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Białka MCM w diagnostyce różnicowej guzów nadnerczy

Rozprawa doktorska

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Wykaz prac naukowych wchodzących w skład cyklu:

1. Minichromosome maintenance proteins MCM-3, MCM-5, MCM-7, and Ki-67 as proliferative markers in adrenocortical tumors.
   Michał Aporowicz, Piotr Czopnik, Eliza Kubicka, Aleksandra Piotrowska, Piotr Dzięgiel, Marek Bolanowski, Paweł Domosławski.
   Anticancer Research 2019 Vol. 39 no. 3; s.1151-1159
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2. Perioperative complications of adrenalectomy – 12 years of experience from a single center/teaching hospital and literature review.
   Michał Aporowicz, Paweł Domosławski, Piotr Czopnik, Krzysztof Sutkowski, Krzysztof Kaliszewski.
   Archives of Medical Science 2018 Vol. 14 no. 5; s.1010-1019
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3. The level of accordance between preoperative and postoperative diagnosis in patients undergoing adrenalectomy – a study of 230 consecutive cases.
   Michał Aporowicz, Paweł Domosławski, Krzysztof Kaliszewski, Krzysztof Sutkowski, Eliza Kubicka, Marek Bolanowski.
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Spis treści:

1. Wstęp i cele badań

Zmiany guzowate gruczołów nadnerczowych są rozpoznawane względnie często, stanowiąc 5 – 9 % wszystkich guzów w organizmie człowieka. Postęp w diagnostyce obrazowej, jaki dokonał się w ostatnich latach, szczególnie w zakresie dostępności badania ultrasonograficznego oraz tomografii komputerowej, przyczynił się do znacznego zwiększenia ich wykrywalności. Przypadkowo zdiagnozowane guzy nadnerczy (tzw. incidentaloma) występują u 2 – 4 % ogólnej populacji, zaś w grupie osób starszych (po 70 r. ż.) – nawet do 7 %. Rozpoznanie to stawiane jest również pośmiertnie – jako rezultat 1 – 9 % badań sekcyjnych. [1-4]

Spośród pacjentów ze zdiagnozowanymi guzami nadnerczy do leczenia operacyjnego kwalifikowane są dwie grupy chorych. U pierwszej występują tzw. wskazania endokrynologiczne (nadczynność wydzielnicza guza), u drugiej – tzw. wskazania onkologiczne (podejrzenie nowotworu złośliwego). W pooperacyjnych badaniach histopatologicznych większość usuwanych zmian okazuje się łagodna – najczęściej rozpoznawany jest gruczolak kory nadnercza. Nowotwór złośliwy, jakim jest rak kory nadnercza, rozpoznawany jest bardzo rzadko, jednak znaczna większość przypadków cechuje się złym rokowaniem i w krótkim czasie prowadzi do zgonu. Wynika to po części z agresywności biologicznej samego nowotworu, jak również z braku wczesnych objawów i – związanego z tym – rozpoznawaniu większości przypadków w wysokim stopniu zaawansowania klinicznego. Decydującym kryterium rozpoznawczym zmian łagodnych i złośliwych pozostaje wciąż klasyczne badanie patomorfologiczne, wsparte wynikiem indeksu proliferacyjnego Ki-67. W związku z tym poszukiwane są obecnie nowe cząsteczki chemiczne, mogące stanowić wiarygodne markery, wspomagające proces diagnostyczny i wczesne ustalenie prawidłowego rozpoznania – w szczególności potwierdzenie bądź wykluczenie nowotworu złośliwego. [1-3,5-6,7]

Białka z rodziny MCM (minichromosome maintenance proteins) opisane i scharakteryzowane zostały w latach 90’. Biorą one w komórce udział m. in. w procesie replikacji materiału genetycznego i utrzymaniu integralności genomu. Wykrywane są jedynie w komórkach dzielących się i wykazują wzmożoną ekspresję w wielu rodzajach guzów, co pozwala na traktowanie ich jako potencjale markery proliferacji. Zastosowanie białek MCM

(dokładnie: MCM-2) jako wskaźników rozpoznawczych złośliwych guzów nadnerczy poruszone zostało dotychczas w zaledwie jednej publikacji naukowej. [8-10]

Ustalenie wskazań do inwazyjnego leczenia guza nadnercza stawia chirurga przed wyborem właściwego dostępu operacyjnego, w zależności od wstępnego rozpoznania klinicznego, rozmiarów zmiany, przebytych przez chorego w przeszłości zabiegów operacyjnych oraz indywidualnych umiejętności operatora. Od właściwej decyzji zależy skuteczne przeprowadzenie doszczętnego zabiegu chirurgicznego, jak również uniknięcie powikłań okołooperacyjnych, których odsetek wg dostępnej literatury oscyluje w szerokich granicach 1,7 – 30,7 %. Wystąpienie ciężkich powikłań jest w stanie zniweczyć wyniki nawet najbardziej poprawnie technicznie wykonanej procedury chirurgicznej. [11-12]

Obserwowany jest również problem niezgodności wstępnego rozpoznania charakteru guza nadnercza, ustalonego na podstawie przedoperacyjnych badań klinicznych, laboratoryjnych i obrazowych, z ostateczną diagnozą, stawianą przez patomorfologa w wyniku badania mikroskopowego preparatu pooperacyjnego. Przykładowo, wg danych z piśmiennictwa, odsetek rozpoznań fałszywie dodatnich w przypadku guza chromochłonnego (pheochromocytoma) może sięgać aż 33 %, co sygnalizuje potrzebę merytorycznej rewizji stosowanego obecnie schematu postępowania z guzami nadnerczy. [13]

Głównym celem niniejszej rozprawy jest ocena możliwości zastosowania wybranych białek z rodziny MCM (MCM-3, 5 i 7) oraz białka Ki-67 jako markery proliferacyjne w diagnostyce różnicowej łagodnych i złośliwych guzach kory nadnerczy. Badanie prowadzono na materiale archiwalnym preparatów gruczolaków (81) i raków (3) kory nadnerczy, pochodzących od pacjentów operowanych w I Katedrze i Klinice Chirurgii Ogólnej, Gastroenterologicznej i Endokrynologicznej UM we Wrocławiu (UMW) w latach 2004 – 2014. Ocena ekspresji powyższych białek ma na celu weryfikację ich przydatności w rozpoznawaniu, monitorowaniu i prognozowaniu rokowania u chorych z guzami nadnerczy. Poprawa diagnostyki przedoperacyjnej w tym zakresie może przełożyć się na wcześniejsze rozpoznawanie zmian złośliwych, a co za tym idzie – zmniejszenie śmiertelności leczonych z tego powodu pacjentów. Badanie wykonywano we współpracy z: Zakładem Histologii i Embriologii Katedry Morfologii i Embriologii Człowieka UMW, Katedrą i Zakładem Patomorfologii UWM oraz Katedrą i Kliniką Endokrynologii, Diabetologii i Leczenia Izotopami UMW.

Kolejnym celem rozprawy jest przedstawienie wyników leczenia operacyjnego wyżej wymienionych chorych, ze szczególnym uwzględnieniem powikłań okołooperacyjnych i identyfikacją czynników ryzyka tych zdarzeń niepożądanych. Nierozerwalnie związana jest z tym dyskusja nt. wyboru dostępu operacyjnego u pacjentów wymagających inwazyjnego leczenia guzów nadnercza.

Celem dodatkowym rozprawy jest analiza zgodności bądź rozbieżności wstępnego rozpoznania klinicznego u chorych z guzami nadnerczy z ostateczną diagnozą patomorfologiczną. Posłużono się tu danymi klinicznymi pacjentów operowanych w I Katedrze i Klinice Chirurgii Ogólnej, Gastroenterologicznej i Endokrynologicznej UMW w latach 2004 – 2018. Przedstawiono w szczególności przypadki niezgodności rozpoznań i przedyskutowano ich potencjalne przyczyny.

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2. Materiał i metody badawcze

Grupę badaną w ocenie roli białek MCM w guzach kory nadnerczy stanowili pacjenci operowani w I Katedrze i Klinice Chirurgii Ogólnej, Gastroenterologicznej i Endokrynologicznej Uniwersytetu Medycznego we Wrocławiu w latach 2004 – 2015. Badanie zostało pozytywnie zaopiniowane przez Komisję Bioetyczną Uniwersytetu Medycznego we Wrocławiu (UMW) – pismo nr KB 151/2016. Stworzono bazę danych, zawierającą informacje o płci i wieku każdego pacjenta, wskazaniach do adrenalektomii, stronie, po której stwierdzono guz oraz jego wymiarach guza. Na podstawie wymiarów guza oszacowano jego objętość, przyjmując elipsoidalny kształt. Do bazy dołączono wyniki badań hormonalnych pacjentów, w większości hospitalizowanych w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami UMW.

Następnie z archiwum Katedry Patomorfologii UMW wypożyczono odpowiednie bloczki parafinowe (81 gruczolaków i 3 raki kory nadnercza), które przekazano do Zakładu Histologii i Embriologii Katedry Morfologii i Embriologii Człowieka UMW. Po zabarwieniu hematoksyliną i eozyną, stworzono wirtualne preparaty, które, po ocenie przez 2 patologów, posłużyły do skonstruowania mikromacierzy tkankowych (TMA). Te z kolei posłużyły za materiał do wykonania reakcji immunohistochemicznych (IHC) z przeciwciałami skierowanymi przeciwko białkom: MCM-3, MCM-5, MCM-7 i Ki-67. Rezultaty reakcji oceniono z użyciem mikroskopu optycznego Olympus BX40 oraz oprogramowania Cell^D. Wyniki obserwacji dołączono do bazy danych, po czym całość poddano analizie statystycznej.

Do oceny powikłań okołooperacyjnych adrenalektomii przeanalizowano dane pacjentów operowanych w powyższej Klinice w latach 2004 – 2015. Dane zebrano w bazie, zawierającej informacje o płci i wieku pacjentów, wskazaniach do zabiegu, zastosowanym dostępie operacyjnym (bez rozróżnienia pomiędzy laparotomią a konwersją z laparoskopii), stronie wykonywanego zabiegu, wyniku badania histopatologicznego, ewentualnych dodatkowych procedurach chirurgicznych podczas tego samego zabiegu oraz wystąpieniu powikłań chirurgicznych bądź niechirurgicznych. Dane przeanalizowano statystycznie i przestawiono w formie tabel.

Do oceny zgodności rozpoznania przedoperacyjnego z pooperacyjnym wykorzystano dane pacjentów operowanych w powyższej Klinice w latach 2004 – 2018. Dane demograficzne chorych, wyniki przedoperacyjnych badań laboratoryjnych i obrazowych, historię choroby oraz wynik ostatecznego badania histopatologicznego zebrano w formie bazy danych i poddano analizie statystycznej. Przed przyjęciem do oddziału chirurgicznego pacjenci odbywali pobyt diagnostyczny w oddziale endokrynologii, celem ustalenia wstępnego rozpoznania i potwierdzenia wskazań do leczenia inwazyjnego; zdecydowana większość chorych była diagnozowana w Klinice Endokrynologii, Diabetologii i Leczenia Izotopami UMW. Podstawą rozpoznania było przynajmniej jedno badanie obrazowe jamy brzusznej (preferowano tomografię komputerową), w połączeniu z wynikami laboratoryjnymi (morfologia krwi, stężenia elektrolitów, dobowy profil kortyzolu, wydalanie wolnego kortyzolu w dobowej zbiórce moczu [DZM], poziom aldosteronu i aktywność reninowa osocza – obydwa w spoczynku i po pionizacji, wydalanie aldosteronu w DZM, metoksypochodne amin katecholowych [MAK] w DZM). W wybranych przypadkach zlecane były również dodatkowe testy. Na tej podstawie guzy nadnerczy i pacjentów podzielono na 8 kategorii: 1) guz bez objawów nadczynności hormonalnej, 2) torbiel nadnercza, 3) guz chromochłonny bądź jego podejrzenie, 4) objawy zespołu Cushinga, 5) objawy zespołu Conna, 6) objawy zespołu Conna ze współistniejącą hiperkortyzolemią, 7) guz przerzutowy bądź jego podejrzenie, 8) nawrót uprzednio usuniętego guza. Wyniki pooperacyjnych badań histopatologicznych i immunohistochemicznych usuniętych guzów zestawiono z rozpoznaniami przedoperacyjnymi. Wśród wyników pooperacyjnych wyróżniono 14 kategorii: [łagodne] 1) gruczolak kory nadnercza, 2) przerost guzkowy, 3) gruczolak bądź przerost guzkowy (rozróżnienie niemożliwe z powodu fragmentacji materiału), 4) guz chromochłonny, 5) nadnercze bez uchwytnych zmian patologicznych, 6) myelolipoma, 7) krwiak, 8) torbiel, 9) naczyniak, 10) ganglioneuroma, [złośliwe]: 11) przerzut nowotworowy do nadnercza, 12) rak kory nadnercza, 13) chłoniak, 14) schwannoma. Następnie oszacowano czułość diagnostyczną i dodatnią wartość predykcyjną dla każdego z rozpoznań przedoperacyjnych.

3. Wyniki badań

W ocenie przydatności białek MCM w roli markerów złośliwości guzów kory nadnerczy przeanalizowano 84 chorych – 81 (96.4%) z gruczolakami kory nadnerczy i 3 (3.6%) – z rakami kory nadnerczy. W grupie tej było 68 (81.0%) kobiet and 16 (19.0%) mężczyzn, w wieku 57.0 ± 10.1 lat. Średnia średnica guza wyniosła 3.89 ± 1.93 cm, a średnia oszacowana na tej postawie objętość: 38.7 ± 57.4 cm^3. Nie wykazano istotnych statystycznie różnic w płci i wieku chorych z gruczolakami i rakami kory nadnerczy. Jednakże guzy złośliwe cechowały się większym rozmiarem i objętością (p=0.017 dla obu porównań), jak również wyższymi wartościami indeksów proliferacyjnych białka Ki-67 (p=0.005), MCM-3 (p=0.005) i MCM-7 (p=0.008), choć nie MCM-5 (p=0.069). Nie znaleziono różnic w wartościach indeksów proliferacyjnych powyższych białek pomiędzy gruczolakami bez nadczynności hormonalnej, z objawami zespołu Cushinga czy Conna (Ki-67, p=0.161; MCM-3, p=0.810; MCM-5, p=0.117; MCM-7, p=0.639). Analiza wzajemnych korelacji pomiędzy wartościami badanych markerów wykazała pozytywną korelację we wszystkich przypadkach. Korelacja ta była najsilniejsza ($r \approx 0.6 – 0.7$) pomiędzy Ki-67 i MCM-3 ($r = 0.61$, $p < 0.001$), Ki-67 i MCM-7 ($r = 0.59$, $p < 0.001$) oraz MCM-3 i MCM-7 ($r = 0.74$, $p < 0.001$). Nie znaleziono żadnych istotnych statystycznie powiązań pomiędzy poziomami badanych markerów a największym wymiarem guza, jego oszacowaną objętością, jak również wiekiem pacjentów. Indeksy proliferacyjne powyższych markerów nie korelowały z żadnych z wyników badań hormonalnych, z 4 wyjątkami. W oparciu o poszczególne wartości ocenianych markerów w każdym przypadku gruczolaka bądź raka, wykreślono dla każdego z nich krzywą ROC (receiver operating characteristic). Analizując kształt krzywych, stwierdzono, że białka Ki-67, MCM-3 i MCM-7 są wiarygodnymi markerami różnicującymi łagodne i złośliwe guzy kory nadnerczy (wypukły kształt krzywych, pole pod krzywą [AUC] > 0.9). Z kolei białko MCM-5, o krzywej leżącej niemal na przekątnej wykresu, o AUC 0.820, miało w najlepszym wypadku jedynie 51.07-procentową czułość i 88.00-procentową specyficzność, co dyskwalifikuje je z roli użytecznego markera.

Zgłębiając problem powikłań okołooperacyjnych, przeanalizowano informacje o 177 zabiegach wykonanych u 170 pacjentów (pięcioro z nich operowano dwukrotnie, a jedną pacjentkę – 3 razy). Zidentyfikowano 18 (10.2%) powikłań śródoperacyjnych i pooperacyjnych,

12 (6.8%) chirurgicznych oraz 6 (3.4%) nie-chirurgicznych. Powikłania chirurgiczne podzielono głębiej na 3 grupy – krwotoczne, związane z uszkodzeniem otaczających struktur anatomicznych oraz pozostałe, nie mieszące się w żadnej z wcześniejszych kategorii. Płeć pacjenta, rozpoznanie przedoperacyjne, czy strona ciała, po której wykonywany był zabieg nie były istotnymi czynnikami ryzyka powikłań. Za to wybrany dostęp operacyjny okazał się być istotnym statystycznie czynnikiem ryzyka powikłań nie-chirurgicznych i całościowych. W zasadzie wszystkie powikłania nie-chirurgiczne wystąpiły po zabiegach laparotomii bądź konwersji z laparoskopii do laparotomii. Jednoczasowe zabiegi bardziej rozległe niż sama adrenalektomia, jak również operacje przeprowadzane przez mniej doświadczonych chirurgów cechowały się istotnie wyższym odsetkiem powikłań nie-chirurgicznych. Powikłania krwotoczne występowały częściej podczas adrenalektomii prawostronnych, podczas gdy uszkodzenia struktur anatomicznych otaczających nadnercza – podczas zabiegów lewostronnych (różnica na granicy istotności statystycznej).

Badając zgodność rozpoznanie przedoperacyjnego z pooperacyjnym, przeanalizowano dane 214 chorych – 150 (70.1%) kobiet i 64 (29.9%) mężczyzn, w wieku 21 do 81 lat (średnia: 56.0 ± 11.3). Guzy bez objawów nadczynności hormonalnej okazywały się w badaniach histopatologicznych być najczęściej gruczolakami kory nadnerczy (62, 48.8%), przerostami guzkowymi (14, 11.0%) bądź myelolipoma (13, 10.2%). Spośród 8 torbieli nadnerczy, jedynie 3 zostały tak rozpoznane przed zabiegiem (czułość 37.5%). Z 35 przypadków podejrzeń guza chromochłonnego potwierdziło się jedynie 21 (dodatnia wartość predykcyjna [PPV] 60.0%). Natomiast spośród 31 potwierdzonych guzów chromochłonnych, jedynie 21 zostało poprawnie rozpoznanych przed operacją (czułość 67.7%). W grupie zespołu Cushinga znaleziono tylko 1 niezgodność na 26 (1 nadnercze bez uchwytnej patologii; PPV 96.2%). W grupie zespołu Conna wystąpiły 2 niezgodności na 20 (1 nadnercze bez uchwytnej patologii i 1 krwiak; PPV 90.0%). Spośród 17 podejrzeń przerzutów nowotworowych do nadnerczy potwierdziło się 14 (PPV 82.4%), choć jednocześnie 3 guzy, które okazały się być przerzutami, zostały wstępnie uznane za łagodne guzy bez nadczynności hormonalnej (czułość 82.4%). Spośród 3 raków kory nadnerczy, 2 zostały wstępnie sklasyfikowane jako łagodne guzy bez nadczynności hormonalnej, 1 przypadek to wznowa uprzednio usuniętej zmiany. Rozpoznanie zespołu Cushinga było częstsze wśród kobiet ($p = 0.009$), podczas gdy przerzutów do nadnerczy – u mężczyzn ($p = 0.001$).

4. Dyskusja

Guz kory nadnercza stanowi względnie częste rozpoznanie kliniczne, którego występowanie dodatkowo wzrasta z wiekiem. Rak kory nadnercza, choć stosunkowo rzadki, oznacza złe rokowanie i ograniczone możliwości terapeutyczne. Z tego powodu różnicowanie łagodnych i złośliwych zmian guzowatych nadnerczy ma szczególnie istotne znaczenie. Proces różnicowania opiera się współcześnie na klasycznych kryteriach morfologicznych, wspieranych wartością indeksu proliferacyjnego Ki-67. Wyniki prezentowanej rozprawy potwierdzają różnicującą role indeksu proliferacyjnego Ki-67 (p=0.005), choć wskazana byłaby raczej wyższa wartość odcięcia niż wskazana w dostępnej literaturze (10 % zamiast 5 %). Podobnie indeksy proliferacyjne białek MCM-3 i MCM-7 stanową wiarygodne markery różnicujące guzy łagodne od złośliwych (odpowiednio: p=0.005 and 0.008). Ich wartości są niezależne od rozmiaru i objętości guza, wieku i stanu hormonalnego pacjenta.

Białka MCM-3, 5 i 7 były uprzednio badane przez innych autorów jako potencjalne markery proliferacyjne w guzach innych gruczołów wydzielania wewnętrznego. Lee i współautorzy badali białko MCM-3 w celu identyfikacji raka brodawkowego tarczycy i uznali, że jest ono pod tym względem bardziej wiarygodne od Ki-67. Podobnie Guida i współautorzy badali białka MCM-5 i 7 w odniesieniu do przypadków brodawkowego i anaplastycznego raka tarczycy, również zauważając statystycznie istotne różnice pomiędzy tkankami nowotworowymi i zdrowymi. Coli i współautorzy przedstawili wyniki badania białka MCM-7 jako markera prognostycznego w guzach przysadki, również stwierdzając, iż jest ono bardziej wiarygodne od Ki-67.

Według dostępnej literatury częstość powikłań okołooperacyjnych adrenalektomii waha się w dosyć szerokich granicach: 1,7 – 30,7 %, co plasuje opisywany ośrodek w środkowej części listy. Wykazano, że płeć pacjenta, rozpoznanie przedoperacyjne, jednoczasowy zabieg obustronnego usunięcia nadnerczy, jak również strona ciała wykonywanego zabiegu nie są czynnikami ryzyka powikłań okołooperacyjnych. Natomiast dostęp operacyjny poprzez laparotomię, jednoczesne zabiegi bardziej rozlegle niż sama adrenalektomia oraz niskie doświadczenie operatora stanowiło czynnik ryzyka powikłań nie-chirurgicznych.

Adrenalektomia prawostronna wydaje się być związana z większym ryzykiem powikłań krwotocznych, zaś lewostronna – z uszkodzeniem przyległych struktur anatomicznych.

Największy odsetek pacjentów wymagał adrenalektomii z powodu guza nadnercza bez objawów nadczynności hormonalnej (127/230; 55.2%). Szczegółowe wskazania obejmowały: duży rozmiar guza (≥ 4 cm), szybki wzrost w powtarzalnych badaniach obrazowych, hiperdensyjność w badaniu TK (nietypowa dla łagodnego gruczolaka), inne podejrzane cechy, jak obecność zwapnień bądź martwicy w obrębie guza. Większość zmian guzowatych (116/127; 91.3%) okazała się być łagodna w pooperacyjnym badaniu histopatologicznym. Drugim w kolejności wskazaniem do zabiegu była nadczynność hormonalna guza (47/230; 20.4%). Pacjenci z zespołem Cushinga byli statystycznie częściej kobietami (p = 0.009). Guz chromochłonny jest w większości przypadków zmianą łagodną, co potwierdziły nasze obserwacje. To rozpoznanie cechowało się najniższą PPV (60.0%), co może być wynikiem rozmaitych trudności w procesie diagnostycznym. Wiele potraw, napojów, używek i leków podwyższa wynik MAK w DZM, co sugeruje ich wcześniejsze odstawienie na kilka tygodni przed wykonywaniem testów hormonalnych. Torbiele nadnerczy stanowią 5 – 8 % incidentaloma nadnerczy, co potwierdziły nasze obserwacje. Rozpoznanie to cechowało się idealną PPV (100%), jednak stosunkowo niską czułością (37.5%), co wskazywałoby, że wiele z tych zmian jest początkowo diagnozowana jako łagodne guzy nieczynne hormonalnie. Nadnercza są powszechnym miejscem docelowym przerzutowania złośliwych nowotworów płuca, nerki, piersi, przewodu pokarmowego, raka wątrobowokomórkowego oraz czerniaka skóry. Rozpoznanie to było statystycznie istotnie częstsze u mężczyzn (p = 0.001). Rak kory nadnercza występuje częściej u kobiet, co potwierdziły nasze obserwacje. 2 przypadki zostały wstępnie zakwalifikowane jako łagodne guzy nieczynne hormonalnie (stanowiąc 1,6 % w tej grupie), zaś 1 przypadek to wznowa uprzednio usuniętego guza nadnercza – co powinno zawsze wzbudzać czujność onkologiczną.

5. Wnioski

Podsumowując, indeksy proliferacyjne białek Ki-67, MCM-3 i MCM-7, jednak nie MCM-5 stanowi wiarygodne czynniki różnicujący łagodne gruczolaki kory nadnerczy od złośliwych raków kory nadnerczy. Na ich wartość nie mają wpływu wymiary, aktywność hormonalna guza, czy wiek pacjenta. Według wiedzy autorów jest to pierwsze tego typu opracowanie w światowej literaturze medycznej.

Nadnercza otoczone są licznymi strukturami anatomicznymi, jak okrężnica, trzustka, śledziona, czy przepona, które mogą zostać uszkodzone podczas zabiegu adrenalektomii. Może to z kolei spowodować ciężkie powikłania pooperacyjne, wymagające ponownego przyjęcia chorego do oddziału chirurgicznego, kolejnego zabiegu, bądź przekazania do oddziału intensywnej terapii. Powikłania laparoskopowej adrenalektomii mogą być wynikiem nieuważnego użycia diatermii jednobiegunowej, jak i nieprawidłowego ułożenia chorego na stole operacyjnym. Minimalnie inwazyjne procedury z dostępu pozaotrzewnowego, wymagające znacznie wyższych ciśnień insuflacji, mogą prowadzić do powstania rozedmy podskórnej tułowia.

Większość wstępnych rozpoznań guzów nadnerczy cechuje się zarówno wysoką czułością, jak i dodatnią wartością predykcyjną. Wyjątek pod tym względem stanowi guz chromochłonny (odpowiednio: 67.7% i 60.0%). Rozpoznanie torbieli nadnercza zostaje rutynowo potwierdzone pooperacyjnie (PPV 100%), jednakże sporo zmian wstępnie kwalifikowanych jako guzy lite okazuje się być torbielami (czułość 37.5%). Chorzy z zespołem Cushinga to statystycznie częściej kobiety ($p = 0.009$), zaś ci z przerzutami nowotworowymi do nadnerczy to częściej mężczyźni ($p = 0.001$). Rak kory nadnercza bywa wstępnie rozpoznany jako guz lity nieczynny hormonalnie (1.6% przypadków w tej grupie), bądź jako odrost uprzednio wyciętego guza, co powinno zawsze wzbudzać podejrzenie złośliwego nowotworu.

6. Publikacja nr 1:

Minichromosome Maintenance Proteins MCM-3, MCM-5, MCM-7, and Ki-67 as Proliferative Markers in Adrenocortical Tumors

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Minichromosome Maintenance Proteins MCM-3, MCM-5, MCM-7, and Ki-67 as Proliferative Markers in Adrenocortical Tumors

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Abstract. *Background/Aim: Morphological features, combined with Ki-67 proliferative index, remain the standard for discriminating benign and malignant adrenocortical tumors. The aim of this study was to evaluate the role of minichromosome maintenance proteins MCM-3, MCM-5, MCM-7, and Ki-67 as proliferative markers in adrenocortical tumors. Materials and Methods: Specimens of 81 adrenocortical adenomas and 3 adrenocortical carcinomas were stained with antibodies against MCM-3, 5, 7 and Ki-67. Results: Malignant tumors were characterized by a greater size (p=0.017), volume (p=0.017), and higher levels of Ki-67 (p=0.005), MCM-3 (p=0.005), MCM-7 (p=0.008), but not MCM-5 (p=0.069). The markers' levels were independent from the tumors' size and volume, the patient's age and hormonal status. ROC curves showed Ki-67 (AUC 0.984), MCM-3 (AUC 0.984), and MCM-7 (AUC 0.950), but not MCM-5 (AUC 0.820) to be reliable markers. Conclusion: Ki-67, MCM-3, and MCM-7, but not MCM-5 are reliable proliferative and diagnostic markers in discerning benign and malignant adrenocortical tumors.*

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Key Words: Adrenocortical adenoma, adrenocortical carcinoma, Ki-67, MCM-3, MCM-5, MCM-7.

Adrenal gland tumors (AGTs) are relatively common and constitute 5-9% of all human tumors. The greater accessibility to diagnostic imaging in recent years, especially ultrasound (US) and computed tomography (CT), has revealed that the rate of AGTs is significantly higher than previously reported. The prevalence of incidentally detected adrenal mass (so-called incidentaloma) is greater with age and ranges from 0.2% (20-29 years old) to 3% (over 50 years of age) and even up to 7% (over 70 years of age) (1, 2). The mean value for the general population is about 2-4% (3, 4). An incidentaloma is typically detected in the right adrenal gland, between the 5th and 7th decade of life (mean age 55 years). AGTs are found on average in 1-8.9% (mean 2.3%) of autopsies, and even in as high as 15% according to some authors (2, 4). Apart from an incidentaloma, adrenal tumors may present symptoms, either of hormonal excess or a mass effect (5).

Following the finding of an AGT, steps are taken to determine its origin (cortical/medullar) and character (benign/malignant) (1, 3, 6). Most AGTs are of cortical origin and benign, adrenocortical adenoma (ACA) is the most frequent diagnosis (70-94%) (2, 5). The majority of ACAs do not display hormonal activity. The most common functioning ACA is aldosterone-producing adenoma (APA), followed by cortisol-producing adenoma (CPA) (7). A malignant AGT can be either a primary adrenal lesion or a metastasis (3). Primary malignancies consist of cortical (adrenocortical carcinoma – ACC) and medullar lesions (malignant pheochromocytoma – about 10% (2.5-26%) of all pheochromocytomas) (6). Metastatic AGTs vary in origin, including lung, renal, breast, gastrointestinal (gastric, colorectal), hepatocellular carcinoma and melanoma (1, 3, 5, 8). The presence of an extra-adrenal primary malignancy increases significantly the odds of an

AGT being malignant from 0.1% to 50-75%. Adrenal incidentaloma may be a sole manifestation of a previously undiagnosed cancer in 0.2% of cases (2).

In contrast to frequently occurring ACAs, adrenocortical carcinoma (ACC) is a very rare neoplasm, with an incidence of 0.5-2/million/year, accounting for 3% of all endocrine neoplasms and 0.05-0.2% of all cancers (2-5, 9). It is most commonly detected in the 5th decade in women (5:1 *vs.* men). ACC may be found accidentally (constituting 0-16%, mean 4.4% of incidentalomas) or due to local clinical symptoms (combined: 40% ACCs). However, 50-60% of ACCs present hormone excess, mostly rapidly increasing hypercortisolemia (Cushing's syndrome mixed with virilization – 35%, pure Cushing's syndrome – 30%), less often pure virilization (20%), hyperestrogenism (10%) or Conn's syndrome (the rarest) (9). ACC is often an aggressive neoplasm, associated with a poor prognosis. Due to the lack of early symptoms, it is usually diagnosed at an advanced stage (2, 3, 5, 9).

The minichromosome maintenance (MCM) proteins were described over two decades ago. In total, there are 10 proteins in this group, named MCM1-10. MCM proteins 2-9 possess the characteristic ATPase domain (MCM box) and are therefore a part of the wider AAA+ group (ATPases Associated with diverse cellular Activities). MCM proteins 1 and 10 play similar roles, *i.e.* they are involved in DNA replication. However, they do not possess such ATPase domain. MCM 2-9 are very conservative proteins that are expressed in all eukaryotic organisms. The main function of MCM proteins is their involvement in the initiation and elongation of DNA replication, along with other factors (10, 11). They are essential in the formation of the pre-replicative complex (preRC), the replication fork and the initial steps of DNA synthesis (11, 12). MCM 2-7 form a hexameric, ring-shaped complex that acts as one of the pre-replication factors and associates with the chromatin and the proteins of the origin recognition complex (ORC; including Cdt6 and Cdt1.15) at the M-G1 transition (10, 11). The MCM 2-7 complex is a non-active DNA helicase, consisting of a catalytic center (the core being formed by MCM 4, 6 and 7) and regulatory subunits (MCM 2, 3 and 5). By the activity of other proteins (cdc7/dbf4 and cyclin E/cyclin-dependent kinase 2), the MCM2-7 complex becomes an active DNA helicase at the G1-S transition, enabling recruitment of additional factors to pre-RCs, the unwinding of DNA from its supercoiled state, the formation of replication forks and further DNA replication (11-13). Moreover, MCM proteins interact with many other cellular proteins, and are, therefore, involved in cellular processes such as: chromatin remodeling, maintenance of genome integrity, prevention of re-replication once per cell cycle, DNA repair, DNA transcription, RNA processing, ribosome biogenesis and cell-cycle regulation (10, 11).

MCM proteins are vital for the process of DNA replication and are often called replication-licensing proteins. Cellular levels of MCMs depend on the cell's status, being high in proliferating cells and much lower in quiescent, differentiated or senescent cells. This makes MCMs useful markers of cell proliferation. As increased levels of MCMs are observed in dysplastic and neoplastic cells, evaluation of such levels helps detecting various precancerous states, pre-invasive and invasive neoplasms. During the last decade, the assessment of MCM expression levels has been extensively investigated in diverse human malignancies, confirming their usefulness as diagnostic and prognostic factors. Numerous studies point out that MCM are more specific and sensitive than conventional proliferative markers such as Ki-67 and PCNA. PCNA turns out to detect not only proliferating, but also quiescent cells, while Ki-67 plays an important role in ribosomic biosynthesis, but does not act as a main proliferative factor (11, 12).

This study evaluated the levels of three MCM proteins – MCM-3, 5, and 7, as well as Ki-67 in adrenocortical tumors (ACAs and ACCs). To the best of the authors' knowledge, this is the first study concerning these molecules in AGTs. MCM-3, apart from MCM-2, is the only MCM family member that possesses an NLS domain indicating nuclear location. MCM-3 phosphorylated by cyclin B/CDK1 plays a regulatory role in MCM2–7 complex, while phosphorylation by cyclin E/CDK2 triggers the S phase checkpoint activation (11, 13). MCM-5, in cooperation with cyclin E, associates with the centrosome and regulates its duplication (13). MCM-7 is also located within the cell nucleus. It interacts with many molecules involved in cell-cycle regulation, including pRb, Mat-1 and FLH. It also participates in mRNA transcription and DNA damage regulation. Similar to MCM-3, MCM-7 can be phosphorylated by cyclin E/CDK2 or cyclin B/CDK1. Present in excess, phosphorylated MCM-7 blocks S phase entry. The phosphorylation of MCM-7 is essential for a proper mitotic exit (11, 13).

Materials and Methods

Patients, clinical data and specimens. All patients included in this study were operated on in the 1st Department and Clinic of General, Gastroenterological and Endocrine Surgery, Wroclaw Medical University (WMU). This study has been approved by the Bioethics Committee of the Wroclaw Medical University (opinion nr KB 151/2016). This is a retrospective study, conducted on already available biological material, therefore formal consent was not necessary.

A database was created containing personal data, sex, age, indication for surgery, laterality of the tumor and the dimensions of the tumor for each case. Based on the size of a tumor, the volume was calculated, assuming an elliptical shape. Each patient was hospitalized in an endocrinology ward for comprehensive evaluation of their hormonal status and establishing/confirming an indication for surgery prior to admittance to our department. The vast majority

of patients were referred to our clinic by the Department and Clinic of Endocrinology, Diabetology and Isotope Therapy, of WMU. Based on discharge papers, the hormonal status was investigated and added into the database (serum cortisol profile, free cortisol excretion in 24-h urine collection, aldosterone, and renin plasma activity – both in resting and supine position, and aldosterone excretion in 24-h urine collection). Catecholamines and derivatives, as well as androgens' metabolism markers were out of our interest; there were no androgen-producing ACAs in our observations. Formalin-fixed paraffin-embedded specimens of the respective AGTs were retrieved from the archive of the Department and Division of Pathomorphology, WMU, and passed down to Division of Histology and Embryology, Department of Human Morphology and Embryology, WMU, for laboratory processing. Four specimens of ACA (out of 85) were found severely damaged and therefore excluded from the examination.

Tissue microarrays (TMA), immunohistochemistry (IHC) and evaluation. The material for the study consisted of 81 archived paraffin embedded samples of ACA and 3 of ACC. 7-μm thick paraffin sections were stained with hematoxylin and eosin, then scanned utilizing the histologic scanner Pannoramic MIDI (3DHistech, Budapest, Hungary) under 20× magnification to create virtual slides. The scans were examined by two independent pathologists and representative spots were selected to create microarrays (3 spots per block, 1.5 mm diameter each). Tissue microarrays (TMAs) were created by using the automatic system TMA Grand Master (3DHistech).

Immunohistochemical reactions were performed by using the Autostainer Link 48 (Dako, Glostrup, Denmark) on 4-μm paraffin sections obtained from the TMA blocks. To deparaffinize, rehydrate and unmask the antigens, the sections were boiled in Target Retrieval Solution High pH (for MCM-3, MCM-5, MCM-7 antibodies) or in Target Retrieval Solution Low pH (for Ki-67 antibody) using PTLink (97˚C, 20 min). All IHC reactions were performed using the Dako EnVision FLEX+, Mouse, High pH (Link) (Dako, cat. number K8002) visualization system. Firstly, endogenous peroxidase was blocked using EnVision FLEX Peroxidase-Blocking Reagent for 5 min. Subsequently, the slides were incubated with the primary antibodies: Ki-67 (MIB-1 clone, ready-to-use, Dako, cat. number IR626), MCM-3 (101 clone, 1:50, Dako, cat. number M7263), MCM-5 (1:100, Santa Cruz Biotechnology, (Dallas, TX, USA) cat. number sc-165994), and MCM-7 (1:50, Santa Cruz Biotechnology, cat. number sc-9966) for 20 min. Afterwards, the secondary antibodies conjugated with horseradish peroxidase (HRP) were applied (EnVision FLEX /HRP) for 20 min. Finally, a substrate for HRP (3,3'-diaminobenzidine (DAB)) was added to a 10-min incubation. All the sections were counterstained with EnVision FLEX Hematoxylin (Dako) for 5 min. The slides were washed in distilled water, then dehydrated using graded ethanol solutions (70%, 96%, 99.8%) and xylene. Subsequently, the preparations were mounted in SUB-X Mounting Medium (Dako) using a Coverslipper (Dako).

The reactions were evaluated using an Olympus BX40 microscope, 400× magnification, and the Cell^D imaging system (Olympus). The percentages of positive IHC reactions were calculated and added into the database.

Statistical analysis. Qualitative data were presented as percentages, and as mean±standard deviation. The Shapiro-Wilk test was used to determine the normality of the distribution. To evaluate correlations, the Spearman Rho test was used. For the comparison of qualitative data chi-square test was used. For the comparison of quantitative data within 2 groups, the *t*-test was used for normal distribution, and the Mann-Whitney *U*-test in other cases. For the comparison of the quantitative data within >2 groups, the analysis of variance (ANOVA) was used for normal distribution, and the Kruskal-Wallis test in other cases. All tests were 2-tailed (except for the Shapiro-Wilk test). The threshold of statistical significance was set at 0.05.

Table I. *Demographic data and markers' levels in ACA and ACC groups*.

	ACA	ACC	All	*p*-Value
Gender	66 F/15 M	2 F/1 M	68 F/16 M	0.521
Age	56.7±9.8	68.0±14.1	57.0±10.1	0.151
Size	3.76±1.76	9±1.41	3.89±1.93	0.017*
Volume	33.2±45.9	261.4±15.2	38.7±57.4	0.017*
Ki-67	4.80±2.70%	18.66±10.29%		0.005*
MCM-3	7.50±5.43%	28.54±10.72%		0.005*
MCM-5	4.84±2.64%	8.79±4.32%		0.069
MCM-7	11.48±7.01%	38.60±23.73%		0.008*

*Statistically significant.

Results

In total, 84 patients – 81 (96.4%) diagnosed with ACA and 3 (3.6%) with ACC – were included in this study. There were 68 females (81.0%) and 16 males (19.0%), 57.0±10.1 years. The mean tumor size was 3.89±1.93 cm and the mean calculated tumor volume was 38.7±57.4 cm^3. The details for each group are presented in Table I. There were no significant differences between the ACA and the ACC patients in terms of sex and age. However, malignant tumors in the ACC group had a greater size (p=0.017) and volume (p=0.017) than those in the ACA group. Similarly, malignant tumors in the ACC group were characterized by higher levels of Ki-67 (p=0.005), MCM-3 (p=0.005), and MCM-7 (p=0.008) than those in the ACA group. There was no difference between the ACA and the ACC groups in the level of MCM-5 (p=0.069). The specimens with the lowest and highest levels of the investigated markers are presented in Figure 1.

Among the ACA group, 55 patients (67.9%) were diagnosed with non-functioning ACA (NFA), 15 (18.5%) had aldosterone-producing adenoma (APA), and 11 (13.6%) had cortisol-producing adenoma (CPA). The details of each group are presented in Table II. Females were dominant in each group, although not equally: they constituted 100% of patients with CPA, 83.6% patients with NFA, and 60.0% of patients with APA. There were statistically significant differences between these groups (p=0.027); females were less dominant in the APA group compared to the NFA group

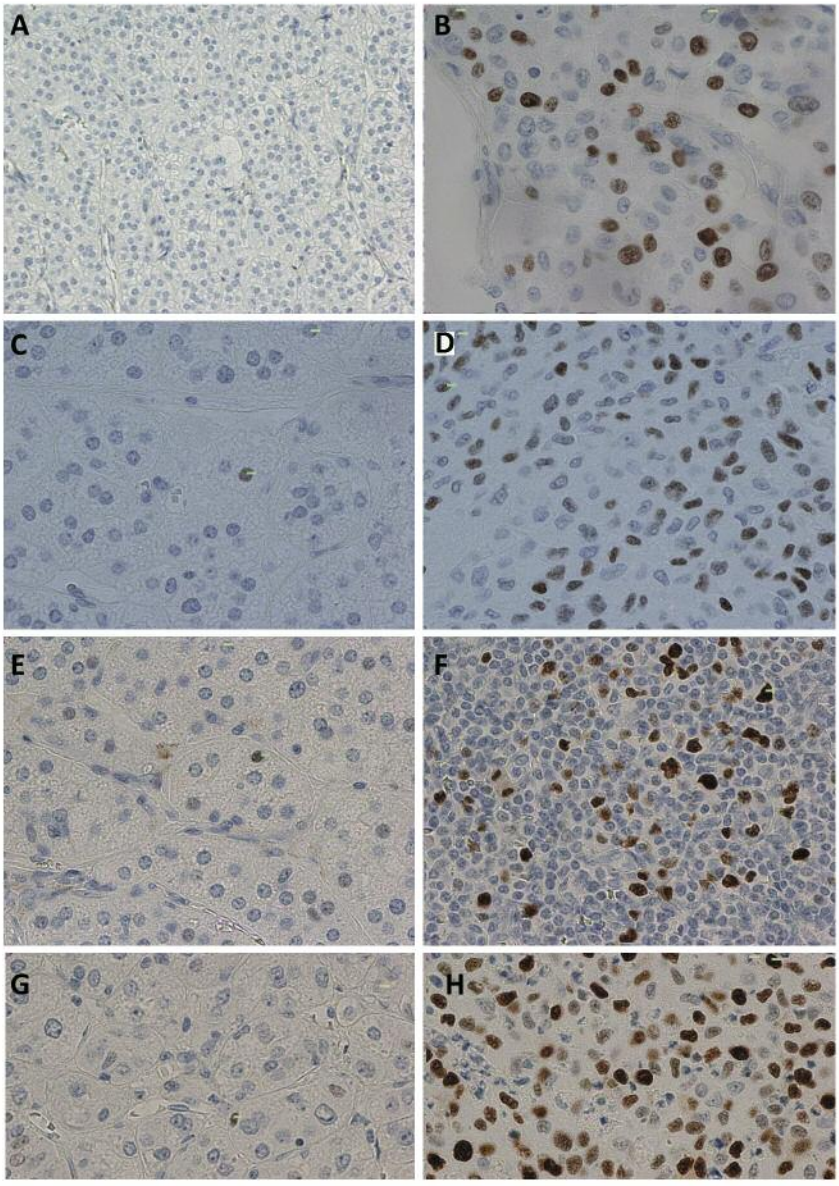

Figure 1. *Specimens with the lowest and highest values of the investigated markers. A: ACA, Ki-67: 0%, 100×. B: ACC, Ki-67: 29.9%, 400×. C: ACA, MCM-3: 1.6%, 400×. D: ACC, MCM-3: 40.6%, 400×. E: ACA, MCM-5: 1.0%, 400×. F: ACC, MCM-5: 13.5%, 400x. G: ACA, MCM-7: 1.1%, 400×. H: ACC, MCM-7: 64.3%, 400×.*

(p=0.048) and the CPA group (p=0.017). There were also differences in age between these groups (p=0.004). Patients with NFA were older than those with APA (p=0.030) and CPA (p=0.047), while no differences between the APA and the CPA groups were observed (p=0.930). When APA and CPA patients were combined into a single group, the difference between NFA and functioning adenomas was even more significant (p=0.004).

There were significant differences in tumor size between groups (p=0.009). NFAs had a significantly greater size than APAs (p=0.007), but not CPAs (p=0.084). APAs and ACAs did not differ in size (p=0.072). The combined group of functioning adenomas had a statistically smaller size than the NFAs (p=0.003). Similar differences were found in terms of calculated volume (p=0.004). NFAs had significantly greater volume than APAs (p=0.002), but CPAs (p=0.057), as well as APAs and CPAs did not differ in volume (p=0.176). The combined group of functioning adenomas had a statistically smaller volume than the NFAs (p=0.001).

There were no differences in the markers' levels between the NFAs, APAs and CPAs (Ki-67, p=0.161; MCM-3, p=0.810; MCM-5, p=0.117; MCM-7, p=0.639). Besides,

Table II. *Demographic data and markers' levels in NFA, APA and CPA groups.*

	NFA	APA	CPA	*p*-Value
Gender	46 F/9 M (83.6/16.4%)	9 F/6 M (60.0/40.0%)	11 F/0 M (100.0/0.0%)	0.027*; N *vs*. A 0.048* N *vs*. C 0.149; A *vs*. C 0.017*
Age	59.1±8.2	51.3±12.0	51.7±10.5	0.004*; N *vs*. A 0.030*
		51.5±11.2		N *vs*. C 0.047* ; A *vs*. C 0.930 N *vs*. A+C 0.004*
Size	4.1±1.6	2.9±2.3	3.3±1.3	0.009*; N *vs*. A 0.007*
		3.1±1.9		N *vs*. C 0.084 ; A *vs*. C 0.072 N *vs*. A+C 0.003*
Volume	38.6±42.1	26.4±68.1	15.5±13.1	0.004*; N *vs*. A 0.002*
		21.8±51.9		N *vs*. C 0.057; A *vs*. C 0.176 N *vs*. A+C 0.001*
Ki-67	4.36±2.08%	6.07±4.47%	5.29±1.77%	0.161; N *vs*. A+C 0.057
MCM-3	7.19±4.54%	8.44±8.83%	7.75±3.58%	0.810; N *vs*. A+C 0.739
MCM-5	4.66±2.61%	4.48±2.67%	6.25±2.57%	0.117; N *vs*. A+C 0.331
MCM-7	11.48±7.10%	12.64±8.47%	9.90±3.99%	0.639; N *vs*. A+C 0.944

*Statistically significant.

after combining APAs and CPAs into a single group, there were no differences in terms of markers' levels between the NFAs and the functioning adenomas (Ki-67, $p=0.057$; MCM-3, $p=0.739$; MCM-5, $p=0.331$; MCM-7, $p=0.944$).

Reciprocal correlations between the markers' levels are presented in Table III. Each marker's level positively correlated with one another. The correlation was strongest (r≈0.6-0.7) between Ki-67 and MCM-3 (r=0.61, $p<0.001$), Ki-67 and MCM-7 (r=0.59, $p<0.001$), and MCM-3 and MCM-7 (r=0.74, $p<0.001$). The correlations between the levels of MCM-5 and other markers were also positive and significant, but weaker (r≈0.3-0.4). This reflects the fact that the levels of MCM-5 did not differ between ACAs and ACCs, while these of other markers did.

Table IV presents the correlations between the markers' levels and the tumors' size and volume, as well as the age of patients. No significant correlation was found between any marker's level and the greatest dimension of the tumor, both in the ACA subgroup and in all the tumors combined. An analysis of ACCs alone was not possible, since only 2 values were available (1 ACC was inoperative and only a surgical biopsy was taken, therefore we could not measure the tumors' size and volume; calculations based on preoperative imaging studies were also impossible). Similarly, there were no significant correlations between any marker's level and either the calculated volume of the tumor or the patient's age, both in the ACA subgroup and in all the tumors combined. Interestingly, there was a significant, positive but weak correlation between the patient's age and the tumor volume in the combined group of ACAs and ACCs (r=0.245, $p=0.025$).

Table V presents the correlations between the markers' and the hormones' levels in all the ACAs combined, as well as limited to solely APAs or CPAs. In general, the markers' levels

Table III. *Reciprocal correlations between markers' levels.*

	MCM-3	MCM-5	MCM-7
Ki-67	r=0.61 $p<0.001$*	r=0.42 $p<0.001$*	r=0.59 $p<0.001$*
MCM-3	x	r=0.39 $p<0.001$*	r=0.74 $p<0.001$*
MCM-5	x	x	r=0.31 $p=0.004$*

*Statistically significant.

did not correlate with any of hormonal parameters, neither in the ACA nor the APA/CPA groups, with 4 exceptions. In the APA group, MCM-3 levels correlated significantly, positively and strongly with resting aldosterone (r=0.829, $p=0.042$) and with aldosterone in supine position (r=0.75, $p=0.02$). Ki-67 levels correlated significantly, positively and weakly with aldosterone in the supine position in the combined ACA group (r=0.348, $p=0.032$). MCM-5 levels correlated significantly, negatively and strongly with aldosterone excretion in 24-h urine collection in the APA group (r=–0.829, $p=0.042$).

Based on the individual values of the markers' levels in each case of ACA and ACC, receiver operating characteristic (ROC) curves were created for each evaluated marker (presented on Figure 2). Ki-67 and MCM-3, followed by MCM-7, seem to be reliable markers for discriminating between ACA and ACC (convex curves, area under curve (AUC) >0.9). MCM-5 (curve almost diagonal, AUC=0.820) with a cut-off point at 7.84% only has 51.07% sensitivity and 88.00% specificity. MCM-5 levels were not different, between ACA and ACC ($p=0.069$).

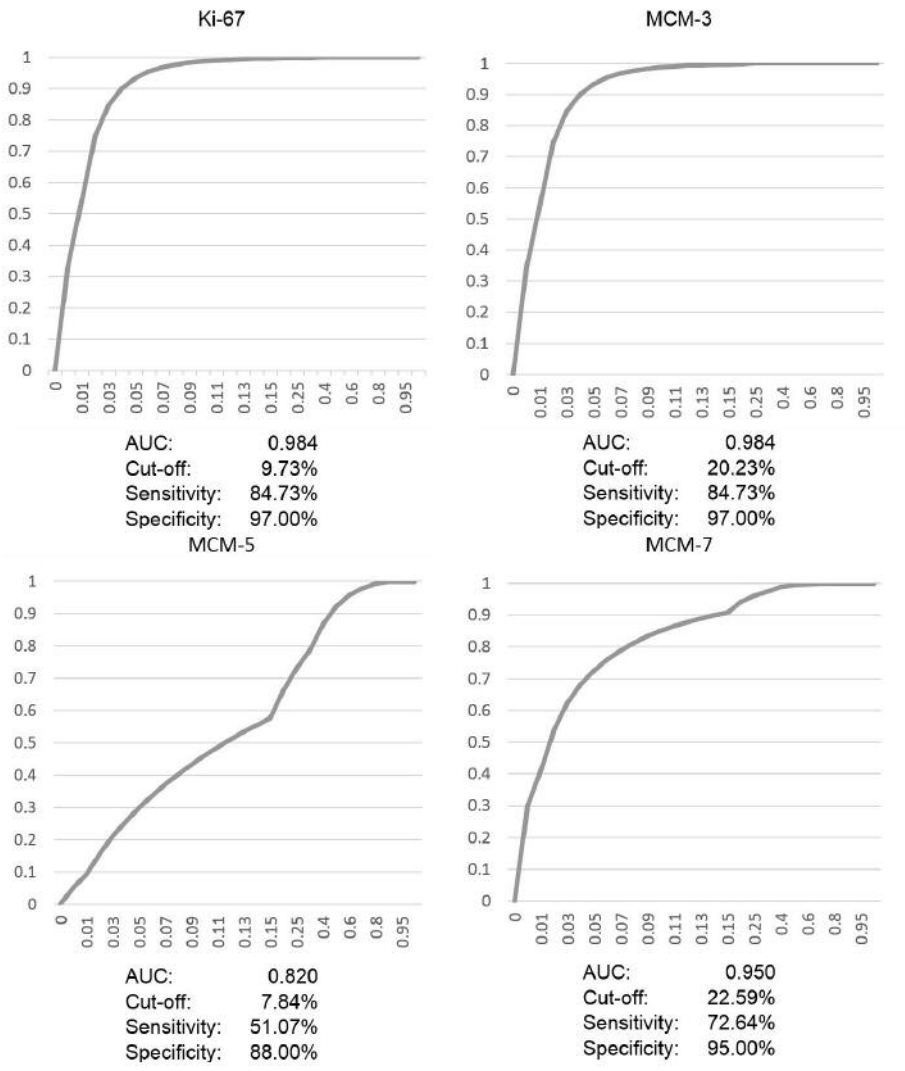

Figure 2. *Receiver operating characteristic (ROC) curves created for the investigated markers.*

Table IV. *Correlations between markers' levels, tumor's dimension and volume and patient's age.*

	Ki-67	MCM-3	MCM-5	MCM-7	Age
Greatest dimension	ACA: r=–0.080	ACA: r=–0.043	ACA: r=0.090	ACA: r=–0.178	ACA: r=0.148
	p=0.492	*p*=0.705	*p*=0.423	*p*=0.111	*p*=0.187
	all: r=–0.002	all: r=0.029	all: r=0.118	all: r=–0.104	all: r=0.204
	p=0.982	*p*=0.794	*p*=0.288	*p*=0.346	*p*=0.064
Calculated volume	ACA: r=–0.114	ACA: r=–0.104	ACA: r=–0.020	ACA: r=–0.171	ACA: r=0.192
	p=0.312	*p*=0.356	*p*=0.860	*p*=0.127	*p*=0.085
	all: r=–0.037	all: r=–0.029	all: r=0.014	all: r=–0.098	all: r=0.245
	p=0.743	*p*=0.795	*p*=0.901	*p*=0.379	*p*=0.025*
Age	ACA: r=–0.144	ACA: r=–0.034	ACA: r=0.026	ACA: r=0.116	X
	p=0.198	*p*=0.763	*p*=0.818	*p*=0.303	
	all: r=–0.083	all: r=0.016	all: r=0.038	all: r=0.150	
	p=0.451	*p*=0.886	*p*=0.728	*p*=0.174	

*Statistically significant.

Table V. *Correlations between markers' and hormones' levels.*

	Ki-67	MCM-3	MCM-5	MCM-7
Serum cortisol 5-6 a.m.	all: r=–0.083	all: r=–0.119	all: r=–0.207	all: r=–0.087
	p=0.629	p=0.488	p=0.226	p=0.612
	CPA: r=0.261	CPA: r=–0.232	CPA: r=0.058	CPA: r=–0.290
	p=0.618	p=0.658	p=0.913	p=0.577
Serum cortisol 8-10 a.m.	all: r=–0.038	all: r=–0.215	all: r=–0.236	all: r=–0.176
	p=0.801	p=0.146	p=0.110	p=0.237
	CPA: r=0.036	CPA: r=0.523	CPA: r=–0.090	CPA: r=0.162
	p=0.939	p=0.229	p=0.848	p=0.728
Serum cortisol 4-8 p.m.	all: r=–0.142	all: r=–0.250	all: r=–0.006	all: r=–0.168
	p=0.345	p=0.094	p=0.967	p=0.263
	CPA: r=0.649	CPA: r=0.198	CPA: r=0.018	CPA: r=0.198
	p=0.115	p=0.670	p=0.969	p=0.670
Serum cortisol 11-12 p.m.	all: r=–0.067	all: r=–0.251	all: r=0.099	all: r=–0.080
	p=0.691	p=0.128	p=0.554	p=0.635
	CPA: r=–0.087	CPA: r=0.754	CPA: r=–0.580	CPA: r=0.464
	p=0.870	p=0.084	p=0.228	p=0.354
Free cortisol excretion	all: r=–0.004	all: r=–0.250	all: r=–0.048	all: r=–0.137
in 24-h urine collection	p=0.983	p=0.130	p=0.774	p=0.412
	CPA: r=0.541	CPA: r=0.234	CPA: r=–0.018	CPA: r=0.018
	p=0.210	p=0.613	p=0.969	p=0.969
PRA resting	all: r=0.102	all: r=–0.103	all: r=–0.143	all: r=–0.211
	p=0.559	p=0.556	p=0.413	p=0.224
	APA: r=0.154	APA: r=–0.154	APA: r=–0.667	APA: r=–0.051
	p=0.805	p=0.805	p=0.219	p=0.935
PRA supine	all: r=0.147	all: r=–0.080	all: r=–0.079	all: r=–0.191
	p=0.391	p=0.641	p=0.646	p=0.266
	APA: r=0.058	APA: r=0.464	APA: r=–0.754	APA: r=0.377
	p=0.913	p=0.354	p=0.084	p=0.461
Aldosterone resting	all: r=0.164	all: r=0.046	all: r=–0.181	all: r=–0.081
	p=0.354	p=0.798	p=0.306	p=0.647
	APA: r=0.486	APA: r=0.829	APA: r=0.600	APA: r=0.657
	p=0.329	p=0.042*	p=0.208	p=0.156
Aldosterone supine	all: r=0.348	all: r=0.044	all: r=–0.064	all: r=0.072
	p=0.032*	p=0.792	p=0.702	p=0.668
	APA: r=0.383	APA: r=0.750	APA: r=0.017	APA: r=0.433
	p=0.309	p=0.020*	p=0.966	p=0.244
Aldosterone excretion	all: r=0.096	all: r=0.282	all: r=–0.122	all: r=0.258
in 24-h urine collection	p=0.602	p=0.118	p=0.505	p=0.154
	APA: r=–0.543	APA: r=–0.429	APA: r=–0.829	APA: r=–0.314
	p=0.266	p=0.397	p=0.042*	p=0.544

*Statistically significant.

Discussion

AGTs are relatively common, with their prevalence rising along with age. Taking into account the current demographic changes in western societies, the management of AGTs will soon become a more important issue. Adrenocortical carcinoma, although rare, is associated with a very poor prognosis and few therapeutic options. Therefore, the question of discerning benign and malignant adrenocortical tumors is of great importance.

The malignant character of AGTs is mostly determined by morphologic criteria, while IHC staining plays a limited role, with the exception of Ki-67 and the mitotic indices as diagnostic and prognostic factors. Ki-67 is a non-histonic protein of the nuclear matrix, detected in the G1-, S-, G2- and M-phases of the cell cycle, and is, therefore, supposedly involved in DNA replication (4). A combination of Ki-67 expression and histopathological features is, to date, the best method for discerning ACCs from ACAs (4, 8, 14). Ki-67 proliferation index (>5%) is indicative of ACC. However, ACCs with a low proliferation index may occur (5). The results of this research confirm the role of Ki-67 in distinguishing between ACAs and ACCs (p=0.005), but a higher cut-off value may be more appropriate (10% instead of 5%).

The presented results indicate as well that MCM-3 and MCM-7 are reliable diagnostic markers in detecting malignant

ACTs. MCM-3 and MCM-7 levels differed significantly between ACAs and ACCs (p=0.005 and 0.008, respectively). They were independent from the tumor's size and volume, the patient's age, as well as the hormonal status (with 1 exception: MCM-3 and aldosterone). MCM-3 and MCM-7 indices (≥20% and ≥23%, respectively) are indicative of ACC. Ki-67, MCM-3 and MCM-7 levels correlated significantly, positively and strongly with one another, as shown in previous studies (11).

MCM-5 seems not to be a reliable marker for ACC. MCM-5 levels did not differ significantly between ACAs and ACCs (p=0.069). The ROC curve for MCM-5 was almost diagonal and with a cut-off point of 7.84% MCM-5 has only a 51.07% sensitivity in detecting ACCs (close to complete randomness). MCM-5 correlated weakly with the other investigated markers.

The roles of MCM 3, 5, and 7 have never been investigated in adrenocortical tumors before. However, Szajerka *et al*. assessed MCM-2 expression in normal adrenal tissue, ACA and ACC. MCM-2 levels turned out to be significantly higher in ACCs when compared to ACAs and normal controls (p<0.05 in both cases). A positive and significant correlation was found between Ki-67 and MCM-2 levels in the malignant tumors group (r=0.136, p<0.05). The authors concluded that MCM-2 is a reliable marker for adrenocortical dysplasia and malignancy (4).

MCM proteins 3, 5, and 7 were previously investigated as proliferation markers in tumors of other endocrine glands, including the thyroid and the pituitary gland. Lee *et al*. studied MCM-3 as a potential proliferation marker in papillary thyroid carcinoma (PTC) using immunohistochemical and western blot analyses. They concluded that MCM-3 could be a more reliable prognostic marker than Ki-67 in PTC. MCM-3 levels were very low or absent in normal thyroid tissues, while they were overexpressed in PTC cells. Additionally, MCM-3 levels correlated positively and significantly with tumor size and extrathyroidal extension (p=0.031 and 0.037, respectively) (15).

Similarly, Guida *et al*. investigated MCM-5 and 7 in papillary and anaplastic thyroid carcinoma (ATC) using immunohistochemistry and northern blotting. The expression of both MCM proteins was negligible in normal thyroid tissue and PTC, but significantly higher in 65% of ATC cases. Moreover, the transcription rate of MCM-7 was up-regulated in ATC, since the MCM-7 promoter activity was at least 10-fold higher in ATC cells compared with normal thyroid cells (16).

Coli *et al*. examined MCM-7 as a prognostic marker in pituitary adenomas (PAs) and found it to be more reliable and informative than Ki-67. Among patients with invasive tumors, a high nuclear expression of MCM-7 (>15%), but not Ki-67, was associated with a much higher (7.7-fold) risk of recurrence or progression. The overexpression of MCM-7 was a factor indicating poor clinical outcome, and might be a candidate marker for surveillance in PA patients (17).

To date, numerous studies present MCM proteins to be better proliferative and diagnostic markers for various malignancies than the classic Ki-67 and PCNA. Indeed, the potential for DNA replication seems to be dependent on the MCM complex. However, MCM proteins take part in many other cellular activities, and most of them are found in sites other than DNA synthesis. Additionally, a reduction of 90% of the cellular amount of MCM proteins seems not to hinder DNA replication or cell cycle progression; this phenomenon is called the "MCM paradox" (10). Perhaps future studies will characterize the position of MCM proteins as proliferative and diagnostic markers more comprehensively.

Moreover, our study confirmed that ACCs are significantly greater in terms of size and volume than ACAs (p=0.017). This is in accordance with other investigators' observations that most ACCs are generally larger than 5-6 cm in diameter and 100 g in weight (1, 2, 5). In our study, two measureable ACCs had 10 and 8 cm in the greatest dimension, and 272 and 250 cm^3 in volume, respectively. We also detected that functioning ACAs are smaller in both size and volume (p=0.003, and 0.001, respectively), and are detected in younger patients (p=0.004) than non-functioning ones. There is no consensus among other researchers about the differences in size between functioning and non-functioning ACAs. Maurea *et al*. found NFAs to be larger than functioning ACAs, but not significantly (18). In turn, Hara *et al*. stated that CPAs are larger than APAs (19). We observed a similar relation, but not significant (p=0.072). Vasilev *et al*. observed that NFAs are smaller than CPAs, and similar in size to APAs, which is in complete opposition to our results (20). In the combined group of all the examined AGTs, the tumors of younger patients had smaller volume (p=0.025).

The most important limitation of this study was the insufficient amount of ACC. However, taking into account the low prevalence of ACC, this seems to be justified.

In conclusion, Ki-67, MCM-3, and MCM-7, but not MCM-5 are reliable proliferative and diagnostic markers in discerning benign and malignant adrenocortical tumors.

Conflicts of Interest

The Authors declare that they have no conflict of interest regarding this study.

Authors' Contributions

MA, PDz, MB and PDo designed the study. MA, PC, EK and AP collected the data. MA performed statistical analysis. MA, PC, AP and MB prepared the manuscript. MA and MB searched the literature. MA, PDz and PDo collected funds. All authors interpreted the data and corrected the manuscript.

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7. Publikacja nr 2:

Perioperative complications of adrenalectomy – 12 years of experience from a single center / teaching hospital and literature review

Perioperative complications of adrenalectomy – 12 years of experience from a single center/teaching hospital and literature review

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Abstract

Introduction: The perioperative complication rate of adrenalectomy varies between 1.7% and 30.7% in the medical literature. This study presents outcomes of adrenalectomy in our center and tries to point out risk factors for perioperative problems.

Material and methods: We retrospectively analyzed all patients who underwent adrenalectomy in our department from January 2004 to June 2015. Patient's sex, indication for procedure, tumor laterality, surgical approach and surgeon's case volume were taken into consideration as possible risk factors for complications.

Results: There were 177 adrenalectomies performed on 170 patients. We reported 18 (10.2%) perioperative complications, 12 (6.8%) surgical and 6 (3.4%) medical. Laparotomy was a significant risk factor for medical ($p < 0.01$) and overall problems ($p = 0.02$). Operations more expansive than just adrenalectomy were associated with higher risk of medical complications ($p = 0.01$). Procedures performed by surgeons with higher volume were associated with smaller risk of medical complications ($p < 0.01$). Right and left adrenalectomies seem to be related to different kinds of risk – bleeding on the right, injury of surrounding structures on the left ($p = 0.05$). Patient's sex, indication for procedure, bilateral procedure and side of operation were not statistically significant risk factors for complications.

Conclusions: Adrenal glands are surrounded by various anatomic structures (colon, pancreas, spleen, diaphragm) that may be injured during adrenalectomy. Complications following a laparoscopic procedure may arise from the use of monopolar coagulation and the patient's position on the operating table. High insufflation pressure during retroperitoneoscopic procedures may cause subcutaneous emphysema.

Key words: complications, laparoscopy, adrenalectomy, teaching, adrenal gland neoplasms.

Introduction

Adrenalectomy is considered to be a relatively safe surgical procedure. However, its perioperative complication rate varies between 1.7% and 30.7% [1, 2]. In the past many features were assessed as possible risk factors for complications. Patient's sex, indication for procedure, tumor laterality, surgical approach and surgeon's case volume were among them. This study presents outcomes of adrenalectomy in our center and

tries to point out some significant risk factors for perioperative problems.

Material and methods

We retrospectively analyzed all patients who underwent adrenalectomy at the 1st Department and Clinic of General, Gastroenterological and Endocrine Surgery of Wroclaw Medical University in the period from January 2004 to June 2015. All patients' data were stored in a clinical database. To identify risk factors for complications we compared their rates in different groups of patients. The χ^2 test and Spearman correlation were used to calculate *p*-values. A *p*-value < 0.05 was considered as statistically significant. All tests were two-tailed.

The number of performed procedures in each year, sex and age of patients, indication for adrenalectomy, surgical approach, side of procedure and histopathological report are summarized in Table I. We treated laparotomy and conversion equally in analysis of complications. Simultaneous additional abdominal procedures were performed in 13 (7.3%) cases. When no material was obtained for histopathological study, it was due to extensive adhesions or associated bleeding (scheduled resection was aborted, but the procedure was associated with the same possible complications as adrenalectomy; therefore we included 4 such cases in our analysis).

The techniques for open and laparoscopic approaches were as follows. For open adrenalectomy the patient was placed in a supine position. Following induction of general anesthesia, the operative field was prepared and draped. A transverse subcostal (Kocher) incision was made below the right or left costal arch, depending on the side of the tumor. After opening the peritoneal cavity, the parietal peritoneum was incised, mobilizing the hepatic or splenic flexure of the colon, respectively. The adrenal gland tumor was identified above the superior pole of the kidney and dissected from surrounding structures. The adrenal vein was doubly ligated and transected between sutures. Dissection was continued further around the tumor, until it had been completely freed, then the specimen was removed from operative field. After inspection of the hemostasis, the abdominal cavity was drained and closed layer by layer. A sterile dressing was placed on the operative wound.

For laparoscopic adrenalectomy the patient was put under general anesthesia, then placed in a lateral position, lying on the side opposite to the tumor. The Veress needle was introduced to the peritoneal cavity about 3–4 cm below the costal arch, medially to the anterior axillary line, on the side of the tumor. The peritoneal cavity was then insufflated with carbon dioxide to 12 mm Hg and the first port with a 30-degree camera was introduced at the same point as the needle. For left adrenalectomy 2 additional ports (10 mm and 5 mm) were placed below the costal arch in a linear pattern. For a right-sided procedure 3 additional ports (two 10 mm and 5 mm) were used; an extra fourth 10 mm port was necessary to introduce the liver retractor. On the right side, the right triangular hepatic ligament was incised, then the parietal peritoneum incised to free the hepatic flexure of the colon. On the left side, splenorenal and colorenal ligaments were dissected and the splenic flexure was mobilized. After visualization of the adrenal tumor, it was dissected using both blunt dissection and monopolar coagulation. The adrenal vein was identified, doubly secured using metal clips and transected between them. The tumor was further dissected then removed from the abdominal cavity using a plastic bag. After inspection of the hemostasis, the abdominal cavity was drained (through the 5 mm port), desufflated and port sites closed. Sterile dressings were applied on port-site wounds.

Results

During the analyzed period 177 adrenalectomies were performed on 170 patients. Five patients were operated on twice and one three times. Five patients underwent two-step bilateral adrenalectomy. In 1 case there was a residual hormonally active adrenal remnant on one side and the patient required a third procedure. In the other case the previously removed tumor recurred after 2 years and the patient required a second operation on the same side; the tumor turned out to be an adrenocortical carcinoma. In our analysis these cases were treated as separate and independent procedures.

We encountered 18 (10.2%) intra- and postoperative complications, 12 (6.8%) surgical, directly related to the procedure, and 6 (3.4%) medical. In some cases the problems resulted from a simultaneous abdominal procedure rather than the adrenalectomy itself. Each complication, its management, type (bleeding, lesion, others), severity (in the Clavien-Dindo classification for postoperative problems) and patient's data are presented in Table II [3].

To identify risk factors we analyzed complication rates in different groups of patients. Table III presents overall, surgical and medical problem rates. Patient's sex, indication for adrenalectomy, site of operation, method and scope of procedure as well as case volume of the performing surgeon were taken into consideration as potential risk factors. Surgical complications were further divided into: bleeding, lesions of surrounding structures and others, and their rates were com-

Table I. Demographic, surgical and pathologic profile of patients and procedures

Year	Number of procedures	Side of procedure	Value	
2004	6	Unilateral:	171 (96.6%)	
2005	5	Right	79 (46.2%)	
2006	7	Left	92 (53.8%)	
2007	8	Simultaneous bilateral	6 (3.4%)	
2008	19	Total	177	
2009	18	**Histopathological report**	**Value**	
2010	15	Adrenocortical adenoma (ACA)	85 (48.0%)	Benign: 156 (88.1%)
2011	13	Nodular hyperplasia (NH)	22 (12.4%)	
2012	14	ACA/NH (distinction impossible)	3 (1.7%)	
2013	31	Pheochromocytoma	19 (10.7%)	
2014	22	No pathology	7 (4.0%)	
2015 (until June)	19	Myelolipoma	7 (4.0%)	
Total	177	Hematoma	4 (2.3%)	
Sex of patients	***N* (%)**	Cyst	6 (3.4%)	
Females	126 (71.2)	Hemangioma	2 (1.1%)	
Males	51 (28.8)	Ganglioneuroma	1 (0.6%)	
Age of patients	**Value**	Metastases	12 (6.8%)	Malignant: 17 (9.6%)
18–19	0 (0.0%)	Adrenocortical carcinoma (ACC)	3 (1.7%)	
20–29	4 (2.3%)	Lymphoma (diffuse large B cell l)	1 (0.6%)	
30–39	11 (6.2%)	Schwannoma	1 (0.6%)	
40–49	28 (15.8%)	No material obtained	4 (2.3%)*	
50–59	67 (37.9%)	Total	177	
60–69	53 (29.9%)	**Indication for adrenalectomy**	**Value**	
70–79	13 (7.3%)	Tumor, non-functioning	99 (55.9%)	
80+	1 (0.6%)	Cyst, non-functioning	3 (1.7%)	
Mean	55.9 ±10.9	Pheochromocytoma or suspicion	24 (13.6%)	
Surgical approach	**Value**	Cushing syndrome	18 (10.2%)	
Retroperitoneal, open	2 (1.1%)	Conn syndrome	19 (10.7%)	
Laparotomy	52 (29.4%)	Cushing and Conn syndrome	1 (0.6%)	
Laparoscopy	105 (59.3%)	Metastases or suspicion	12 (6.8%)	
Laparoscopy with conversion	9 (5.1%)	Recurrent tumor	1 (0.6%)	
Retroperitoneoscopic	9 (5.1%)	Total	177	
Total	177			

**Scheduled resection was aborted due to extensive adhesions or associated bleeding.*

Table II. Description of each complication: patient's data, type (bleeding, lesion, others), severity (in Clavien-Dindo classification) and management

Sex	Age	Side	Comorbidities	Previous surgery or simultaneous procedure	Indication	Approach	Complication	Type	Severity	Management	Histopath. report
M	65	R	Arterial hypertension	–	Tumor	O	Abscess of surgical site and right pleural effusion	S/other	III b	3 relaparotomies: lavage, drainage adhesions release, right pleural drainage	Myelolipoma
F	73	L	–	–	Tumor	L → conv	Iatrogenic perforation of the left (splenic) flexure of the colon	S/lesion		Conversion to laparotomy, 2-layer suture of colon wall	ACA
M	64	R	–	–		L	Surgical site hematoma	S/bleed	III b	Readmission → laparotomy, evacuation of hematoma and subhepatic drainage	ACA
F	35	L	–	Simultaneous: tumor removed with the distal part of pancreas	Tumor	L	Surgical site hematoma and hemorrhagic infarction of the spleen due to splenic vein thrombosis	S/lesion	III b	Readmitted about 10 days later because of abdominal pain → US → laparotomy, evacuation of hematoma and splenectomy; see **Figure 1** – arrows indicate necrotic parts of spleen	ACA, normal pancreas; partial splenic necrosis
F	54	L	Rectal cancer	Simultaneous: LAR, bil adnexectomy, appendectomy, splenectomy	Tumor	O	Localized peritonitis due to sigmo-rectal fistula	S/other	IV a	Relaparotomy (new sigmo-rectal anastomosis) → transfer to ICU → readmission → discharged	Adrenal hyperplasia, rectal ca
F	68	L	Acromegaly	Previous: removal of brainstem tumor 14 y before, partial gastrectomy and thyroidectomy 1 y before	Tumor	L	4-mm diaphragm perforation during removing adhesions after gastrectomy; small left pneumothorax	S/lesion		Laparoscopic Z-shaped suture without conversion to laparotomy; pneumothorax did not require chest tube	ACA
F	43	R	–	–	Cushing syndrome	R	Subcutaneous emphysema of the torso due to high insufflation pressure	S/other	I	Emphysema gradually disappeared over days on its own	ACA
F	55	L	–	–	Tumor	L	Bleeding, hemoperitoneum	S/bleed	III b	Laparotomy, source of bleeding was torn capsule of the spleen → splenectomy	ACA
F	55	R	Type 2 diabetes	Previous: left nephrectomy and adrenalectomy due to renal cancer	Tumor (meta susp.)	L	Paresis of left upper extremity ← temporary dysfunction of brachial plexus ← position on the left side during surgery	S/other	I d	Rehabilitation, physiotherapy	ACA

Table II. Cont.

Sex	Age	Side	Comorbidities	Previous surgery or simultaneous procedure	Indication	Approach	Complication	Type	Severity	Management	Histopath. report
F	52	R	–	Previous: LAR 6 y before	Tumor	O	Surgical wound hematoma	S/other	I	Drainage at bedside	ACA
F	53	R	–	–	Cushing syndrome	L → conv	Tear of right adrenal vein, massive hemorrhage from IVC	S/bleed		Rapid conversion to laparotomy, ligation of bleeding vein, hemostasis	ACA
M	52	L	–	Simultaneous, not planned: splenectomy and left nephrectomy	Tumor (malignancy susp.)	L → conv	Removal of left adrenal gland with spleen and left kidney due to infiltration	S/lesion	IV b	Respiratory and circulatory insufficiency → ICU → readmission → discharged; ↑ creatinine → ambulatory counseling	Adrenal cyst, hemangioma?; no malignancy
F	54	R + L	MEN 2 syndrome	Previous: thyroidectomy due to medullar thyroid cancer (MTC)	Pheochromocytoma (bilateral)	O	Left-side pneumonia	M	II	Antibiotics	Pheochromocytoma
F	58	L	–	Simultaneous: tumor removed with tail of pancreas	Tumor	O	Anemia despite no signs of bleeding from surgical site	M	II	Transfusion of 2 packed red blood cells	Clear-cell ACA*, normal pancreas
F	55	L	–	–	Tumor	O	Thrombophlebitis: superficial in left lower extremity and deep in left upper one	M	II	Subcutaneous injections of particulate heparin	Large (7 × 5 cm) ACA
F	73	L	–	Previous: LHC 2 y before due to colon cancer	Tumor (meta susp.)	O	Pneumonia	M	II	Antibiotics	ACA
M	56	L	Arterial hypertension, paroxysmal tachycardia	–	Tumor (pheo susp.)	O	Postoperative tachycardia up to 120 bpm, hyperthermia up to 38.4°C	M	II	Antibiotic (ampicillin) and β-blocker (propranolol)	ACA 2.5 cm
M	38	R	Pancreatic head tumor	Simultaneous: Whipple pancreatoduodenectomy	Tumor	O	Epidural catheter → hematoma of spinal canal → compression of spinal cord → paraplegia	M	V	Laminectomy and cord decompression – unsuccessful; permanent paraplegia, vast decubitus ulcers, chronic renal failure, various septic complications; death 4 years later due to brain abscess	Adrenal nodular hyperplasia; chronic pancreatitis

**It is an adenoma composed of neoplastic clear cells containing intracytoplasmic lipid droplets [20].*

Table III. The analysis of possible risk factors for complications

Variable	All patients (1)	All complications (2)	Surgical complications (3)	Medical complications (4)	Statistical significance
Sex:					$p = 0.92$ (2)
Female	126 (71.2%)	13 (10.3%)	9 (7.1%)	4 (3.2%)	$p = 0.76$ (3)
Male	51 (28.8%)	5 (9.8%)	3 (5.9%)	2 (3.9%)	$p = 0.80$ (4)
Indication:					
Tumor or cyst	102 (57.6%)	13 (12.7%)	9 (8.8%)	4 (3.9%)	$p = 0.41$ (2)
Hyperactivity	62 (35.0%)	4 (6.5%)	2 (3.2%)	2 (3.2%)	$p = 0.38$ (3)
Metastases/recidivation	13 (7.4%)	1 (7.7%)	1 (7.7%)	0	$p = 0.76$ (4)
Uni-/bilateral:					$p = 0.59$ (2)
Unilateral	171 (96.6%)	17 (9.9%)	12 (7.0%)	5 (2.8%)	$p = 0.50$ (3)
Bilateral	6 (3.4%)	1 (16.7%)	0	1 (16.7%)	$p = 0.07$ (4)
Laterality (for unilateral):					$p = 0.66$ (2)
Right	79 (46.2%)	7 (8.9%)	6 (7.6%)	1 (1.3%)	$p = 0.78$ (3)
Left	92 (53.8%)	10 (10.9%)	6 (6.5%)	4 (4.3%)	$p = 0.23$ (4)
Method:					
Laparotomy or conversion	61 (34.5%)	12 (19.7%)	6 (9.8%)	6 (9.8%)	$p = 0.02$ (2) (*)
Retroperitoneal, open	2 (1.1%)	0	0	0	$p = 0.57$ (3)
Laparoscopic	105 (59.3%)	5 (4.8%)	5 (4.8%)	0	$p < 0.01$ (4) (*)
Retroperitoneoscopic	9 (5.1%)	1 (11.1%)	1 (11.1%)	0	
Scope:					$p = 0.11$ (2)
Only adrenalectomy	164 (92.7%)	15 (9.1%)	11 (6.7%)	4 (2.4%)	$p = 0.89$ (3)
Additional procedures	13 (7.3%)	3 (23.0%)	1 (7.7%)	2 (15.4%)	$p = 0.01$ (4) (*)
Operator:					
No. 1	75 (42.4%)	5 (6.7%)	4 (5.3%)	1 (1.3%)	$p = 0.24$ (2)
No. 2	35 (19.8%)	5 (14.3%)	5 (14.3%)	0	$p = 0.92$ (3)
No. 3	11 (6.2%)	2 (18.2%)	1 (9.1%)	1 (9.1%)	$p < 0.01$ (4) (*)
No. 4 (resident)	10 (5.6%)	0	0	0	$r = –0.10$, $p = 0.72$ (2)
No. 5	10 (5.6%)	1 (10.0%)	1 (10.0%)	0	$r = 0.31$, $p = 0.29$ (3)
No. 6	8 (4.5%)	0	0	0	$r = –0.22$, $p = 0.44$ (4)
No. 7	5 (2.8%)	2 (40.0%)	1 (20.0%)	1 (20.0%)	Resident vs. rest:
No. 8	5 (2.8%)	1 (20.0%)	0	1 (20.0%)	$p = 0.27$ (2)
No. 9	4 (2.3%)	0	0	0	$p = 0.38$ (3)
No. 10	3 (1.7%)	0	0	0	$p = 0.54$ (4)
No. 11	3 (1.7%)	0	0	0	
No. 12	2 (1.1%)	1 (50.0%)	0	1 (50.0%)	
No. 13	2 (1.1%)	1 (50.0%)	0	1 (50.0%)	
No. 14	2 (1.1%)	0	0	0	
No. 15	1 (0.6%)	0	0	0	
No. 16	1 (0.6%)	0	0	0	
Laterality:		Bleeding	Lesion	Others	$p = 0.47$ (2)
Right	79 (46.2%)	2 (2.5%)	0 (0.0%)	4 (5.1%)	$p = 0.06$ (3)
Left	92 (53.8%)	1 (1.1%)	4 (4.3%)	1 (1.1%)	$p = 0.05$ (2 vs. 3) (*)

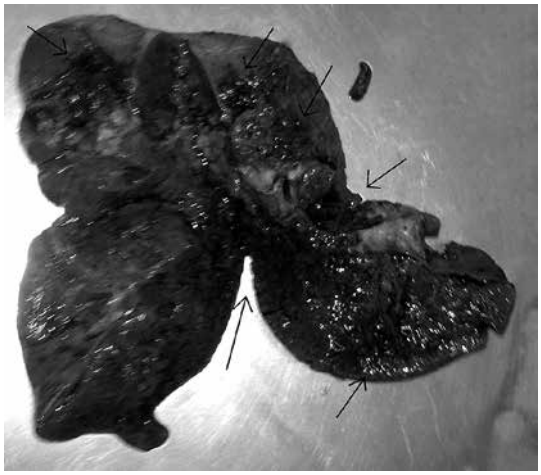

Figure 1. Postoperative specimen, splenectomy due to infarction. Arrows show necrotic parts of the spleen

pared during right- and left-side procedures. Statistically significant values were indicated with an asterisk.

In our analysis patients' sex was not a risk factor for complications. Surgical complications tended to be more common in females while medical ones more common in males. The total complication rate was similar in these groups. Differences were not statistically significant.

Type of adrenal pathology diagnosed by the referring physician was also not a risk factor. Both surgical and medical complications were observed mostly after resection of a benign, non-functioning tumor or cyst, but no differences were statistically significant.

Side of operation was not a risk factor either. All surgical problems followed unilateral procedures. Medical and overall complications were more common after bilateral adrenalectomy, but these differences were not significant.

We observed a higher rate of surgical complications after right adrenalectomy, but it was not statistically significant. Non-surgical problems seemed to be more common after the left-side procedure, also insignificantly. Overall complication rates were similar. Tumor laterality was not a risk factor for complications.

Surgical approach proved to be an important risk factor for medical and total complications. All non-surgical problems followed laparotomy or conversion. Laparotomy was also related to the highest rate of overall problems. Both differences were statistically significant. The highest rate of surgical complications was observed in the retroperitoneoscopic group, but the differences were insignificant.

Operations more expansive than simple adrenalectomy had significantly higher rate of medical complications. Surgical and overall complications were also more common after broader operations, but the differences were not significant.

In our material operations were performed by 16 different surgeons. Only medical complication rate depended on the surgeon case volume. Differences in rates of surgical and overall complications were not statistically significant. There was a weak, inversely proportional dependency between surgical case volume and total and medical complication rates, but statistically insignificant as a correlation.

Among presented surgeons there was one resident (operator no. 4), who performed 10 procedures without any type of complications. His complication rates seemed therefore lower than those of some senior surgeons, but the differences were statistically insignificant.

We compared complication rates in three subgroups of surgical complications (bleeding, lesions of surrounding structures and others) after right and left adrenalectomy. Bleeding was more common on the right side, while lesions occurred more often on the left; the differences were insignificant. When comparing bleeding vs lesions on both sides, the difference was also on the threshold of significance.

Discussion

The occurrence of perioperative complications in adrenal gland surgery varies from 1.7% to 30.7% in the medical literature [1, 2]. Complication rates from our observations place our clinic in the middle class of surgical centers.

We did not find an association between patient's sex and complication rate either in our material or in reviewed articles [4, 5].

Many authors report that surgical and center case volumes are predictors of postoperative complications, but others report no such correlation [6–9]. In our material surgical case volume proved to be an important risk factor for medical complications.

The impact of participation of surgical trainees on perioperative outcomes is interesting. Adrenalectomies performed with residents or fellows take a little longer time (about 16 min), regardless of operative approach. Teaching hospitals may have greater risk of complications. Other studies do not find a correlation between resident participation and perioperative morbidity. One study even found the participation of trainees to decrease the odds of complications, but failed to explain why. In our observation complication rates of residents are at least comparable to those of senior operators [8–10].

Bilateral adrenalectomy is associated with a higher complication rate (23% vs. 15%; even 26% according to Wong) [7, 8, 11, 12]. In our study medical and overall complications were more common after bilateral adrenalectomy. There were no

surgical problems following bilateral procedures, but we had only 6 of them in our material.

Most reviewed publications did not find the side of the operation to be a risk factor for risk of complications [4, 5]. However, due to asymmetry of the abdomen, left and right adrenalectomy may be associated with different types of risk. Both vascular and parenchymal structures should be considered.

The left adrenal vein is 2–4 cm long, passes behind the pancreas, unites with the left inferior phrenic vein and drains into the left renal vein. The right adrenal vein is shorter (1–5 mm) and directly enters the inferior vena cava (IVC). Both length of the right vein and its proximity to the IVC create major risk of iatrogenic injury and bleeding. Therefore it should be early ligated and divided. Loss of control of the vein may cause severe hemorrhage and require immediate IVC repair [11, 13]. Such an event happened to our patient no. 11 – see Table II.

During transperitoneal left adrenalectomy intraoperative injury of the spleen, splenic vessels and colon is possible. The left adrenal gland is also in close proximity to the pancreatic tail, so careless dissection in this area may cause pancreatic injury leading to fistula and/or pseudocyst. The prevalence of pancreatic fistula is 2.3% according to Alesina. We encountered one iatrogenic perforation of the colon, one hemorrhagic infarction of the spleen, one diaphragm perforation and one hemoperitoneum caused by torn capsule of the spleen – see patients no. 2, 4, 6 and 8 [1, 5, 7, 12, 14, 15].

Few studies compare outcomes of adrenalectomy between benign and malignant or secreting and non-secreting tumors. Kiernan concluded that type of pathology influences neither the complication rate nor the necessity of transfusions [14]. Porpiglia found no differences in perioperative variables between secreting vs. non-secreting and malignant vs benign tumors [5].

Pheochromocytoma may have adverse operative outcomes regardless of operative approach. Such tumors may be larger than average, more vascularized and friable. There is always a risk of both hypotensive and/or hypertensive crisis, despite preoperative preparation with adrenolytics. The most dangerous after-effects of hypertensive crisis are cardio-respiratory arrest and cerebral infarction. Early control of the adrenal vein and minimal tumor manipulation are essential for an uneventful procedure [11, 14–16].

Cushing's disease/syndrome makes adrenalectomy more difficult and increases the risk of metabolic complications and respiratory insufficiency. Due to anti-inflammatory and immune-suppressive effects of cortisol, the risk of postoperative infections increases. Inhibition of collagen synthesis and increased blood coagulability make these patients susceptible to poor wound healing, deep venous thrombosis, and pulmonary embolism [12].

Adrenocortical carcinoma is quite rare but also likely to have adverse therapeutic outcomes [14]. Metastases to adrenal glands are much more common and are mainly of mammary, pulmonary, cutaneous or renal origin [17]. According to Hauch *et al.*, malignant tumors have significantly more adverse outcomes (23.1% vs. 13.2%) [8].

Adrenalectomy may utilize several surgical approaches. Anterior laparotomy, open posterior retroperitoneal technique, anterior or lateral laparoscopy and lateral and posterior retroperitoneoscopic methods can be distinguished.

Anterior laparotomy provides good exposure and a wide operative field. It is therefore preferred in cases of large and/or malignant tumors. Myśliwiec recommends classical adrenalectomy for tumors bigger than 10 cm or infiltrating surroundings. Cooper describes longer survival of patients with ACC after an open procedure than in the endoscopic group [1, 6, 14, 17–19].

Despite its usefulness, the open approach is the most invasive and requires the largest incision. This may not be desired in patients with healing problems, such as older or obese patients or those suffering from Cushing's syndrome. Open adrenalectomy or conversion is associated with significantly greater perioperative morbidity, regardless of adrenal pathology [7, 12, 14, 17].

Very few studies compare posterior retroperitoneal open adrenalectomy with other approaches. Wong reports frequent occurrence of neuromuscular complaints and chronic back pain after such procedures, probably due to nerve damage [12].

Laparoscopic adrenalectomy is recommended for small benign adrenal masses (< 6 cm in diameter and < 100 g in weight) and surgical treatment of adrenal metabolic disorders. It is less invasive than an open procedure and thus enables the elderly, the obese or those suffering from circulatory diseases to qualify for treatment. Laparoscopic procedures are associated with lower postoperative morbidity (8% vs. 13–20%) [6, 11, 12, 14, 15, 17, 18].

Laparoscopy is contraindicated for tumors larger than 6 cm, with suspicion of malignancy and in case of extensive adhesions from prior surgery. Laparoscopic resection of malignant masses increases the risk of intraoperative tumor spillage and incomplete clearance. Position of laparoscopic excision of adrenal malignancies is controversial and debatable [6, 11, 12, 14, 15, 17, 18].

Compared with the retroperitoneoscopic technique, laparoscopy offers a larger operative field and working space. Surgeons are generally more familiar with the anatomy and procedures concerning the peritoneal cavity than the retroperitoneal space and thus laparoscopic adrenalectomy is easier for them to learn. Laparoscopy is recom-

mended in the case of a simultaneous abdominal operation, ectopically located adrenal glands and after nephrectomy on the ipsilateral side. Alesina pointed out that splenic injuries and intra-abdominal abscesses occurred only after laparoscopic procedures, while relaxation and/or hypoesthesia of the abdominal wall was observed only after retroperitoneoscopic ones [2, 7, 12, 15, 17].

Minimally invasive retroperitoneal adrenalectomy is recently becoming the gold standard for surgical treatment of small (≤ 6 cm) and benign adrenal tumors, as well as isolated small solitary metastases. It provides the most direct access to the adrenal gland; hence there is no risk of injury to intraperitoneal organs. No bowel manipulation equals no postoperative paralytic ileus. The retroperitoneal approach requires no dissection of adhesions, which makes it suitable for patients with previous laparotomy. Bilateral adrenalectomy, even simultaneous, is possible without repositioning the patient. Insufflation of the retroperitoneal space has a lesser effect on hemodynamic and respiratory parameters than laparoscopy. High insufflation pressure (up to 28 mm Hg) provides better hemostasis due to compression of small vessels [2, 11, 12, 17, 18].

Retroperitoneoscopic adrenalectomy offers limited operative space, more difficult dissection and an inverted anatomic perspective. Therefore it is not suitable for larger tumors (> 7–8 cm), patients with a short distance between the ribs and the iliac crest and/or high BMI, such as those suffering from Cushing's syndrome. It is also contraindicated if a simultaneous intra-abdominal procedure is necessary. In the event of major bleeding immediate conversion is impossible due to the patient's position. High insufflation pressure provokes ejection of catecholamines, increases end-tidal CO_2 pressure, the risk of hemodynamic instability, deep venous thrombosis and gas embolism. Subcostal (Th12) nerve injury may occur in 9% of patients [1, 2, 11, 12, 17–20].

In conclusion, adrenal glands are surrounded by important anatomic structures (such as the colon, pancreas, spleen and diaphragm) that may be injured during careless dissection. That in turn may cause serious postoperative complications, requiring readmitting the patient and reoperation.

Complications after laparoscopic adrenalectomy can arise from the use of monopolar coagulation as well as the patient's position on the operating table. Videoscopic extraperitoneal procedures require higher insufflation pressure to create the working space than laparoscopy; this additional pressure may cause subcutaneous emphysema.

Complication rates depend on type of surgical approach, scope of operation and surgeon's case volume. Laparotomy is a significant risk factor for medical and overall problems. In our observations all non-surgical complications occurred after open procedures.

The second significant risk factor is the scope of the surgical procedure. Operations more expansive than just adrenalectomy are associated with much higher risk of postoperative medical complications.

Individual experience of the performing surgeon proved to be a risk factor for non-surgical complications. Procedures performed by surgeons with higher volume were associated with smaller risk of complications. Perioperative outcomes of residents are at least comparable to those of senior operators.

Patient's sex, type of pathology diagnosed by referring physician, bilateral procedure and side of operation are not statistically significant risk factors for complications.

Different types of complications are associated with right and left adrenalectomy. Risk of bleeding dominates on the right, while injury of surrounding structures occurs mostly on the left.

Conflict of interest

The authors declare no conflict of interest.

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The level of accordance between preoperative and postoperative diagnosis in patients undergoing adrenalectomy – a study of 230 consecutive cases

The level of accordance between preoperative and postoperative diagnosis in patients undergoing adrenalectomy – a study of 230 consecutive cases

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Abstract

Introduction: Patients are qualified for an adrenalectomy due to endocrine or oncologic reasons. Final histopathological diagnoses include a wide spectrum of more than a dozen entities. The aim of this study was to compare preoperative and postoperative diagnoses of patients undergoing adrenalectomy to determine the level of diagnostic accuracy, as well as sex and age of patients.
Material and methods: A group of 214 patients (230 specimens in total) operated on in a single center was studied and their demographic and pathological data were investigated.
Results: The majority of diagnoses were characterized by both high positive predictive value and sensitivity, excluding pheochromocytoma (60.0% and 67.7%, respectively) and adrenal cyst (100% and 37.5%, respectively). Patients operated on due to Cushing's syndrome were statistically significantly more often females (p = 0.009), while those with metastases (diagnosed both pre- and postoperatively) were more often males (both p = 0.001). Patients qualified due to non-functioning tumors were older than those with Cushing's or Conn's syndrome (p = 0.044 and p = 0.002, respectively).
Conclusions: The lowest diagnostic accuracy is observed in cases of pheochromocytoma and adrenal cyst. Meticulous preparation of the patient for hormonal tests, including discontinuation of certain medications, is essential for obtaining accurate results. The diagnosis of Cushing's syndrome is more prevalent in females, while metastasis syndrome is more prevalent in males. Adrenocortical carcinoma may initially be diagnosed as a non-functioning tumor (1.6% of such cases) or a recurrence of a previously resected tumor, which should always raise a suspicion of a malignant neoplasm.

Key words: adrenal gland neoplasms, adrenocortical tumors, pheochromocytoma, adrenalectomy, histopathology,

Introduction

An adrenalectomy is a surgical resection of an adrenal gland. Patients are most commonly qualified for adrenalectomy due to a diagnosis of an

adrenal tumor, either symptomatic or incidentally detected during imaging studies (so-called incidentaloma). Symptoms of an adrenal tumor may be associated with its size (a mass effect) or an excessive secretion of hormones [1]. Indications for adrenalectomy may therefore be divided into two large groups, namely endocrine and oncologic ones. Endocrine indications include basically all hypersecretion syndromes: Cushing's syndrome (ACTH-independent or ACTH-dependent, but with contraindications or refractory to transsphenoidal surgery), primary hyperaldosteronism (Conn's syndrome), hyperandrogenic syndrome of adrenal origin (very rare) and a pheochromocytoma (PHEO; or even a suspicion of one). Oncologic indications appear when an adrenal tumor is of high risk of being malignant, either metastatic or primary. Adrenal glands are common sites of metastases, while primary adrenal malignancies (adrenocortical carcinoma (ACC) and malignant PHEO)) are less common. Oncologic recommendations for adrenalectomy include certain radiologic features of a tumor (high density in unenhanced CT (> 30 Hounsfield units (HU)), slow contrast medium washout (< 50% in the 10th minute) and low lipid content in MR) as well as tumor size greater than 5 cm and/or its fast growth assessed during consecutive imaging studies [2–5].

Preoperative preparations for adrenalectomy depend on the initial diagnosis. Patients diagnosed with hypersecretion syndromes require specific management, which consists of normalization of blood pressure and heart rate using α- and (sometimes) β-blockers (PHEO), control of hypercortisolemia with steroidogenesis inhibitors, e.g. ketoconazole (Cushing's syndrome), or normalization of blood pressure and hypokalemia using spironolactone and potassium (Conn's syndrome) [2, 6].

Following the operation, the histopathological examination of the excised specimen ultimately determines the tumor's character and verifies the preoperative diagnosis. The scope of pathological diagnoses varies widely, including more than a dozen entities, with benign adrenocortical adenoma (ACA) being the most common one [1, 6, 7]. Various pathologic techniques can be used, ranging from classic staining with hematoxylin and eosin (H-E), through different scales designed to assist in the diagnostic process (e.g. the Weiss system), to immunohistochemistry (IHC), which allows one to stain the specimen with antibodies in order to look for a specific protein (antigen). In the case of an adrenal tumor, the most commonly investigated proteins include melan-A (MART-1), inhibin α, chromogranin A (CgA), synaptophysin, neurofilament, protein S-100, calretinin, vimentin, cytokeratins, D2-40, steroidogenic factor-1 (SF-1, Ad4BP), epithelial membrane antigen (EMA) and glycoprotein HMFG-2.

The aim of our study was to compare the initial and final diagnoses of patients undergoing adrenalectomy to determine the level of coherence between them. Based on these data, we estimated the accuracy (sensitivity and positive predictive value (PPV)) for each diagnosis. We also compared the sex and age of patients in every group.

Material and methods

The data of all patients undergoing adrenalectomy, regardless of operation technique, in the 1st Department and Clinic of General, Gastroenterological and Endocrine Surgery, Wroclaw Medical University (WMU), from 2004 to 2018, were investigated. Patients' demographic data, the results of preoperative laboratory and imaging studies, as well as postoperative histopathological reports, were gathered in a database.

Prior to admittance to the surgical department, patients were diagnosed in an endocrine department for comprehensive evaluation of their hormonal status and establishing or confirming an indication for surgical treatment. The vast majority of patients were referred to our clinic by the Department and Clinic of Endocrinology, Diabetology and Isotope Therapy, WMU. The standard preoperative evaluation included at least one imaging study (computed tomography was preferred) and the following laboratory parameters: complete blood count (CBC), electrolytes (Na^+ and K^+), serum cortisol profile, free cortisol excretion in 24-hour urine collection, aldosterone and plasma renin activity (PRA) – both in resting and supine position, aldosterone excretion in 24-hour urine collection, metanephrines in 24-hour urine collection. In selected patients, additional tests were performed, including androgen metabolism markers (dehydroepiandrosterone sulfate (DHEA-S), testosterone, androstenedione, sex hormone-binding globulin (SHBG)), dexamethasone test (with small (2 mg) or large (8 mg) dose), adrenocorticotropic hormone (ACTH), catecholamines in plasma and excretion in 24-hour urine collection, vanillylmandelic acid (VMA), chromogranin A (CgA). Based on clinical presentation, imaging studies and laboratory test, patients were qualified for surgical treatment in 8 categories: 1) tumor without hormonal hypersecretion, 2) adrenal cyst, 3) pheochromocytoma or suspicion of one, 4) Cushing's syndrome, 5) primary hyperaldosteronism (Conn's syndrome), 6) Conn's syndrome coexisting with hypercortisolemia, 7) metastatic tumor or suspicion of one and 8) recurrence of previously removed adrenal tumor.

After adrenalectomy, the postoperative specimens were fixed with 10% formalin, transferred to

the Department and Division of Pathomorphology, WMU, then processed into paraffin-embedded blocks. Slices from blocks were evaluated using classic H-E staining, as well as IHC techniques, depending on the pathologist's choice. Definitive pathological diagnoses included 14 positions, namely: (benign) ACA, nodular hyperplasia (NH), ACA/NH (distinction impossible), PHEO, adrenal gland without any detected pathology, myelolipoma, hematoma, adrenal cyst, hemangioma, ganglioneuroma; and (malignant) metastasis, ACC, lymphoma and schwannoma.

When evaluating the PPV and sensitivity for specific preoperative diagnoses, the following assumptions were made: 1) a non-functioning tumor can turn out to be anything on postoperative pathology, apart from an adrenal gland with no pathology or a cyst; 2) a cyst, a pheochromocytoma and a metastasis can only be a cyst, a pheochromocytoma or a metastasis, respectively; 3) Cushing's or Conn's syndrome or both combined can be an ACA, NH, ACA/NH or ACC; 4) a recurrent tumor can be any type of malignancy. The assessment of sensitivity was possible only for those preoperative diagnoses that had the same postoperative equivalent, that is: a cyst, PHEO and metastasis (as only then could a false negative (FN)) value be calculated).

Statistical analysis

For statistical comparisons of patients' sex, the χ^2 test was used. The Shapiro-Wilk test was used to check the normality of distribution of the age of patients. For further comparisons of the age of patients between groups, the Kruskal-Wallis test with post-hoc Conover test was used.

Results

There were 214 patients undergoing adrenalectomy from January 2004 to December 2018 in the studied department and clinic. There were 150 (70.1%) females and 64 (29.9%) males, from 21 to 81 years old (mean age: 56.0 ±11.3). The majority of patients underwent a single resection, but 6 of them were operated on twice and one was operated on three times (222 operations in total). The laparoscopy was the preferred surgical approach (141 procedures, 63.5%), followed by laparotomy (55, 24.8%), laparoscopy with conversion to laparotomy (15, 6.8%), retroperitoneoscopic (9, 4.1%) and open extraperitoneal method (2, 0.9%). The left adrenalectomy was slightly more common than the right one (109 (49.1%) vs. 105 (47.3%)); 8 (3.6%) procedures were a simultaneous bilateral adrenalectomy. Therefore 230 postoperative specimens were evaluated in total. Among this group there were no cases of a malignant PHEO or an androgen-producing tumor. Perioperative complications that accompanied some of these procedures were described in the previous paper [8].

The comparison between preoperative and postoperative diagnoses is summarized in Table I. Specimens indicated as non-functioning tumors had a variety of pathological diagnoses, both benign and malignant. The ACA was the most common one (62, 48.8%), followed by NH (14, 11.0%) and – interestingly – myelolipoma (13, 10.2%). Only 8 cases did not match the assumed scope of diagnoses – 3 adrenal glands without any pathology and 5 cysts; therefore the PPV was 93.7%. Three specimens diagnosed as adrenal cysts had their diagnosis confirmed (PPV 100%). However, for 8 cysts recognized in total, only 3 were described as one before an operation (sensitivity 37.5%). Out of 35 cases indicated as PHEO, only 21 were confirmed by pathology (PPV 60.0%). At the same time, for 31 PHEOs diagnosed in total, only 21 had the same preoperative diagnosis (sensitivity 67.7%); the remaining 10 were marked as non-functioning tumors. In the Cushing's syndrome group, there was only 1 mismatch per 26 (1 adrenal gland without pathology; PPV 96.2%). Similarly in the Conn's syndrome group, there were 2 mismatches per 20 (1 adrenal gland without pathology and 1 hematoma; PPV 90.0%). One single case of Conn's syndrome with coexisting hypercortisolemia turned out to be an adrenal gland without pathology. Out of 17 cases of suspected metastases 14 were confirmed (PPV 82.4%), yet 3 actual metastases were initially classified simply as non-functioning tumors (sensitivity 82.4%). The origin of metastases was: lung cancer (7 cases, 41.2%), renal cancer (4, 23.5%), adenocarcinoma (site unknown; 2, 11.8%) digestive tract neoplasm (suspicion; 2, 11.8%), skin melanoma (1, 5.9%) and neoplasm of unknown origin (1, 5.9%). One single case of a recurrence of a tumor in a lodge of a previously removed adrenal gland was an ACC; the pathological report from the first procedure was "borderline adenoma" and a close clinical follow-up was indicated. Out of 3 recognized ACCs, 2 were initially diagnosed as non-functioning tumors.

A high PPV signifies a high chance of confirmation of preoperative diagnosis. Most of them were characterized by a high PPV: adrenal cyst and recurrent tumor – both 100%, Cushing's syndrome – 96.2%, non-functioning tumor – 93.7%, Conn's syndrome – 90.0%, suspected metastases – 82.4%. Only pheochromocytoma had considerably lower PPV (only 60.0%), indicating that the remainder (40.0%) were over-diagnosed.

The lower the sensitivity, the greater was the fraction of patients with said condition that was not properly diagnosed (under-diagnosed). Suspected metastases had the highest value (82.4%),

Table I. Comparison of preoperative and postoperative diagnoses

	TU	Cyst	PHEO	Cushing	Conn	C + C	Meta	REC	SUM
ACA	62		9	13+3s	16		1		104
NH	14		4	6	2		2		28
ACA/NH				3					3
PHEO	10		21						31
AG w/o pathol.	3		1	1	1	1			7
Myelolipoma	13								13
Hematoma	5				1				6
Cyst	5	3							8
Hemangioma	2								2
Ganglioneuroma	2								2
Meta	3						14		17
ACC	2							1	3
Lymphoma	1								1
Schwannoma	1								1
Non-diagnostic	4								4
SUM	127	3	35	26	20	1	17	1	230
TP	119	3	21	25	18	0	14	1	
FP	8	0	14	1	2	1	3	0	
FN		5	10				3	2	
Sensitivity		37.5	67.7				82.4		
PPV	93.7	100	60.0	96.2	90.0	0	82.4	100	

TU – non-functioning tumor, PHEO – pheochromocytoma, C + C – Conn's and Cushing's syndrome, META – metastasis, REC – recurrence of a removed tumor, AG w/o pathol. – adrenal gland without any detected pathology, TP – true positive, FP – false positive, FN – false negative, PPV – positive predictive value; diagnostic mismatches are marked by filled (grey) boxes.

followed by pheochromocytoma (67.7%), and adrenal cyst (37.5%).

Regarding the initial (preoperative) diagnosis, there was a statistically significant difference in terms of patients' sex (p = 0.001) – Table II. Further analysis showed that the diagnosis of Cushing's syndrome was more common in females (p = 0.009) and, in turn, the diagnosis of metastases to adrenal glands was more common in males (p = 0.001). There was also a statistically significant difference in terms of patients' age (p = 0.008). Patients diagnosed with Conn's syndrome were significantly younger than those diagnosed with PHEO (p = 0.005), non-functioning tumor (p = 0.002) and recurrent tumor (p = 0.043). Patients diagnosed with a recurrent tumor were also older than those diagnosed with an adrenal cyst (p = 0.045) and simultaneous Cushing's and Conn's syndrome (p = 0.028). Patients diagnosed with non-functioning tumor were older than those with Cushing's syndrome (p = 0.044).

Regarding the final (postoperative) diagnosis, there was a statistically significant difference in terms of patients' sex (p = 0.016). Metastases to adrenal glands were more common in males (p = 0.001). There was no statistically significant difference in terms of patients' age (p = 0.112).

Discussion

The majority of patients in our study were qualified for an adrenalectomy due to a non-functioning adrenal tumor (127/230; 55.2%). Specific indications for surgical resection included: sufficiently large tumor size (≥ 4 cm, associated with an increased risk of malignancy), its fast growth in consecutive imaging studies, high density in CT (not typical for a benign adenoma) or other suspicious CT features, such as calcifications or necrosis – Figures 1 A and B [5, 9]. Most of the tumor in this group (116/127; 91.3%) turned out to be benign in postoperative histopathological eval-

Table II. Demographic characteristics of studied groups

Parameter	Female	Male	SUM	*P*-value (sex)	Age	*P*-value (age)
Initial diagnosis:				0.001		0.008
TU	95 (74.8%)	32 (25.2%)	127	n/s	57.7 ±11.1	
Cyst	1 (33.3%)	2 (66.7%)	3	n/s	42.7 ±10.8	
PHEO	24 (68.6%)	11 (31.4%)	35	n/s	57.8 ±11.0	
Cushing	24 (92.3%)	2 (7.7%)	26	0.009	52.7 ±10.7	
Conn	11 (55.0%)	9 (45.0%)	20	n/s	49.7 ±11.1	
Cushing and Conn	1 (100.0%)	0 (0.0%)	1	n/s	31.0	
Meta	5 (29.4%)	12 (70.6%)	17	0.001	55.3 ±10.9	
REC	1 (100.0%)	0 (0.0%)	1	n/s	70.0	
Final diagnosis:				0.016		0.112
ACA	80 (76.9%)	24 (23.1%)	104	n/s	56.7 ±11.2	
NH	18 (64.3%)	10 (35.7%)	28	n/s	56.0 ±11.0	
ACA/NH	3 1(00.0%)	0 (0.0%)	3	n/s	43.0 ±11.3	
PHEO	24 (77.4%)	7 (22.6%)	31	n/s	54.9 ±11.0	
AG w/o pathol.	6 (85.7%)	1 (14.3%)	7	n/s	54.7 ±11.0	
Myelolipoma	8 (61.5%)	5 (38.5%)	13	n/s	55.2 ±11.2	
Hematoma	5 (83.3%)	1 (16.7%)	6	n/s	62.7 ±11.1	
Cyst	5 (62.5%)	3 (37.5%)	8	n/s	51.8 ±10.9	
Hemangioma	1 (50.0%)	1 (50.0%)	2	n/s	56.5 ±10.6	
Ganglioneuroma	1 (50.0%)	1 (50.0%)	2	n/s	35.0 ±9.9	
Meta	5 (29.4%)	12 (70.6%)	17	0.001	57.0 ±11.0	
ACC	2 (66.7%)	1 (33.3%)	3	n/s	68.0 ±12.4	
Lymphoma	0 (0.0%)	1 (100.0%)	1	n/s	55.0	
Schwannoma	0 (0.0%)	1 (100.0%)	1	n/s	25.0	
Non-diagnostic	4 (100.0%)	0 (0.0%)	4	n/s	66.0 ±10.9	

n/s – non-significant, TU – non-functioning tumor, PHEO – pheochromocytoma, Meta – metastasis, REC – recurrence of a removed tumor, AG w/o pathol. – adrenal gland without any detected pathology.

uation. Polish guidelines from 2002 recommend surgical removal of an adrenal tumor measuring ≥ 4 cm and such an approach was taken in most of the cases described in this paper. Yet an American algorithm (National Institute of Health, 2003) allows observation of such tumors, recommending adrenalectomy only for tumors ≥ 6 cm, unless other radiological features of malignancy are present. This relies on data indicating that the estimated risk of malignancy is about 2% in an adrenal tumor < 4 cm, but rises up to 25% in lesions ≥ 6 cm [10]. Similarly, the updated Polish guidelines from 2016 recommend 5 cm to be a more suitable cut-off value, but only some portion of the studied patient group was operated on after 2016 [2]. We also detected that patients in the non-functioning tumor group were older than those in Cushing's or Conn's syndrome groups ($p = 0.044$ and $p = 0.002$, respectively).

The second main indication for adrenalectomy was hypersecretion of an adrenal cortex (47/230; 20.4%). Patients qualified due to Cushing's syndrome were significantly more often females ($p = 0.009$). Patients in the Conn's syndrome group were significantly younger than those in PHEO ($p = 0.005$), non-functioning tumor ($p = 0.002$) and recurrent tumor groups ($p = 0.043$). The important fact in a preoperative diagnosis of Conn's syndrome is the discontinuation of specific drugs (including ACE inhibitors, angiotensin receptors blockers, progestogens, diuretics, spironolactone and eplerenone) for up to several weeks prior to

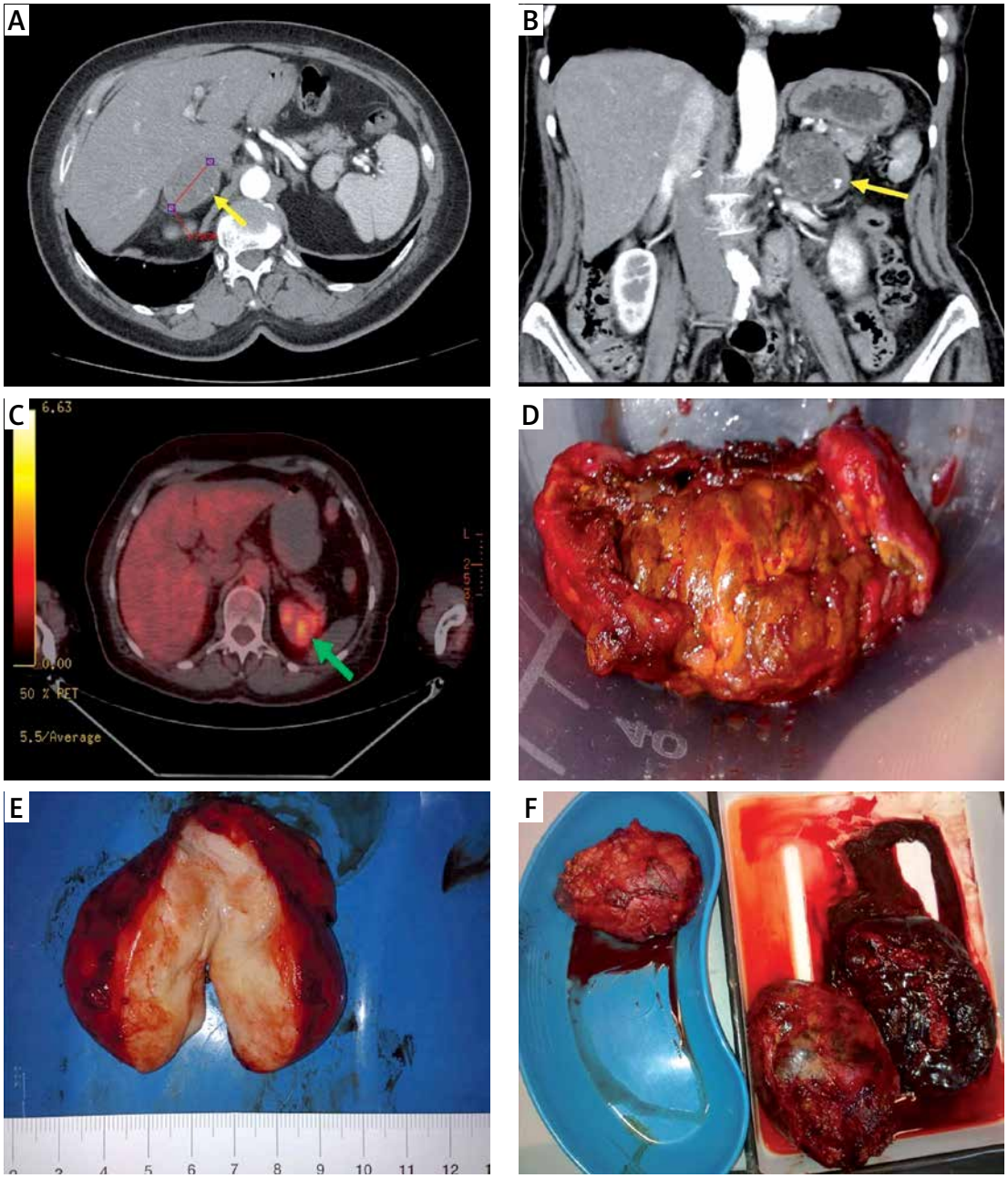

Figure 1. Preoperative imaging studies and postoperative specimens of adrenal tumors. **A** – Computed tompography (CT), transverse scan: right adrenal gland tumor, 47 mm in greatest dimension, 72-year-old woman; postoperative histopathologic report: hematoma of a medullar part. **B** – CT, coronal scan: left adrenal gland tumor, 50 mm in greatest dimension, heterogenicity and calcifications seen within the tumor; 69-year-old woman; postoperative histopathologic report: adrenocortical adenoma 5 × 4 × 2,5 cm. **C** – PET-CT, transverse scan: left adrenal gland tumor, 85 mm in greatest dimension, suspicion of metastasis (73-year-old man who underwent right nephrectomy 5 years before due to clear-cell renal cell carcinoma (CCRCC)); postoperative histopathologic report: CCRCC metastasis to adrenal gland. **D** – Adrenocortical adenoma (typical golden color), around 4 cm, 52-year-old woman with Cushing's syndrome. **E** – Ganglioneuroma, around 8 cm, 43-year-old woman with non-functioning tumor. **F** – Metastases of lung cancer to both adrenal glands, around 10 and 11 cm (size comparable to a spleen, shown in a lower right corner), 67-year-old man

any hormonal workup, since these substances may alter the aldosterone-to-renin ratio (ARR) [2, 5, 10].

The final determination as to the character of an excised adrenal mass is established by a histopathological examination [6, 11]. The scope of possible diagnoses includes at least a dozen options.

Adrenocortical adenoma (ACA) is the most frequent diagnosis (70–94%). Typically ACAs are small (less than 50 g in weight), well-separable from surrounding tissues and golden/yellow in color (Figure 1 D). Most ACAs are hormonally inactive. Among hormonally active ACAs, aldoste-

rone-producing ACAs (APAs) are more common than cortisol-producing ACAs (CPAs), while androgen-producing ACAs are very rare [1, 4, 7, 12]. This is in accordance with our results: there were 72 (69.2%) hormonally inactive ACAs, 16 (15.4%) APAs, 16 (15.4%) CPAs and no androgen-producing ACAs.

Another form of hormonally active adrenocortical growth is nodular hyperplasia (NH). In our observation there were 20 (71.4%) non-functioning NHs, 6 (21.4%) causing Cushing's syndrome and 2 (7.1%) causing Conn's syndrome [13]. In 3 cases discrimination between ACA and NH was impossible due to fragmentation of a specimen.

In a group referred to as non-functioning tumors, the third most commonly recognized pathology was myelolipoma (13 cases, 10.2%). This was similar to the results of Al Harthi *et al*., who estimated the detection of this pathology to be 7% of adrenal incidentalomas. To date it remains unknown how this kind of tumor actually develops. It is usually a small, asymptomatic and non-functional lesion, a benign neoplasm containing mature adipose tissue and hemopoietic elements (but not producing actual blood cells) [14].

PHEO is usually a benign, sporadic, catecholamine-producing adrenal tumor. However, in about 9–23% of cases (and even more in children) it develops in extraadrenal tissues close to sympathetic ganglia (so-called paraganglioma). Also up to 25% of patients may present with the hereditary form of PHEO (e.g. in the course of multiple endocrine neoplasia type 2 (MEN-2) or von Hippel-Lindau (VHL) syndrome), with a tendency for extraadrenal and multifocal tumors. Additionally, in up to 26–35% of cases it is malignant, and at the time of diagnosis, about 10% of patients with PHEO present with a metastatic (disseminated) disease [15]. This is in opposition to our findings, as we did not observe a single case of malignant PHEO. Perhaps such patients were referred to other departments for surgical treatment. According to Myśliwiec *et al.*, biochemical diagnosis of PHEO is associated with a significant percentage of false positive (FP) results, reaching 33%. We can fully substantiate that statement, as in our observation PHEO had the lowest PPV (60.0%), making the remaining 40.0% of cases FP (over-diagnosed). Such a high amount of FP diagnoses may result from various possible distractions in the diagnostic process. Diverse substances, including meals (bananas, chocolate, cocoa, citrus fruits, vanillin), drinks (coffee, tea), drugs (e.g. α- and β-blockers, sympathicomimetics, calcium channels antagonists, aminophylline, disulfiram, L-DOPA, methyldopa, insulin, tetracycline, erythromycin) and narcotics (cocaine, amphetamine, (pseudo)ephedrine) may artificially elevate levels of urinary metanephrines and should definitely be discontinued before performing such diagnostics. The next reason for overdiagnosis may be a routine evaluation of urinary 24-hour metanephrines only and not plasma free metanephrines in a suspicion of PHEO. Testing for plasma metanephrine levels is slightly more sensitive and specific, yet it is also more expensive and less available; therefore the urinary metanephrines remain the most commonly performed test. Moreover, the test for plasma metanephrines became available just a few years ago and our research dates back before this time. The diagnosis of PHEO should also be supported by imaging studies – in a CT PHEO exhibits a non-adenoma pattern (higher density: > 10 HU, usually > 30 HU, compared to < 10 HU for adenoma) [2, 10, 16]. One last issue here is the human factor, that is the fear of omission (under-diagnosis) of PHEO. Since it may be associated with serious complications, physicians are anxious about overlooking a single case of PHEO and may be prone to rather exaggeratedly qualify a patient for an operation than to risk letting go a dubious case.

Adrenal cysts are diagnosed in about 5–8% of incidentalomas, usually unilaterally (> 80%) and more commonly in females – as was the case in our observations (5 females and 3 males). Several types of cysts can be distinguished, including endothelial (congenital or retentive, hemangiomas and lymphangiomas), epithelial (associated with a parasitic infection) and pseudocysts (usually resulting from a hemorrhage or necrosis). Some fraction of lesions classified as hemorrhagic pseudocysts may originate from an angioma, which structure has been destroyed by an internal hemorrhage. Adrenal cysts are associated with about 7% risk of malignancy [17, 18]. In our study a diagnosis of an adrenal cyst had a perfect PPV (100%), yet a relatively poor sensitivity (37.5%), indicating that most of these lesions are initially recognized as solid non-functioning tumors.

Ganglioneuroma is a rare, benign, and typically asymptomatic tumor of the autonomic nerve fibers (consisting of ganglion cells, Schwann cells and fibrous tissue). Apart from adrenal glands, it may be found within the retroperitoneal space, posterior mediastinum, head and neck. Macroscopically it is usually a firm, solid, white tumor (Figure 1 E). Only symptomatic cases require surgical treatment. We had 2 ganglioneuromas in our observation, both initially diagnosed as non-functioning adrenal masses.

Adrenal glands are common sites of metastases from various malignancies of other locations. In about 0.2% of cases an adrenal incidentaloma turns out to be the only manifestation of a previously undiagnosed cancer. If an extra-adrenal malignancy is present, the risk of a co-existing

adrenal tumor being malignant increases from 0.1% to 50–75% [7]. According to the literature, the most common sources of metastases to adrenal glands are: lung, renal, breast, gastrointestinal tract (gastric, colorectal) and liver cancers (hepatocellular carcinoma – HCC) as well as skin melanoma [19]. We observed similar proportions, as the source of most metastases was lung cancer (7; 41.2%), followed by renal cancer (4; 23.5%; Figure 1 C), adenocarcinoma (site unknown; 2; 11.8%) digestive tract neoplasm (2; 11.8%), skin melanoma (1; 5.9%) and a neoplasm of unknown origin (1; 5.9%). In our observation the metastasis group had a high PPV as well as sensitivity (both 82.4%). We also detected that metastases were diagnosed predominantly in males, both pre- and postoperatively (both $p = 0.001$). Taking into account that the most common sources of metastases are lung and renal cancer, which are more prevalent in males, this seems justified. Metastatic adrenal tumors may reach especially large dimensions, such as those of lung cancer presented in Figure 1 F, measuring 10 and 11 cm (comparable to the removed spleen).

ACC is a malignant neoplasm originating from an adrenal cortex. It is found mostly in older females, as we observed (2 females vs. 1 male; mean age 68.0 ±12.4). Despite its rarity (incidence 0.5–2/million/year), ACC is an aggressive neoplasm, lacking early symptoms and therefore often diagnosed at an advanced stage and associated with a poor prognosis. More than half of patients (43–69%, mean 54%) present with stage III/IV and almost half of them (38–40%) have metastases; therefore the overall 5-year survival ranges between only 16 and 44% (mean: 30%) [7]. In our study 2 ACCs were found in the non-functioning tumor group (constituting 1.6% of this group) and 1 was initially diagnosed as a recurrent adrenal tumor (100% of this group). The regrowth of a previously resected adrenal tumor should always raise a suspicion of a malign neoplasm.

To sum up, the advantage of our study is a presentable group of examined patients and specimens (214 and 230, respectively), since adrenalectomy is not routinely performed in local primary care surgical centers and such material is not easy to obtain. On the other hand, the limitation of our study is both the retrospective design and a significant time period of observation (2004–2018), during which much has changed in the area of qualifications as well as surgical approach to adrenalectomy.

In conclusion, the majority of diagnoses of adrenal tumors are characterized by both high PPV and sensitivity. Yet PHEO is characterized by both much lower PPV and sensitivity (60.0% and 67.7%, respectively), indicating that many cases are either over- or under-diagnosed, and closer preoperative diagnostics are necessary to improve these rates. Meticulous preparation of a patient for hormonal tests, including discontinuation of certain medications, is essential for obtaining accurate results. The diagnosis of an adrenal cyst is always confirmed postoperatively (PPV 100%), but some masses initially recognized as solid tumors also turn out to be cysts (sensitivity: 37.5%). Patients qualified for an adrenalectomy due to Cushing's syndrome are significantly more often females ($p = 0.009$), while those with metastases (diagnosed both pre- and postoperatively) are more often males (both $p = 0.001$). Patients qualified due to non-functioning tumors are older than those with Cushing's or Conn's syndrome ($p = 0.044$ and $p = 0.002$, respectively). Adrenocortical carcinoma may initially be diagnosed as a non-functioning tumor (1.6% of such cases) or a recurrence of a previously resected tumor, which should always raise a suspicion of a malignant neoplasm.

Conflict of interest

The authors declare no conflict of interest.

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9. Białka MCM w diagnostyce różnicowej guzów nadnerczy

Streszczenie rozprawy doktorskiej

Guzy nadnerczy rozpoznawane są względnie często, stanowiąc 5 – 9 % wszystkich guzów w organizmie człowieka. Postęp w diagnostyce obrazowej, jaki dokonał się w ostatnich latach, przyczynił się do znacznego zwiększenia ich wykrywalności. Przypadkowo zdiagnozowane guzy nadnerczy (tzw. incidentaloma) występują u 2 – 4 % ogólnej populacji, zaś w grupie osób starszych (po 70 r. ż.) – nawet do 7 %.

Spośród pacjentów z guzami nadnerczy do leczenia operacyjnego kwalifikowani są ci, u których występują wskazania endokrynologiczne (nadczynność wydzielnicza guza), bądź wskazania onkologiczne (podejrzenie nowotworu złośliwego). W pooperacyjnych badaniach histopatologicznych większość usuwanych zmian okazuje się łagodna – najczęściej rozpoznawany jest gruczolak kory nadnercza. Rak kory nadnercza rozpoznawany jest bardzo rzadko, jednak cechuje się złym rokowaniem, co wynika m. in. z braku wczesnych objawów i – związanego z tym – rozpoznawaniu większości przypadków w wysokim stopniu zaawansowania klinicznego. Decydującym kryterium różnicującym zmiany łagodne i złośliwe pozostaje wciąż klasyczne badanie patomorfologiczne, wsparte wynikiem indeksu proliferacyjnego Ki-67. W związku z tym poszukiwane są obecnie nowe wiarygodne markery, mogące wspomóc wczesne ustalenie prawidłowego rozpoznania.

Białka z rodziny MCM (minichromosome maintenance proteins) biorą w komórce udział m. in. w procesie replikacji materiału genetycznego i utrzymaniu integralności genomu. Wykrywane są jedynie w komórkach dzielących się i wykazują wzmożoną ekspresję w wielu rodzajach guzów, co pozwala na traktowanie ich jako potencjalne markery proliferacji.

Ustalenie wskazań do inwazyjnego leczenia guza nadnercza stawia chirurga przed wyborem właściwego dostępu operacyjnego. Od właściwej decyzji zależy skuteczne przeprowadzenie doszczętnego zabiegu chirurgicznego, jak również uniknięcie powikłań okołooperacyjnych.

Obserwowany jest również problem niezgodności wstępnego rozpoznania charakteru guza nadnercza, ustalonego na podstawie przedoperacyjnych badań klinicznych, laboratoryjnych i obrazowych, z ostateczną diagnozą, stawianą w wyniku badania

histopatologicznego. Wg danych z piśmiennictwa, odsetek fałszywych rozpoznań sięga w wybranych przypadkach 33 %.

Niniejsza rozprawa doktorska składa się z cyklu trzech powiązanych tematycznie artykułów.

Głównym celem pierwszej publikacji jest ocena możliwości zastosowania wybranych białek z rodziny MCM (MCM-3, 5 i 7) oraz białka Ki-67 jako markerów proliferacyjnych w diagnostyce różnicowej w łagodnych i złośliwych guzach kory nadnerczy. Badanie prowadzono na materiale archiwalnym preparatów gruczolaków (81) i raków (3) kory nadnerczy, pochodzących od pacjentów operowanych w I Katedrze i Klinice Chirurgii Ogólnej, Gastroenterologicznej i Endokrynologicznej UM we Wrocławiu (UMW) w latach 2004 – 2014. Preparaty przeniesiono na mikromacierze tkankowe (TMA), które następnie wybarwiono metodą immunohistochemiczną (IHC) z użyciem przeciwciał przeciwko MCM-3, 5, 7 oraz Ki-67.

Celem drugiego artykułu jest przedstawienie powikłań okołooperacyjnych i identyfikacja ich czynników ryzyka. Analiza objęła 170 pacjentów (177 zabiegów) poddanych adrenalektomii w powyższej Klinice w latach 2004 – 2015. Płeć pacjenta, wskazania do zabiegu, operowana strona ciała, dostęp chirurgiczny oraz doświadczenie operatora (wyrażone jako łączna liczba wykonanych resekcji nadnerczy) wzięto pod uwagę jako możliwe czynniki ryzyka powikłań okołooperacyjnych.

Celem trzeciej pracy jest analiza zgodności wstępnego rozpoznania klinicznego z ostateczną diagnozą patomorfologiczną. Przeanalizowano dane 214 pacjentów (230 preparatów) operowanych w powyższej Klinice w latach 2004 – 2018. Przedstawiono w szczególności przypadki niezgodności rozpoznań i przedyskutowano ich potencjalne przyczyny.

W pracy nr 1 wykazano, że złośliwe guzy kory nadnerczy cechowały się większą średnicą ($p=0.017$) i objętością ($p=0.017$) oraz wyższymi wartościami indeksu proliferacyjnego białka Ki-67 ($p=0.005$), MCM-3 ($p=0.005$), MCM-7 ($p=0.008$), ale nie MCM-5 ($p=0.069$). Wartości indeksów były niezależne od wymiarów i czynności hormonalnej guza oraz wieku pacjenta. Krzywe ROC potwierdziły, że białka Ki-67 (AUC 0.984), MCM-3 (AUC 0.984), oraz MCM-7 (AUC 0.950), a nie MCM-5 (AUC 0.820) są wiarygodnymi markerami złośliwości guza.

W pracy nr 2 opisano 18 (10.2%) powikłań okołooperacyjnych, 12 (6.8%) chirurgicznych i 6 (3.4%) nie-chirurgicznych. Dostęp poprzez laparotomię ($p < 0.01$), zabiegi bardziej rozległe niż sama adrenalektomia ($p = 0.01$), bądź wykonywane przez mniej doświadczonych operatorów ($p < 0.01$) wiązały się z większym ryzykiem powikłań nie-chirurgicznych. Adrenalektomia prawo- i lewostronna wydają się być powiązane z ryzykiem odmiennych powikłań chirurgicznych, tj. krwawienia po stronie prawej, oraz uszkodzenia otaczających struktur nienaczyniowych po stronie lewej ($p = 0.05$).

W pracy nr 3 przedstawiono, iż większość rozpoznań zmian guzowatych nadnerczy cechowała się zarówno wysoką dodatnią wartością predykcyjną i czułością, za wyjątkiem guza chromochłonnego (odpowiednio: 60.0% i 67.7%) i torbieli nadnercza (odpowiednio: 100% i 37.5%). Chorzy z zespołem Cushinga byli częściej płci żeńskiej ($p = 0.009$), podczas gdy ci z przerzutami nowotworu do nadnerczy – płci męskiej ($p = 0.001$). Pacjenci z guzami nieczynnymi hormonalnie byli starsi niż ci z zespołem Cushinga czy Conna (odpowiednio: $p = 0.044$ i $p = 0.002$).

Białka Ki-67, MCM-3 i MCM-7, a nie MCM-5, są wiarygodnymi markerami diagnostycznymi w różnicowaniu łagodnych i złośliwych guzów kory nadnerczy. Na ich wartość nie mają wpływu wymiary, aktywność hormonalna guza, czy wiek pacjenta. Według wiedzy autorów jest to pierwsze tego typu opracowanie w światowej literaturze medycznej.

Nadnercza otoczone są przez liczne struktury anatomiczne (jak okrężnica, trzustka, śledziona, czy przepona), które mogą zostać uszkodzone podczas zabiegu adrenalektomii. Powikłania po procedurach laparoskopowych mogą być konsekwencją nieuważnego użycia koagulacji monopolarnej, czy ułożenia chorego na stole operacyjnym. Wysokie ciśnienie insuflacji podczas dostępu zaotrzewnowego minimalnie inwazyjnego może skutkować powstaniem rozedmy podskórnej.

Najniższą zgodność rozpoznania przed- i pooperacyjnego zabiegu zaobserwowano w guzach chromochłonnych i torbielach nadnerczy. Zespół Cushinga rozpoznawany jest częściej u kobiet, podczas gdy przerzuty nowotworowe do nadnerczy – u mężczyzn. Rak kory nadnercza może być początkowo diagnozowany jako guz nieczynny hormonalnie (stanowiąc 1,6 % przypadków w tej grupie), bądź jako nawrót uprzednio usuniętego guza, co zawsze powinno budzić podejrzenie nowotworu złośliwego.

10. MCM proteins in differential diagnosis of adrenal tumors

Summary of dissertation

Adrenal gland tumors (AGTs) are relatively common and constitute 5 – 9 % of all human tumors. Much better accessibility to diagnostic imaging procedures in recent years has revealed that the rate of AGTs is significantly higher than previously reported. The prevalence of incidentally detected adrenal mass (so-called incidentaloma) is about 2 – 4 % for general population and up to 7% in patients over 70 years old.

Among patients with adrenal tumors, two groups are qualified for a surgical treatment – those with endocrine indications (hormonal hypersecretion syndromes) or oncologic indications (suspicion of a malignant neoplasm). The majority of adrenal tumors are benign, with adrenocortical adenoma (ACA) being the most common diagnosis. Although adrenocortical carcinoma (ACC) are diagnosed quite rarely, they are associated with very poor prognosis, which is caused by the lack of early symptoms, that results in most of cases being diagnosed at an advanced clinical stage. To date, morphological features, combined with Ki-67 proliferative index, remain the standard for discriminating benign and malignant adrenocortical tumors. Therefore new reliable markers are searched for, that could be helpful in a diagnostic process.

The minichromosome maintenance (MCM) proteins are involved e. c. in a process of DNA replication and maintaining genome integrity. They can only be detected within dividing cells and are more prominently expressed in many types of tumors, which allows to treat them as possible proliferative markers.

Establishing an indication for an adrenalectomy implies the appropriate selection of operative access by the surgeon. The right decision warrants an effective and radical surgical resection, as well as a low rate of perioperative complications.

Additionally, there is an issue of discrepancy between an initial diagnosis of an adrenal tumor, based on preoperative clinical, laboratory and imaging studies, and the final diagnosis, based on histopathological evaluation. According to the available literature, the rate of false positive diagnoses may be as high as 33% in certain situations.

This dissertation consists of three articles, connected by a common theme.

The main aim of the first paper was to evaluate the role of minichromosome maintenance proteins MCM-3, MCM-5, MCM-7, and Ki-67 as proliferative markers in differential diagnosis of benign and malignant adrenocortical tumors. Archival specimens of 81 adrenocortical adenomas and 3 adrenocortical carcinomas of patients operated from 2004 to 2014 in 1st Department and Clinic of General, Gastroenterological and Endocrine Surgery, Wroclaw Medical University, were retrieved. Then they were transferred on tissue microarrays (TMA), stained with antibodies against MCM-3, 5, 7 and Ki-67, using immunohistochemical (IHC) method.

The aim of the second paper was to present perioperative complications and risk factors of adrenal operations. In total 170 patients (177 procedures) who underwent adrenalectomy between 2004 and 2015 in the above mentioned Department were analyzed. Patient's sex, indication for the procedure, tumor laterality, surgical approach and surgeon's case volume were taken into consideration as possible risk factors for complications.

The aim of the third paper was to analyze the coherence between an initial clinical diagnosis with a final histopathological diagnosis. The analysis included 214 patients (230 specimens)
clinical data of patients operated between 2004 and 2018 in above Department. Cases of inconsistent diagnoses were presented and possible reasons discussed.

In the first paper we proved that malignant tumors were characterized by a greater size ($p=0.017$), volume ($p=0.017$), and higher levels of Ki-67 ($p=0.005$), MCM-3 ($p=0.005$), MCM-7 ($p=0.008$), but not MCM-5 ($p=0.069$). The markers' levels were independent from the tumors' dimensions and hormonal function, as well as patient's. ROC curves showed Ki-67 (AUC 0.984), MCM-3 (AUC 0.984), and MCM-7 (AUC 0.950), but not MCM-5 (AUC 0.820) to be reliable markers.

In the second paper we described 18 (10.2%) perioperative complications, 12 (6.8%) surgical and 6 (3.4%) medical. Access through laparotomy ($p < 0.01$), operations more expansive than just adrenalectomy ($p = 0.01$) or performed by surgeons with smaller case volume ($p < 0.01$) were associated with increased risk of medical complications. Right and left adrenalectomy seem to be associated with a different kinds of risk and complications – a bleeding on the right side and an injury of surrounding structures on the left side ($p = 0.05$).

In the third paper we showed that the majority of diagnoses were characterized by both high positive predictive value and sensitivity, excluding pheochromocytoma (60.0% and 67.7%, respectively) and adrenal cyst (100% and 37.5%, respectively). Patients with Cushing's syndrome were more often females ($p = 0.009$), while those with metastases to adrenal gland – males ($p = 0.001$). Patients with non-functioning tumors were older than those with Cushing's or Conn's syndrome ($p = 0.044$ and $p = 0.002$, respectively).

Ki-67, MCM-3 and MCM-7 proteins, but not MCM-5, are reliable diagnostic markers in differentiation of benign and malignant adrenocortical tumors. These markers' levels are dependent neither from dimensions and hormonal activity of the tumor, nor the patient's age. To the best of authors' knowledge, this is the first such study presented in medical literature worldwide.

Adrenal glands are surrounded by various anatomic structures (such as colon, pancreas, spleen, diaphragm) that may be injured during adrenalectomy. Complications following a laparoscopic procedure may arise from the use of monopolar coagulation and the patient's position on the operating table. High insufflation pressure during retroperitoneoscopic procedures may cause subcutaneous emphysema.

The lowest diagnostic accuracy is observed in pheochromocytomas and adrenal cysts. Cushing's syndrome is more prevalent in females, while metastases to adrenal glands are more prevalent in males. Adrenocortical carcinoma may initially be diagnosed as a non-functioning tumor (1.6% of such cases) or a recurrence of a previously resected tumor, which should always raise a suspicion of a malignant neoplasm.

11. Oświadczenia współautorów określające indywidualny wkład w powstanie prac

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Oświadczam, że w pracy:
Michał Aporowicz, Piotr Czopnik, Eliza Kubicka, Aleksandra Piotrowska, Piotr Dzięgiel, Marek Bolanowski, Paweł Domosławski, 2019, Minichromosome Maintenance Proteins MCM-3, MCM-5, MCM-7, and Ki-67 as Proliferative Markers in Adrenocortical Tumors, Anticancer Research, 39: 1151-1159
mój udział polegał na: współudziale w opracowaniu koncepcji pracy, współudziale w kierowaniu projektem naukowym obejmującym badania opisane w tej pracy, zebraniu danych klinicznych i stworzeniu bazy danych, ocenie ostatecznych preparatów mikroskopowych, opracowaniu części statystycznej, przeglądzie dostępnej literatury, napisaniu wersji roboczej artykułu oraz korekcji i akceptacji ostatecznego tekstu pracy.

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mój udział polegał na: udziale w gromadzeniu danych klinicznych, korekcji i akceptacji ostatecznego tekstu pracy.

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mój udział polegał na: sformułowaniu założeń pracy, współudziale w kierowaniu projektem naukowym obejmującym badania opisane w tej pracy, nadzorze nad przebiegiem badań, korekcji i akceptacji ostatecznego tekstu pracy.

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mój udział polegał na: opracowaniu dostarczonego materiału biologicznego i przeniesieniu go na mikromacierze tkankowe, wykonaniu doświadczeń techniką immunohistochemii, nadzorze nad oceną ostatecznych preparatów, korekcji i akceptacji ostatecznego tekstu pracy.

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