cholelithiasis detectability. Cholelithiasis might be a side effect of somatostatin analogues treatment, thus ultrasound is recommended, especially in pharmacologically treated group. In addition, the medical histories lack the information regarding the performance of AcroQoL, thus we speculate that it has not been conducted. Nowadays recommendations and studies emphasize that life quality assessment is very important in the evaluation of the effectiveness of treatment in acromegaly (10, 11, 14).

CONCLUSIONS

In our population we observed female predominance. Over the years we noticed a reduction in the number of patients with active acromegaly and an increase of patients with

pharmacologically controlled disease. More than 50% of patients demonstrated a coexistence of cardiac, metabolic and endocrine disturbances and what is important to decline only 5% of patients did not suffer from any disease from those main groups. For these reasons, an obtain a strict control of hormone excess is the best strategy to limit the development of complications of acromegaly.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Bioethics Committee of the Wroclaw Medical University, Wrocław, Poland. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

MR, AJ-P, and MB contributed to the study conception and design. Material preparation and data collection were performed by MR, GZ, AJ-P, JH-Ż, MK, and IB. MR created the database. BK performed the statistical analysis. MR, AJ-P, JH-Ż, and BK interpreted the results. The first draft of the manuscript was written by MR and AJ-P and corrected by JH-Ż, BK, GZ, and MB. All authors contributed to the article and approved the submitted version.

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Publikacja 3

Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study

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Is H19 RNA a Useful Marker of Acromegaly and Its Complications? A Preliminary Study

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Abstract: Acromegaly is a rare endocrine disorder caused by somatotroph pituitary adenoma. Besides its typical symptoms, it contributes to the development of cardiovascular, metabolic, and bone comorbidities. H19 RNA is a long non-coding RNA and it is suspected to be involved in tumorigenesis, cancer progression, and metastasis. H19 RNA is a novel biomarker for the diagnosis and monitoring of neoplasms. Moreover, there might be an association between H19 and cardiovascular and metabolic diseases. We enrolled 32 acromegaly patients and 25 controls. We investigated whether whole blood H19 RNA expression is associated with the diagnosis of acromegaly. Correlations between H19 and tumour dimension, invasiveness, and biochemical and hormonal parameters were evaluated. We analysed the coincidence of acromegaly comorbidities with H19 RNA expression. In the results, we did not observe a statistically significant difference in H19 RNA expression between acromegaly patients and the controls. There were no correlations between H19 and the adenoma size and infiltration and patients' biochemical and hormonal statuses. In the acromegaly group, hypertension, goitre, and cholelithiasis were observed more frequently. The diagnosis of acromegaly was a factor contributing to the occurrence of dyslipidaemia, goitre, and cholelithiasis. We found an association between H19 and cholelithiasis in acromegaly patients. To conclude, H19 RNA expression is not a relevant marker for diagnosis and monitoring of acromegaly patients. There is a higher risk of hypertension, goitre, and cholelithiasis related to acromegaly. Cholelithiasis is associated with a higher H19 RNA expression.

Keywords: H19; acromegaly; lncRNA

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1. Introduction

Acromegaly is a disease caused by excess production of growth hormone (GH), mainly due to pituitary somatotroph adenoma [1]. Pituitary adenomas are benign tumours originating from adenohypophysis cells [2,3]. Regardless of the lack of malignant potential, some of them can be locally invasive or have high cell proliferative activity [2], leading to nonradical treatment or a higher risk of recurrence. Somatotroph adenomas are mainly macroadenomas, defined as tumours exceeding 10 mm in the maximal dimension [4]. Due to their size, some of them infiltrate adjacent structures, such as optic chiasm, sphenoid and cavernous sinuses, and bone. The clinical manifestation of acromegaly results from GH and insulin-like growth factor I (IGF-I) overproduction and tumour mass effect. Acromegaly patients present facial and acral enlargement, joint pain, headaches, and bitemporal hemianopsia. Systemic complications, including bone and joint, cardiovascular, respiratory, and metabolic comorbidities, are the major problems affecting patient quality of life and life expectancy [5–7]. Multidisciplinary care is recommended for effective management. Furthermore, a delay in the diagnosis of acromegaly is a common problem due to the variety of symptoms and rare prevalence of the disease [8]. Therefore, novel biomarkers could be helpful tools in diagnosis and monitoring of acromegaly patients.

Recently, there has been a wealth of research presenting hypotheses about the pathogenesis of neoplasms' origin and processes contributing to their progression. The search for novel biomarkers that could be useful in early stage diagnosis, which could optimize management and patient monitoring, is an important matter. Non-coding RNAs are a huge part of the human genome and lack an open reading frame encoding a functional protein [9]. Long non-coding RNAs (lncRNAs) are nucleic acids over 200 nucleotides long. They have a role in the regulation of transcription, translation, and epigenetic processes [10,11]. Among them, H19 lncRNA is the earliest investigated and was first described by Pachnis et al. in 1984 [12]. Human H19 is a 2.3-kb RNA molecule encoded by the H19 gene located on chromosome 11p15.5 [13]. Initially, H19 RNA was reported to be related to embryogenesis, with its high expression in the foetal heart, muscle, and liver. It is also downregulated after birth [12,14]. Further investigations revealed its potential role in tumorigenesis. The contribution of the different mechanisms of H19 to neoplasm origins and progression has been investigated. The H19 gene is expressed only from the maternal allele in the imprinting process [15]. The loss of imprinting and alterations in the methylation pattern of promoter sequences were initially evaluated processes in colorectal, lung, and oesophageal cancers [16,17]. Moreover, research revealed that H19 promotes cell migration, invasion, and angiogenesis and inhibits apoptosis [18]. Further investigations determined the role of H19 as an oncogene [19–22] or, on the contrary, as a tumour suppressor [23,24]. Up to now, the association between H19 and different types of cancers has been revealed for, i.e., breast [25,26], lung [17,22,27], oesophageal [28], colorectal [29,30], and bladder [31,32] cancers. Due to the variable expression of H19 in different types of tumours, its role as a tumour marker was speculated. Moreover, the downregulation of H19 expression in postoperative patients was revealed in some types of cancers [32–34]; hence, the monitoring function of H19 was revealed.

Evidence that H19 lncRNA contributes to tumorigenesis has been shown in many types of malignancies. However, papers concerning H19 and pituitary tumours are limited. The majority of previous studies has been performed on cell lines or animal models. Only one research work has characterized the role of H19 in a large group of patients with pituitary adenomas [35]. There is a lack of studies focused on patients diagnosed with acromegaly.

In some studies, the inhibitory role of H19 in pituitary adenomas has been suggested [36]. The downregulation of H19 RNA in pituitary adenoma tissues compared with the normal pituitary glands has been revealed [37]. H19 has been reported to suppress tumour growth by inhibiting the phosphorylation of 4E-binding protein 1 (4E-BP1). Moreover, the expression of H19 in plasma exosomes of patients with pituitary adenomas was significantly lower than in healthy controls [35]. Additionally, the suppressive influence of H19 on tumour cell proliferation and the negative correlation between H19 and pituitary tumour volume has been shown [35,37]. In recent studies, its synergistic effect with cabergoline on inhibiting tumour growth has been demonstrated for prolactinomas [35,38]. To the best of our knowledge, the interaction between H19 and somatostatin analogues has not been explored.

H19 has also been determined to play a role in the pathogenesis of other diseases. This has been shown for central nervous system disorders, such as epilepsy, Parkinson's, and Alzheimer's diseases [36]. Additionally, H19s associations with osteoarthritis [39], osteoporosis [40], cardiovascular [41,42], and metabolic disorders [43,44] have been revealed.

lncH19 RNA presents itself as a novel biomarker of tumorigenesis, and since the papers focusing on GH-secreting adenomas are limited, the aim of the present study was to investigate whether lncH19 RNA expression could be useful in acromegaly patients. Additionally, we evaluated whether H19 expression depends on the clinical characteristics of the patients, their treatment, tumour invasiveness, or prevalence of acromegaly comorbidities to explore if H19 RNA could be applied in prognostics and monitoring of the status of the disease and its complications.

2. Materials and Methods

The study group consisted of 32 patients with acromegaly (24 women and 8 men; mean age 51.97 ± 13.93 yrs). Twenty-five healthy patients were enrolled in the control group (16 women and 9 men; mean age 41.08 ± 13.79 yrs). The inclusion criterion for the study group was the diagnosis of acromegaly, either currently or in the past, defined as elevated IGF-I levels and unsuppressed GH in OGTT, according to the current recommendations of the Endocrine Society and Polish Society of Endocrinology [1,45]. The exclusion criteria for the control group were pituitary tumours confirmed by MRI or the history of other neoplasms. All the participants were recruited from the Department of Endocrinology, Diabetes and Isotope Therapy, Wroclaw Medical University. The bioethics committee of Wroclaw Medical University approved the protocol of the study (no. KB-36/2020). All subjects signed informed consent forms following the Declaration of Helsinki.

Medical histories were taken from all the participants, including current and past treatment and coexisting diseases. These data were used to categorize the patients into subgroups of the acromegaly de novo, pharmacologically treated or successfully operated patients. We recorded patients' weight and height and calculated their body mass indexes (BMI). Fasting venous blood samples were collected from all the participants. IGF-I levels were expressed in relation to the upper limit of normal (ULN) for age (patient's IGF-I concentration divided by IGF-I upper reference range limit matched for age). A pituitary MRI examination (1.5 Tesla) was performed on all the participants. MRI was not repeated if it had been performed in the past 6 months. According to these results, we classified the pituitary tumours as macro- (if at least one dimension was ≥10 mm) or microadenomas and analysed their extrasellar expansion.

The GH, IGF-I, thyrotropic hormone (TSH), free thyroxine (fT4), prolactin (PRL), follicle-stimulation hormone (FSH), luteinizing hormone (LSH), oestradiol (E2), total testosterone (T), dehydroepiandrosterone sulphate (DHEAS), adrenocorticotropic hormone (ACTH), and cortisol concentrations were assayed using a chemiluminescence immunoassay. Vitamin D levels were also measured by chemiluminescence immunoassays. Serum calcium was assayed using a colorimetric assay with a reference range of 8.4–10.2 mg/dL. Glucose levels were measured using the hexokinase method. The total cholesterol was assayed using a routine enzymatic method with oxidase, esterase, and peroxidase; low-density lipoprotein (LDL) was estimated by the Friedewald Equation; high-density lipoprotein (HDL) was measured by the direct assay precipitation method using a combination of polymer polyanions; and triglycerides were assayed using a routine enzymatic method.

Five millilitres of whole blood samples from all the participants were collected in PAXgene Blood RNA Tubes (Qiagen GmbH, Hilden, Germany) and stored at −70 °C until further processing. The total RNA from collected samples was extracted according to the protocol of PAXgene Blood miRNA (Qiagen GmbH, Hilden, Germany).

The concentration and quality of the purified RNA were evaluated by measuring the absorbance at A260 and A280 nm in a NanoPhotometer Touch UV/VIS (Implen GmbH, München, Germany). The recommended optimal and equal amount of total RNA was reverse transcribed using a RT2 First Strand Kit (Qiagen GmbH, Hilden, Germany).

Two genes, GAPDH and ACTB, were used as the reference genes for the normalization of lncH19 expression in the presence of XpressRef Universal Total RNA control (Qiagen GmbH, Hilden, Germany).

Reactions as controls for cDNA contaminations, NTC (no template control) and NRT (no reverse transcriptase control), were also prepared for all the experimental samples.

A quantitative real-time PCR reaction was performed by using a RT2 qPCR FAST SYBR Green according to the protocol provided by the manufacturer (Qiagen GmbH, Hilden, Germany).

Analyses of gene expression were performed on a Corbett Rotor-Gene Real-Time cycler (Qiagen GmbH, Hilden, Germany). All experiments were run in duplicate. The Ct (cycle threshold) was averaged for each sample. The relative gene expression of lncRNA H19 was evaluated by using the 2-ΔΔCt method.

Statistical analyses were performed using Statistica 13.3 (TIBCO Software, Inc., Palo Alto, CA, USA). Variables were presented as means with standard deviations (SD) and medians with interquartile ranges (IQR). A Shapiro–Wilk test was used to determine the normality of the variables. A student's t-test was applied when normality was indicated. Mann–Whitney and Kruskal–Wallis tests were used for data not normally distributed. Proportional differences were tested using Chi-square Pearson's and Fisher's exact tests. Correlations were determined using Spearman's rank correlation test. Logistic regression was used to test if an interaction between acromegaly and age influenced the occurrence of comorbidities. A robust nonparametric ANCOVA in R using the WRS2 package [46] was used to analyse the association between age and acromegaly diagnosis with H19 expression. p values of <0.05 were considered statistically significant.

3. Results

The general characteristics of the acromegaly patients and the controls are presented in Table 1. The mean weight and median body mass index (BMI) were higher compared to the control group (p = 0.018 and 0.001, respectively), whereas the mean height was lower in the acromegaly group (p = 0.045).

Table 1. General characteristics of acromegaly patients and the control group.

	Acromegaly (N = 32)			Controls (N = 25)			
	Mean ± SD	**Median**	**IQR**	**Mean ± SD**	**Median**	**IQR**	***p***
Sex (F%/M%)		75/25			64/36		0.368
Age (years)	51.97 ± 13.93	48.00	43.00–64.50	41.08 ± 13.79	39.00	30.00–49.00	0.005
Height (cm)	165.11 ± 8.51	164.00	158.5–170.5	170.17 ± 9.05	168.00	164.00–180.00	0.045
Weight (kg)	84.43 ± 18.74	83.50	70.50–99.50	71.63 ± 18.36	69.00	57.00–82.00	0.018
BMI (m^2/kg)	30.92 ± 6.42	31.05	26.37–35.27	24.55 ± 5.14	24.54	19.37–29.39	0.001
IGF-I (ng/mL)	275.31 ± 199.08	181.50	131.00–395.00	136.28 ± 54.29	138.00	93.70–153.00	0.004
IGF-I/ULN	1.12 ± 0.78	0.78	0.65–1.39	0.49 ± 0.16	0.50 ± 0.16	0.37–0.58	0.000

The study group included five patients with acromegaly de novo, fifteen patients during treatment with somatostatin analogues (SSA), and a group of twelve successfully operated patients. Twenty patients underwent one transsphenoidal surgery, three patients were operated on twice, and one patient was operated on three times with this method. One patient had transcranial reoperation. Radiotherapy was performed in four cases. Neither surgery nor radiation of the pituitary gland region were performed in the control group. Among patients who belonged to de novo or SSA-treated subgroups, eleven (55%) patients had macroadenomas (mean maximal dimension 18.7 mm). In the acromegaly de novo group, three of five patients had macroadenomas (mean maximal dimension 17.3 mm). Extrasellar invasion was detected in eight patients (40%). Pituitary insufficiency was diagnosed in fourteen acromegaly patients and six controls. The most frequent impairment in the acromegaly group was secondary hypogonadism. All patients received adequate substitution of hormones.

3.1. H19 Expression

There was no statistically significant difference in H19 expression between women and men. A significant positive correlation between H19 expression and age was obtained in the acromegaly group (p = 0.025; r = 0.396 for $-\Delta\Delta$Ct H19-BACT), which was not observed for the controls. On account of the age difference between acromegaly and control groups, a nonparametric ANCOVA in R with WRS2 package was used. We found no difference in H19 expression between acromegaly patients and the control group (the interaction between

age and the occurrence of acromegaly did not influence variables in H19 expression; the results are illustrated in Table 2).

Table 2. H19 expression in acromegaly and control groups taking into account age (nonparametric ANCOVA in R with the WRS2 package).

	Age	n1	n2	Difference	SE	Lower CI Limit	Upper CI Limit	Test Value	*p*-Value
−ΔΔCt H19-BACT									
	37	18	18	0.00000	0.00030	−0.00080	0.00080	0.06	0.955
	39	16	19	0.00010	0.00030	−0.00070	0.00090	0.30	0.764
	45	19	14	−0.00010	0.00030	−0.00100	0.00090	0.23	0.819
	47	19	13	0.00010	0.00030	−0.00100	0.00110	0.15	0.882
	50	21	12	0.00010	0.00040	−0.00110	0.00130	0.32	0.756
−ΔΔCt H19-GAPDH									
	37	18	18	0.00030	0.00030	−0.00050	0.00120	1.20	0.244
	39	16	19	0.00040	0.00030	−0.00040	0.00120	1.42	0.175
	45	19	14	0.00020	0.00030	−0.00080	0.00110	0.49	0.630
	47	19	13	0.00020	0.00040	−0.00080	0.00120	0.55	0.589
	50	21	12	0.00000	0.00040	−0.00100	0.00110	0.06	0.950

We found no difference in H19 expression between acromegaly patients and the control group (Table S1). Additionally, H19 expression did not correspond with the hormonal statuses of the patients. No significant correlations between H19 and GH, IGF-I, or IGF-I x ULN concentrations or other hormones levels were obtained. We did not observe a variable expression of H19 between the groups of patients without operation or radiotherapy, successfully cured patients, and the controls (Table S2). We also did not observe statistically significant differences in the expression of H19 between successfully treated patients compared to unoperated patients or patients with ineffective surgery, as well as in comparison to the control group (Table S3).

Patients with active acromegaly (de novo or uncontrolled during SSA therapy) did not vary in H19 expression from radically operated or controlled subjects (Table S4). Additionally, SSA-treated patients did not differ in H19 expression between de novo patients and the controls (Table S5). Patients treated with radiotherapy did not present variable H19 expression from de novo patients and the control group (Table S6).

We found no correlation between maximal tumour dimension and H19 expression. The H19 expression was not associated with extrasellar invasion. No variations in H19 expression were observed between patients with pituitary insufficiency among all participants nor between acromegaly and control groups.

No significant correlations were found between H19 expression and biochemical results (glucose, total cholesterol, HDL, LDL, triglycerides, calcium, and vitamin D) in acromegaly patients.

3.2. Comorbidities

The occurrence of comorbidities is presented in Table 3. Goitre, dyslipidaemia, and hypertension were the most frequent comorbid diseases in the acromegaly group. They were observed in at least half of the group. Acromegaly patients suffered from hypertension, goitre, and cholelithiasis more frequently compared to the control group. The difference for dyslipidaemia was on the verge of statistical significance.

We analysed which factor—a diagnosis of acromegaly or age—contributed to those variables. According to the logistic regression, we found that age, not acromegaly, was a significant predictor for the occurrence of hypertension (p = 0.000; OR = 1.152; 95%CI 1.063–1.200; R2 = 0.556). For dyslipidaemia, we found that acromegaly was a significant predictor (p = 0.024; OR = 89.738; 95%CI 1.102–7307.195; R2 = 0.203), independent from age. A similar result was achieved for goitre (p = 0.027; OR = 2328.933; 95%CI 2.366–2,292,682.85;

R2 = 0.458). Cholelithiasis was observed only in acromegaly patients. Age was not associated with the prevalence of this complication ($p = 0.880$).

Table 3. Comorbidities in acromegaly and the controls.

	Acromegaly (N/%)	Controls (N/%)	*p*
Hypertension	16 (50.0)	5 (20.0)	0.020
Dyslipidemia	21 (65.6)	10 (40.0)	0.054
Diabetes	9 (28.1)	2 (8.0)	0.090
Prediabetes	8 (25.0)	1 (4.0)	0.063
Goiter	21 (65.6)	3 (12.0)	0.000
Nephrolithiasis	3 (9.4)	0	0.248
Cholelithiasis	12 (37.5)	0	0.001
Colon polyps	3 (9.4)	0	0.248
Osteoporosis	2 (6.25)	1 (4.0)	1.000
Pituitary insufficiency	14 (43.75)	6 (24.0)	0.121

Patients with cholelithiasis had higher H19 expressions compared to patients without cholelithiasis and the controls. This result was on the verge of statistical significance in the Kruskal–Wallis test ($p = 0.039$) and in post hoc tests (acromegaly with cholelithiasis vs. acromegaly without cholelithiasis $p = 0.068$; acromegaly with cholelithiasis vs. controls: $p = 0.058$). We did not observe any significant variation in H19 expression depending on the occurrence of hypertension, dyslipidaemia, diabetes, prediabetes, osteoporosis, goitre, nephrolithiasis, or colon polyps.

4. Discussion

In our research, we did not find a significant variation in H19 expression between patients with somatotroph adenomas and healthy controls. Our results do not correspond with previous studies, where the inhibitory role of H19 in pituitary adenomas was suggested [35]. An analysis of H19 expression in peripheral whole blood, not pituitary gland tissues or plasma exosomes, might affect the results of our experiment. The majority of previous studies analysed the tissue expression of H19. Upregulated tissue-specific expression of H19 was found in, e.g., breast [26], colorectal [29], and oesophageal [28] cancers. On the contrary, in one of the studies, the diagnostic value of tissue H19 was not presented for breast cancer [47]. For pituitary glands, Wu et al. revealed the downregulation of H19 in tissue samples of pituitary tumours compared to non-neoplastic gland specimens [37]. The plasma expression of H19 was significantly higher, and hence was presented as a useful marker in non-small cell lung [27] and gastric [48] cancers. Additionally, peripheral blood H19 was presented as a useful diagnostic tool in sepsis [49], myocardial infarction [42], osteoarthritis [39], and epilepsy [50]. Exosomal H19 expression has been proven as a marker of breast and bladder cancers [32,34]. Regarding pituitary tumours, Zhang et al. proved a reduced H19 expression in exosomes derived from the blood of patients with all types of pituitary adenomas [35]. For breast cancer, both tumour tissue and plasma H19 expressions were increased compared to healthy controls [33]. Additionally, in diabetes mellitus patients, there was no significant difference between serum and exosomal expression of H19 [51]. There is a lack of studies comparing tissue or exosomal H19 expression with the whole blood on the subject of pituitary adenomas.

We did not find any association between H19 and maximal tumour dimension. In contrast to the above reports, a significantly higher H19 expression was reported in invasive GH tumour samples in comparison to non-invasive ones [52]. However, in our study, we did not observe any differences in H19 expression between patients with invasive and non-invasive adenomas. We also did not observe a distinction in H19 expression between patients de novo and pharmacologically treated. The impacts of dopamine agonists have been evaluated in previous papers [35,38] and there is no information concerning somatostatin analogues to compare. In our study, we did not observe a variation in H19 expression

between the patients after surgery or radiotherapy and the untreated group. To the best of our knowledge, there are no research works analyzing H19 expression in terms of surgical or radiation treatment of pituitary adenoma patients.

Moreover, the lack of a difference in H19 expression between women and men agrees with previous studies [27,39]. We observed a positive correlation between H19 expression and age in acromegaly patients, which is in contrast to some studies that presented no correlation between H19 expression and age [27,53].

Acromegaly patients are at a high risk of cardiovascular diseases, metabolic disorders, and endocrine comorbidities [6]. Excesses of GH and IGF-I lead to remodelling of tissues and organs, including the heart and vessels; increases in lipolysis and insulin resistance; disruption of bone turnover; and predisposition of the patient to thyroid enlargement and colon polyps [54]. The distribution of comorbidities in acromegaly patients detected in this research is similar to the results that we presented in our previous observational study [55]. Dyslipidaemia, goitre, and hypertension were the complications with the highest frequency. Hypertension and dyslipidaemia are also diseases of affluence, with a high prevalence in the general population. However, we revealed that acromegaly was an independent factor contributing to the occurrence of dyslipidaemia and high triglycerides level. No statistical correlation was found between lipid profile and H19. Similar results were presented by Safaei et al. [42]. The occurrence of acromegaly was not a prognostic factor for hypertension, diabetes, or prediabetes, whereas those complications were also frequent in the group of acromegaly. High occurrences of hypertension and metabolic disorders enhance the risk of cardiovascular events. The occurrence of goitre was significantly higher in the study group compared to the controls, and the diagnosis of acromegaly was an independent factor influencing this result. The extremely high OR and an upper limit of 95% confidence could be the result of a small sample size. In the literature, a higher predisposition to goitre, autonomous function, and even thyroid cancer was described [54,56]. Additionally, we observed a higher incidence of cholelithiasis in acromegaly patients. Acromegaly by itself predisposes the patient to this complication, but treatment with somatostatin analogues may enhance the risk [57,58]. Moreover, patients with the co-occurrence of cholelithiasis had higher H19 expressions in comparison to patients without cholelithiasis and the controls. In the literature, there is a lack of information about the association between H19 and cholelithiasis, so this result is a novel finding. Fawzy et al. found an increased H19 expression in the serum of patients with type 2 diabetes [44], whereas contrasting results and a decreased H19 expression were reported by Alfaifi et al. [43]. In our report, we did not observe significant differences in H19 expressions between diabetic and non-diabetic patients. Furthermore, some contrasting results were reported for the H19 expression in postmenopausal osteoporosis patients. In Xiaoling et al.'s research, a significant decrease in H19 was shown [59], whereas Li et al. found significantly a higher expression in this group [60]. We did not obtain a different H19 expression in patients who suffered from osteoporosis. Overexpression of H19 in colorectal cancer has also been reported [61,62]. In our patient group, only benign colon polyps were observed. Patients with colon polyps did not present different H19 expressions.

5. Conclusions

To the best of our knowledge, this is the first study focusing on whole blood lncH19 RNA expression in acromegaly patients. According to the obtained results, the whole blood expression of H19 is not a relevant diagnostic or monitoring marker for acromegaly. H19 is an intriguing biomarker for malignancies; however, we did not find advantages for its use for patients with benign pituitary tumours. Due to the small sizes of the groups, especially of de novo patients, which is a limitation of our study, these are preliminary results, and further investigations should be continued with larger groups. Additionally, association between tissue-specific and circulating H19 expression is a subject that should be explored. Acromegaly patients are at a higher risk of comorbid diseases, especially hypertension,

dyslipidaemia, and goitre. For the first time, an association between cholelithiasis and H19 expression in acromegaly was revealed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biomedicines11041211/s1, Table S1: Difference in H19 expression between acromegaly (1) and the control group (2); Mann-Whitney test; Table S2: Difference in H19 expression between patients without operation and radiotherapy (1), successfully cured patients (2) and the control group (3); Kruskal-Wallis test; Table S3: Difference in H19 expression between patients unoperated/ineffectively operated (1), successfully operated (2) and the controls (3); Kruskal-Wallis test; Table S4: Difference in H19 expression between patients with active acromegaly (1), controlled or cured acromegaly (2) and the control group (3); Kruskal-Wallis test; Table S5: Difference in H19 expression between patients de novo (1), SSA treated (2) and the control group (3); Kruskal-Wallis test; Table S6: Difference in H19 expression between patients de novo (1), treated with radiotherapy (2) and the control group (3); Kruskal-Wallis test.

Author Contributions: Study conception and design, M.R., A.J.-P. and M.B.; conception of the methods, K.K. and A.Z.; patient enrollment, M.R. and A.J.-P.; laboratory analyses, K.K. and A.Z.; data collection, M.R., K.K., and A.Z.; results interpretation, M.R. and A.J.-P.; writing—original draft preparation, M.R.; writing—review and editing, A.J.-P., A.Z. and M.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Wroclaw Medical University (protocol code KB-36/2020, 16.01.2020).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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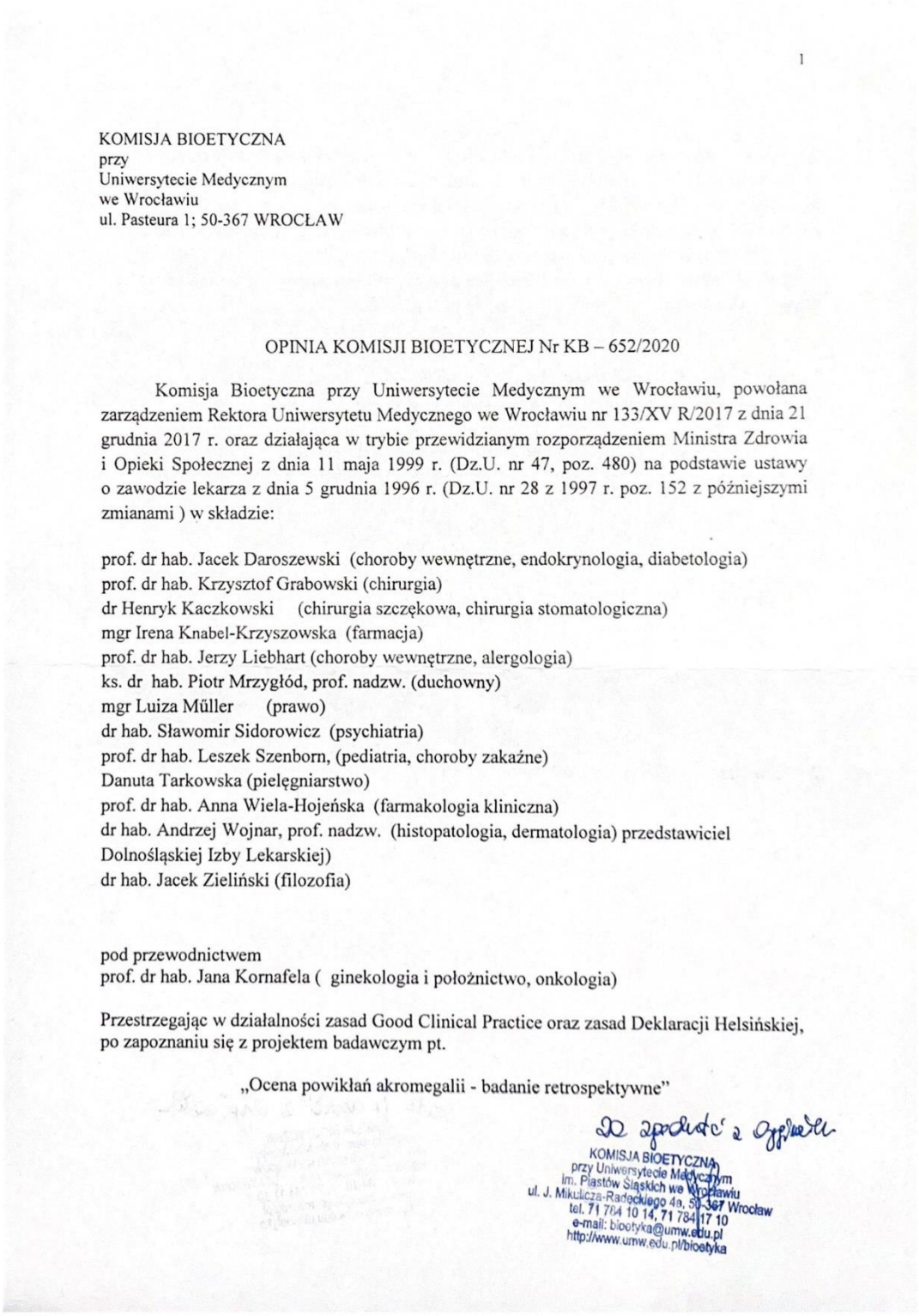
Załączniki

Zgody Komisji Bioetycznej



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KOMISJA BIOETYCZNA
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OPINIA KOMISJI BIOETYCZNEJ Nr KB – 652/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Ocena powikłań akromegalii - badanie retrospektywne"

KOMISJA BIOETYCZNA
przy Uniwersytecie Medycznym
im. Piastów Śląskich we Wrocławiu
ul. J. Mikulicza-Radeckiego 4a, 50-367 Wrocław
tel. 71 784 10 14, 71 784 17 10
e-mail: bioetyka@umw.edu.pl
http://www.umw.edu.pl/bioetyka

zgłoszonym przez **lek. Małgorzatę Rollę** oraz **dr Jowitę Halupczok-Żyłę** zatrudnioną w Katedrze i Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektów badawczych realizowanych poza działalnością statutową
Nr rejestrowy CWN UMW: BW - 65/2020

Opinia jest ważna do dnia 1 grudnia 2022 r.

Wrocław, dnia 21 października 2020 r.
BW

Uniwersytet Medyczny [illegible]wiu
KOMISJA BIOETYCZNA
przewodnicząca
prof. dr hab. Jan Ko[illegible]

Za zgodność z oryginałem
KOMISJA BIOETYCZNA
przy Uniwersytecie Medycznym
im. Piastów Śląskich we Wrocławiu
ul. J. Mikulicza-Radeckiego 4a, 50-367 Wrocław
tel. 71 784 10 14, 71 784 10 10
e-mail: bioetyka@umw.edu.pl
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KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 36/2020

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Jacek Daroszewski (choroby wewnętrzne, endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód, prof. nadzw. (duchowny)
mgr Luiza Müller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
prof. dr hab. Leszek Szenborn, (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar, prof. nadzw. (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.

„Ocena osoczowej ekspresji Inc H19 RNA u pacjentów z gruczolakami przysadki"

zgłoszonym przez **lek. Małgorzatę Rollę** uczestniczkę studiów doktoranckich w Katedrze i Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła wyrazić zgodę na przeprowadzenie badania w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu pod nadzorem prof. dr hab., Marka Bolanowskiego **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności:

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu

Opinia powyższa dotyczy: projektu badawczego będącego podstawą rozprawy doktorskiej

Wrocław, dnia 16 stycznia 2010 r.

BW

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel

Oświadczenia współautorów

Wrocław, 19.04.2023

dr n. med. Aleksandra Jawiarczyk-Przybyłowska
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Katarzyna Kolačkov, Marek Bolanowski, 2021, *H19 in endocrine system tumours*, Anticancer Res., 2021, 41: 557-565

mój udział polegał na nadzorze merytorycznym nad manuskryptem, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Potwierdzam

Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr n. med. Katarzyna Kolačkov
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

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Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Katarzyna Kolačkov, Marek Bolanowski, 2021, *H19 in endocrine system tumours*, Anticancer Res., 2021, 41: 557-565

mój udział polegał na pomocy w tworzeniu koncepcji pracy, wprowadzaniu poprawek językowych do pierwotnej wersji manuskryptu.

Katarzyna Kolačkov

Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII,
DIABETOLOGII I LECZENIA IZOTOPAMI
Kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

prof. dr hab. Marek Bolanowski
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

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mój udział polegał na nadzorze merytorycznym, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Wrocław, 19.04.2023

dr n. med. Aleksandra Jawiarczyk-Przybyłowska
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na pomocy w tworzeniu koncepcji pracy, gromadzeniu danych poddawanych analizie, interpretacji wyników, nadzorze merytorycznym, tworzeniu pierwotnej wersji manuskryptu.

Potwierdzam
Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
Kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr n. med. Jowita Halupczok-Żyła
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Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na gromadzeniu danych poddawanych analizie, interpretacji wyników, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Jowita Halupczok-Żyła

Potwierdzam
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KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr n. med. Marcin Kałużny
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na gromadzeniu danych poddawanych analizie i nadzorze merytorycznym.

Dr n. med. Marcin Kałużny
specjalista chorób wewnętrznych
ENDOKRYNOLOG, DIABETOLOG
2458645

Potwierdzam
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kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr inż. Bogumił Konopka
Katedra Inżynierii Biomedycznej
Politechnika Wrocławska

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na przeprowadzeniu analizy statystycznej, interpretacji wyników, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Bogumił Konopka

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KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
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prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

mgr Izabela Błoniecka
Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytecki Szpital Kliniczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na gromadzeniu danych poddawanych analizie.

Błoniecka Izabela

Potwierdzam
Uniwersytet Medyczny we Wrocławiu
KATEDRA I ZAKŁAD ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
Kierownik
prof. dr hab. n. med. Marek Bolanowski

Warszawa, 19.04.2023

prof. dr hab. Grzegorz Zieliński
Klinika Neurochirurgii
Wojskowy Instytut Medyczny w Warszawie

OŚWIADCZENIE

Oświadczam, że w pracy

Malgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 2021, 12: 642131

mój udział polegał na gromadzeniu danych poddawanych analizie oraz wprowadzaniu poprawek merytorycznych do pierwotnej wersji manuskryptu.

Grzegorz Zieliński

Prof. dr hab. n. med.
Grzegorz Zieliński
spec. neurochirurg
PWZ 2711701

Potwierdzam
Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

prof. dr hab. Marek Bolanowski
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Jowita Halupczok-Żyła, Marcin Kałużny, Bogumil M. Konopka, Izabela Błoniecka, Grzegorz Zieliński, Marek Bolanowski, 2021, *Complications and comorbidities of acromegaly - retrospective study in Polish Center*, Front.Endocrinol., 12: 642131

mój udział polegał na pomocy w tworzeniu koncepcji pracy, gromadzeniu danych poddawanych analizie, nadzorze merytorycznym, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Wrocław, 19.04.2023

dr n. med. Aleksandra Jawiarczyk-Przybyłowska
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Katarzyna Kolačkov, Agnieszka Zembska, Marek Bolanowski, 2023, *Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study*, Biomedicines, 11: 1211

mój udział polegał na pomocy w tworzeniu koncepcji pracy, współudziale w rekrutowaniu i gromadzeniu danych od uczestników badania, interpretacji wyników, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
Kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr n. med. Katarzyna Kolačkov
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

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mój udział polegał na zaplanowaniu metodyki oraz przeprowadzeniu oceny ekspresji H19 RNA w zgromadzonym materiale, współudziale w tworzeniu bazy danych, przygotowaniu literatury niezbędnej do stworzenia manuskryptu.

Katarzyna Kolačkov

Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

dr n. med. Agnieszka Zembska
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Katarzyna Kolačkov, Agnieszka Zembska, Marek Bolanowski, 2023, *Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study*, Biomedicines, 11: 1211

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Zembska

Uniwersytet Medyczny we Wrocławiu
KATEDRA I KLINIKA ENDOKRYNOLOGII, DIABETOLOGII I LECZENIA IZOTOPAMI
Kierownik
prof. dr hab. n. med. Marek Bolanowski

Wrocław, 19.04.2023

prof. dr hab. Marek Bolanowski
Katedra i Klinika Endokrynologii, Diabetologii i Leczenia Izotopami
Uniwersytet Medyczny we Wrocławiu

OŚWIADCZENIE

Oświadczam, że w pracy

Małgorzata Rolla, Aleksandra Jawiarczyk-Przybyłowska, Katarzyna Kolačkov, Agnieszka Zembska, Marek Bolanowski, 2023, *Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study*, Biomedicines, 11: 1211

mój udział polegał na pomocy w tworzeniu koncepcji pracy, nadzorze merytorycznym, wprowadzaniu poprawek merytorycznych i językowych do pierwotnej wersji manuskryptu.

Wrocław, 19.04.2023 r.

Lek. Małgorzata Rolla

Wykaz publikacji

1. Publikacje w czasopismach naukowych

1.1 Publikacje w czasopiśmie z IF

Lp	Opis bibliograficzny	IF	Punkty
1	**Rolla Małgorzata**, Halupczok-Żyła Jowita, Jawiarczyk-Przybyłowska Aleksandra, Bolanowski Marek: Bone densitometry by radiofrequency echographic multi-spectrometry (REMS) in acromegaly patients, Endokrynologia Polska (Polish Journal of Endocrinology), 2020, vol. 71, nr 6, s. 524-531, DOI:10.5603/EP.a2020.0056	1,582	70
2	**Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Kolačkov Katarzyna, Bolanowski Marek: H19 in endocrine system tumours, Anticancer Research, 2021, vol. 41, nr 2, s. 557-565, DOI:10.21873/anticanres.14808	2,435	70
3	**Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Halupczok-Żyła Jowita, Kałużny Marcin, Konopka Bogumil M., Błoniecka Izabela, Zieliński Grzegorz, Bolanowski Marek: Complications and comorbidities of acromegaly - retrospective study in Polish Center, Frontiers in Endocrinology, 2021, vol. 12, art.642131 [10 s.], DOI:10.3389/fendo.2021.642131	6,055	100
4	**Rolla Małgorzata**, Jawiarczyk-Przybyłowska Aleksandra, Kolačkov Katarzyna, Zembska Agnieszka, Bolanowski Marek: Is H19 RNA a useful marker of acromegaly and its complications? A preliminary study, Biomedicines, 2023, vol. 11, nr 4, art.1211 [10 s.], DOI:10.3390/biomedicines11041211	4,757*	100
	Podsumowanie	14,829	340,00

IF 2021

1.2 Publikacje w czasopiśmie bez IF -

1.3 Publikacje w czasopiśmie - prace kontrybutorskie -

2. Monografie naukowe

2.1 Książka autorska -

2.2 Książka redagowana -

2.3 Rozdziały

3. Varia -

3.1 Komentarz -

3.2 Inne -

4. Abstrakty

Lp	Opis bibliograficzny
1	Jawiarczyk Aleksandra, **Rolla Małgorzata**, Halupczok-Żyła Jowita, Kałużny Marcin, Bolanowski Marek: Systemic complications in acromegaly - a retrospective study in Polish centre, W: ENEA Wrocław 2018 - [18th Congress of the European NeuroEndocrine Association. Wrocław, 17-20 October 2018. Abstracts on USB flash drive] 2018, poz.P22
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3	Jawiarczyk-Przybyłowska Aleksandra, Halupczok-Żyła Jowita, Syrycka Joanna, **Rolla Małgorzata**, Zembska Agnieszka, Kubicka Eliza, Bolanowski Marek: Trabecular bone score as a useful tool for assessment of fracture risk in acromegaly, Endocrine Abstracts, 2020, vol. 70, poz.AEP689, [22nd European Congress of Endocrinology. 5-9 September 2020 [online]], DOI:10.1530/endoabs.70.AEP689
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