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Nowoczesne terapie padaczki lekoopornej u pacjentów ze stwardnieniem guzowatym

Rozprawa na stopień doktora w dziedzinie nauk medycznych i nauk o zdrowiu w dyscyplinie nauki medyczne

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2. **Śmiałek D**, Jóźwiak S, Kotulska K. Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study. Journal of Clinical Medicine. 2023;12(1):1-13
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3. **Śmiałek D**, Kotulska K, Duda A, Jóźwiak S. Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Comples Under 2 Years of Age. Neurology and Therapy. 2023:1-16 (ahead-of-print)
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Spis treści

1. Wykaz stosowanych skrótów

AE – *adverse effect*, działanie niepożądane

AML – *angiomyolipoma*, naczyniakomięśniakotłuszczak

ASM – *antiseizure medication,* leki przeciwpadaczkowe

CR – *cardiac rhabdomyoma*, mięśniak prążkowanokomórkowy serca

CTCAE – *Common Terminology Criteria for Adverse Events,* powszechne kryteria terminologii dla zdarzeń niepożądanych

DRE – *drug-resistant epilepsy,* padaczka lekooporna

EMA – *European Medicines Agency*, Europejska Agencja Leków

FDA – *Food and Drug Administration*, Agencja Żywności i Leków

ILAE – *International League Against Epilepsy*, Międzynarodowa Liga Przeciwpadaczkowa

OUN – ośrodkowy układ nerwowy

mTOR – *mechanistic target of rapamycin*, ssaczy cel rapamycyny

mTORi – *mTOR inhibitor,* inhibitor mTOR

SEGA – *subependymal giant cell astrocytoma*, gwiaździak podwyściółkowy olbrzymiokomórkowy

TSC – *tuberous sclerosis complex,* stwardnienie guzowate

2. Streszczenie w języku polskim

Wstęp: Stwardnienie guzowate (TSC) jest chorobą skórno-nerwową dziedziczoną autosomalnie dominująco, występującą u 1 na 6000 dzieci. Mutacja w genie *TSC1* lub *TSC2* doprowadza do nadmiernej aktywacji kompleksu kinazy mTOR i powstania łagodnych zmian guzowatych na wielu narządach, w tym wątrobie, sercu, płucach, oraz ośrodkowym układzie nerwowym. U niemal 80% pacjentów z TSC występują napady padaczkowe, przeważnie pojawiające się w pierwszych miesiącach życia. Prospektywne badania kliniczne wykazały, że prewencyjne włączenie leczenia przeciwpadaczkowego, wigabatryny, przed wystąpieniem pierwszych napadów padaczkowych zmniejsza częstość napadów oraz ryzyko wystąpienia padaczki lekoopornej (DRE). Mimo to, u ponad 60% pacjentów z padaczką napady stają się lekooporne. W ostatnich latach inhibitor mTOR (mTORi), ewerolimus, został zarejestrowany do stosowania u pacjentów z padaczką lekooporną (DRE) powyżej drugiego roku życia, gwiaździakiem podwyściółkowym olbrzymiokomórkowym oraz naczyniakomięśniakotłuszczakiem nerki w przebiegu TSC. Aktualna wiedza na temat skuteczności mTORi, sirolimusu oraz ewerolimusu, w leczeniu padaczki u dzieci poniżej drugiego roku życia jest ograniczona. Pacjenci w tej grupie wiekowej mogą odnieść korzyści z prewencji oraz poprawy kontroli padaczki, ponieważ wczesne wystąpienie napadów, a w szczególności DRE, wiąże się ze zwiększoną częstością niepełnosprawności intelektualnej oraz trudnościami w nauce. Działania niepożądane (AE) związane ze stosowaniem sirolimusu u najmłodszych pacjentów jak dotąd nie zostały w pełni poznane, mimo że jest on częściej stosowany w Polsce niż ewerolimus z uwagi na większą dostępność i przystępne kryteria refundacji.

Cel pracy: Celem niniejszej rozprawy doktorskiej było podsumowanie aktualnego stanu wiedzy na temat czynników ryzyka wystąpienia DRE u pacjentów TSC oraz ocena bezpieczeństwa i skuteczności mTORi w leczeniu padaczki u dzieci przed ukończeniem drugiego roku życia.

Metodologia: Przeprowadzono przegląd elektronicznych baz publikacji naukowych na temat czynników ryzyka wystąpienia lekoopornych napadów padaczkowych u pacjentów z TSC. Analiza została wykonana zgodnie ze schematem PRISMA (ang. *Preferred Reporting Items for Systematic reviews and Meta-Analyses*), a do wyboru artykułów posłużył model PICOS (ang. *Population, Intervention, Comparison, Outcome, Study*). W celu oceny bezpieczeństwa i skuteczności leczenia mTORi u najmłodszych pacjentów przeprowadzono

analizę retrospektywną dokumentacji medycznej dzieci ze potwierdzoną diagnozą TSC, u których zastosowano sirolimus lub ewerolimus po raz pierwszy przed ukończeniem drugiego roku życia. Pacjenci otrzymywali leczenie w latach 2008-2022 w Klinice Neurologii i Epileptologii Instytutu „Pomnik-Centrum Zdrowia Dziecka" oraz w Klinice Neurologii Dziecięcej Warszawskiego Uniwersytetu Medycznego. Przeanalizowano działania niepożądane związane ze stosowaniem sirolimusu w zależności od wieku oraz czasu trwania leczenia. Do oceny skuteczności leczenia włączono pacjentów, u których leczenie mTORi rozpoczęto przed wystąpieniem napadów oraz tych, u których zgłaszano napady padaczkowe w miesiącu poprzedzającym włączenie leku. Zbadano zmianę częstości napadów w obu tych grupach trzy, sześć, dwanaście oraz dwadzieścia cztery miesiące po rozpoczęciu leczenia. Przeprowadzono analizę porównawczą z grupą kontrolną, w której pacjenci nie otrzymali mTORi.

Wyniki: Rozprawa doktorska składa się z jednej pracy przeglądowej oraz dwóch prac oryginalnych. Praca przeglądowa podsumowuje aktualny stan wiedzy na temat parametrów związanych z wystąpieniem DRE u pacjentów z TSC. Wśród najistotniejszych czynników ryzyka DRE wyróżniono trzy: obecność mutacji w genie *TSC2*, napady zgięciowe oraz liczbę guzków korowych. Ponadto, zauważono zależność pomiędzy wczesnym wiekiem wystąpienia napadów padaczkowych a lekoopornością. Do retrospektywnej analizy danych medycznych wstępnie zakwalifikowano 529 pacjentów z obu ośrodków. Po uszczegółowieniu kryteriów dotyczących leczenia mTORi rozpoczętego przed ukończeniem drugiego roku życia, do badania włączono 21 osób (21/24, 87,5%) leczonych sirolimusem oraz 3 ewerolimusem (3/24, 12,5%). W pierwszej pracy oryginalnej oceniono bezpieczeństwo stosowania sirolimusu w grupie wszystkich 21 pacjentów, którzy otrzymali lek. Działania niepożądane wystąpiły u wszystkich pacjentów leczonych sirolimusem, jednak zostały określone jako reakcje o niewielkim lub średnim nasileniu, nie powodujące zagrożenia życia lub zdrowia. Przeważnie odnotowywano nieprawidłowe wyniki badań laboratoryjnych: anemię, zaburzenia lipidowe oraz nadpłytkowość. Pacjenci nie wymagali istotnych interwencji, ani wdrażania dodatkowego leczenia. Druga praca oryginalna przedstawia wpływ sirolimusu oraz ewerolimusu włączonych przed ukończeniem drugiego roku życia na przebieg padaczki u pacjentów z TSC. Dziewięcioro pacjentów (9/21, 42,9%) otrzymało mTORi przed wystąpieniem napadów, a u dwunastu osób (12/21, 57,1%) zaobserwowano napady padaczkowe w miesiącu poprzedzającym włączenie leku. Troje pacjentów zostało wykluczonych z tej części analiz z uwagi na wystąpienie ostatniego napadu ponad miesiąc

przed włączeniem leczenia. U większości pacjentów leczonych prewencyjnie napady pojawiły się jednorazowo, a 78,8% z nich nie prezentowała napadów padaczkowych w momencie zakończenia obserwacji. W grupie pacjentów, u których występowały napady padaczkowe przed włączeniem mTORi, po dwudziestu czterech miesiącach redukcja średniej liczby napadów była istotna statystycznie ($p = 0,031$). Dwa lata po rozpoczęciu leczenia w grupie pacjentów leczonych mTORi, częstość napadów padaczkowych uległa znaczącej poprawie w stosunku do grupy kontrolnej ($p = 0,0079$).

Wnioski: Przegląd systematyczny literatury wykazał, że czynniki ryzyka DRE u pacjentów z TSC są przeważnie niemodyfikowalne. Regularne monitorowanie pacjentów ze stwierdzonymi czynnikami ryzyka pozwala na wczesne i skuteczne wdrożenie leczenia przeciwpadaczkowego. Uzyskane wyniki badań wskazują na poprawę kontroli napadów padaczkowych u pacjentów z włączonym mTORi przed ukończeniem drugiego roku życia. Działania niepożądane sirolimusu w tej grupie wiekowej są częste, ale o niewielkim lub średnim nasileniu. Dalsze prospektywne badania kliniczne powinny zostać przeprowadzone, aby potwierdzić uzyskane wyniki na temat skuteczności i bezpieczeństwa stosowania mTORi w grupie najmłodszych pacjentów z TSC.

3. Streszczenie w języku angielskim

Title: “Modern therapies for refractory epilepsy in patients with tuberous sclerosis complex”

Introduction: Tuberous sclerosis complex (TSC) is an autosomal dominant neurocutaneous disorder affecting 1 in 6,000 children. Mutation in the *TSC1* or *TSC2* gene leads to overactivation of the mTOR kinase complex and formation of benign tumors on multiple organs, including the liver, heart, lungs, and central nervous system. Nearly 80% of patients with TSC develop epileptic seizures, most of them in the first months of life. Prospective clinical trials have shown that the preventive antiepileptic treatment with vigabatrin reduces seizure frequency and the risk of drug resistance. Nonetheless, seizures become refractory in two-thirds of patients with epilepsy. Recently, a mTOR inhibitor (mTORi), everolimus, has been approved for use in patients with drug-resistant epilepsy (DRE) older than two years, subependymal giant cell astrocytoma, and renal angiomyolipoma in TSC. Current knowledge of the efficacy of mTORi, sirolimus, and everolimus in epilepsy treatment in children under two years of age is limited. Patients in this age group may benefit from prevention and epilepsy control improvement, as early-onset seizures, particularly DRE, are associated with an increased incidence of intellectual disability and learning difficulties. Adverse effects (AEs) of sirolimus in the youngest patients have not been established yet, although it is more frequently used in Poland than everolimus due to its availability and more preferable reimbursement criteria.

Objective: The aim of this dissertation was to summarize the current state of knowledge on risk factors for DRE in patients with TSC and to evaluate the effect and safety of mTORi in treating epilepsy in children under two years of age.

Methodology: Electronic databases of medical publications on risk factors for drug-resistant epileptic seizures in patients with TSC were searched. The analysis was conducted according to the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines, and the PICOS (Population, Intervention, Comparison, Outcomes and Study) framework was used to select articles. A retrospective analysis of medical records of children with a confirmed diagnosis of TSC who initiated sirolimus or everolimus treatment before the age of two was used to evaluate the safety and effect of mTORi. All patients were treated in years 2008-2022 in the Department of Neurology and Epileptology, The Children’s Memorial Health Institute, and the Department of Pediatric Neurology, the Medical University of Warsaw, Poland. AEs associated with the use of sirolimus in this group of

patients were analyzed, depending on the age and the treatment duration. Children who received mTORi before the onset of seizures and those who reported epileptic seizures in the month prior to the treatment introduction were included in the evaluation of mTORi efficacy. The change in seizure frequency in both groups was analyzed three, six, twelve, and twenty-four months after treatment initiation. A comparative analysis was performed with a control group in which patients did not receive a mTORi.

Results: The doctoral dissertation consists of a systematic review and two original papers. The review paper summarizes the current state of knowledge based on the available literature on the risk factors associated with the development of DRE in patients with TSC. According to the literature, three parameters, the presence of a mutation in the *TSC2* gene, infantile spasms, and the number of cortical tubers, are the most significant risk factors for DRE. In addition, the early age at onset of epileptic seizures also increases the risk of refractoriness. Initially, 529 patients from two clinical centers were included in the retrospective data analysis. After specifying the inclusion criteria regarding mTORi treatment initiation before the age of two, 21 patients (21/24, 87.5%) treated with sirolimus and 3 with everolimus (3/24, 12.5%) were included in the study. The first original paper evaluates the safety of sirolimus in all 21 patients who received this mTORi. AEs occurred in all patients treated with sirolimus but were described as of mild to moderate severity, not life- or health-threatening. The most common findings were abnormal blood test results, such as anemia, hyperlipidemia, and thrombocytosis. Patients did not require major interventions, or additional treatment. The second original paper describes the effects of sirolimus and everolimus on the course of epilepsy in patients with TSC. Nine patients (9/21, 42.9%) received mTORi before the onset of seizures, and twelve patients (12/21, 57.1%) reported epileptic seizures in the month prior to drug initiation. Three patients were excluded from this part of the analysis due to the last seizure occurring more than a month before the treatment introduction. In most patients who received preventive mTORi treatment, seizures occurred only once, and 78.8% of them did not report epileptic seizures at the end of follow-up. In the group of patients who had seizures prior to mTORi treatment initiation, after twenty-four months, the reduction in the mean number of seizures was statistically significant ($p = 0.031$). After two years of follow-up, the frequency of epileptic seizures in the group of all patients who received mTORi improved significantly compared to the control group ($p = 0.0079$).

Conclusions: The systematic review demonstrated that most risk factors for DRE in patients with TSC are non-modifiable. Regular monitoring of TSC patients with known

parameters associated with refractoriness allows early and effective initiation of antiepileptic treatment. The findings from the study indicate an improvement in epileptic seizure control in patients with mTORi treatment initiated before the age of two. AEs of sirolimus in this age group are common, yet of mild to moderate severity. Further prospective clinical trials on larger groups of patients should be conducted to confirm the results from our study on the efficacy and safety of mTORi in the youngest patients with TSC.

4. Wstęp

4.1. Stwardnienie guzowate

Stwardnienie guzowate (ang. *tuberous sclerosis complex*, TSC), lub też choroba Bourneville-Pringle'a, jest chorobą nerwowo-skórną dziedziczoną w sposób autosomalnie dominujący, ze zmienną ekspresją. Około 60% przypadków powstaje w wyniku mutacji *de novo*. Występuje u 1:6000 żywych urodzeń [1]. Choroba spowodowana jest mutacją w genie *TSC1* lub *TSC2*. Gen *TSC1* znajduje się na długim ramieniu chromosomu 9 (9q34), a gen *TSC2* na krótkim ramieniu chromosomu 16 (16p13.3) [2]. Geny te kodują białka, odpowiednio hamartynę oraz tuberynę, należące do genów supresorowych nowotworów, które w prawidłowych warunkach tworzą kompleks hamujący działanie kinazy białkowej treoninowo-serynowej mTOR (ang. *mechanistic target of rapamycin*, ssaczy cel rapamycyny). Mutacja w jednym z genów powoduje utratę właściwości supresyjnych, a tym samym nadmierną aktywację szlaku mTOR. Brak inhibicji kinazy skutkuje niekontrolowaną proliferacją oraz powstaniem licznych łagodnych zmian nowotworowych [3].

Najczęściej zmiany te lokalizują się na skórze w formie naczyniakowłókniaków, tzw. guzków Pringle, plam typu *cafe au lait* czy też plam odbarwieniowych [4]. U większości pacjentów widoczne są zmiany ośrodkowym układzie nerwowym (OUN) [5]. Podwyściółkowe guzki okołokomorowe, a w szczególności gwiaździak podwyściółkowy olbrzymiokomórkowy (ang. *subependymal giant cel astrocytoma*, SEGA), czyli guz powstający z transformacji guzków podwyściółkowych, mogą prowadzić do wodogłowia poprzez utrudnianie odpływu płynu mózgowo-rdzeniowego przez otwór międzykomorowy [6]. Ponadto, guzki korowo-podkorowe w korze mózgu oraz móżdżku oraz pasmowate linie migracji w istocie białej mózgu, są najczęściej stwierdzanymi zmianami w badaniach obrazowych [7].

U około 80% pacjentów z TSC wykrywane są naczyniakomięśniakotłuszczaki nerek (ang. *angiomyolipoma*, AML), oraz pojedyncze lub też liczne torbiele [5,8]. W pobliżu locus na chromosomie 16 znajduje się gen *PKD1*, związany z wielotorbielowatością nerek typu 1, zatem przy większych delecjach choroba ta współistnieje z TSC. Mięśniaki prążkowanokomórkowe (ang. *cardiac rhabdomyoma*, CR) stwierdzane są w mięśniu sercowym u niemal połowy dzieci z TSC [5,9]. Zaobserwowano samoistne zmniejszanie się guzów wraz z wiekiem pacjentów, zatem są one prawdopodobnie jeszcze częstsze u noworodków [10,11]. Guzy serca stanowią najwcześniejszy możliwy do wykrycia objaw

TSC, często widoczny w rutynowo wykonywanym USG prenatalnym [12]. Większość z nich jest bezobjawowa, jednak w zależności od umiejscowienia, mogą powodować zaburzenia rytmu serca. U 40-50% pacjentów z TSC występują hamartoma astrocytarne, bezobjawowe guzy siatkówki [13,14].

Aktualne kryteria rozpoznania stwardnienia guzowatego pochodzą z 2021 roku [15]. Diagnoza może zostać postawiona na podstawie kryteriów klinicznych lub genetycznych. Kryteria genetyczne obejmują identyfikację patogennej mutacji w genie *TSC1* lub *TSC2*. Są one szczególnie istotne, jako że obraz choroby jest zróżnicowany, od ciężkich do skąpoobjawowych postaci klinicznych [16].

Kryteria kliniczne przestawione zostały w Tabeli 1. Rozpoznanie kliniczne TSC jest pewne, gdy spełnione zostaną dwa kryteria kliniczne duże albo jedno kryterium duże oraz dwa małe. Rozpoznanie TSC uznaje się za prawdopodobne, gdy spełnione zostaje jedno kryterium kliniczne duże albo dwa i więcej kryteriów małych. U około 10% pacjentów spełniających kryteria kliniczne rozpoznania TSC nie zostaje wykryty wariant patogenny w genie *TSC1* ani *TSC2* za pomocą konwencjonalnych testów genetycznych [17]. Kryteria genetyczne oraz kliniczne uzupełniają się, a tym samym umożliwiają odpowiednią wczesną diagnostykę oraz ustalenie dalszego postępowania.

Tabela 1. Kryteria kliniczne duże oraz małe w diagnostyce stwardnienia guzowatego [15].

Kryteria kliniczne duże	**Kryteria kliniczne małe**
Plamki odbarwieniowe (≥3; co najmniej 5 mm średnicy)	Zmiany skórne typu „confetti”
Naczyniakowłókniaki twarzy (≥3) lub włókniaki płaski okolicy czołowej	Ubytki w szkliwie zębowym (≥3)
Włókniaki okołopaznokciowe (≥2)	Włókniaki dziąseł (≥2)
Ogniska skóry szagrynowej	Hamartoma pozanerkowe
Mnogie hamartoma siatkówki	Mnogie torbiele nerek
Mnogie guzki korowe lub linie migracji	Zmiany sklerotyczne kości
Guzki podwyściółkowe (≥2)	Plamy achromatyczne siatkówki
Podwyściółkowy gwiaździak olbrzymiokomórkowy	
Mięśniak prążkowanokomórkowy serca	
Lymfangioleiomiomatoza	
Naczyniakomięśniakotłuszczaki (≥2)	

4.2. Padaczka w stwardnieniu guzowatym

U około 80% pacjentów ze stwardnieniem guzowatym występują napady padaczkowe, przeważnie po raz pierwszy zaobserwowane pomiędzy trzecim a piątym miesiącem życia [3,18]. Wystąpienie pierwszych napadów klinicznych poprzedzone jest zmianami w zapisie EEG [19]. Napady w przebiegu TSC występują często pod postacią napadów zgięciowych, częściowo złożonych, uogólnionych toniczno-klonicznych, oraz mioklonicznych. Podejrzewa się, że istotną rolę w epileptogenezie pełnią guzki korowe, których usunięcie skutkuje zmniejszeniem liczby oraz ciężkości napadów [20].

W Europie wigabatryna jest lekiem przeciwpadaczkowym (ang. *antiseizure medication,* ASM) pierwszego wyboru u pacjentów z TSC niezależnie od rodzaju napadu [15]. Pomimo nowoczesnych, dobrze tolerowanych ASM, które mogą być włączane w drugiej kolejności w przypadku braku kontroli napadów, u ponad 60% pacjentów napady stają się lekooporne [21].

Zgodnie z definicją Międzynarodowej Ligi Przeciwpadaczkowej (ang. *International League Against Epilepsy*, ILAE) z 2010 r. padaczkę lekooporną (ang. *drug-resistant epilepsy*, DRE) rozpoznaje się, gdy dwa właściwie dobrane i odpowiednio stosowane leki przeciwpadaczkowe nie skutkują trwałym ustąpieniem napadów [22]. U pacjentów z DRE, poza dodaniem kolejnych leków przeciwpadaczkowych oraz zwiększaniem ich dawki, stosowane jest również leczenie niefarmakologiczne. Metody niefarmakologiczne obejmują przede wszystkim leczenie chirurgiczne, oraz wdrożenie diety ketogennej lub diety Atkinsa [23]. Wszczepienie stymulatora nerwu błędnego jest zalecane w razie braku skuteczności zabiegu chirurgicznego lub obecnych przeciwskazań do jego przeprowadzenia. Dieta ketogenna reguluje mechanizmy epigenetyczne i szlak mTOR, a jej zastosowanie pozwala na znaczącą redukcję liczby napadów u pacjentów z TSC [24,25]**.** Również kannabidiol ma mechanizm działania przeciwdrgawkowego, prawdopodobnie poprzez szlak mTOR [23,26].

Ryzyko niepełnosprawności intelektualnej oraz jej nasilenie wzrasta u pacjentów z padaczką o wczesnym początku, w szczególności u tych, u których napady stają się lekooporne [27]. Zaburzenia neuropsychiatryczne oraz inne powikłania TSC, a przede wszystkim napady padaczkowe, znacząco obniżają jakość życia zarówno pacjentów, jak i ich opiekunów [28]. Pokazuje to, jak istotną rolę pełni odpowiednia diagnostyka, a tym samym zapobieganie napadom i ich leczenie.

Prewencyjne włączenie wigabatryny przed wystąpieniem pierwszych napadów klinicznych zmniejsza ryzyko wystąpienia napadów w kolejnych miesiącach oraz ich lekooporności [29,30]. W wieloletnim nierandomizowanym prospektywnym badaniu klinicznym, do którego włączono 14 pacjentów z TSC leczonych prewencyjnie oraz 31 pacjentów w grupie kontrolnej, różnica w częstości napadów oraz ilorazie inteligencji pomiędzy grupami była istotna statystycznie (odpowiednio $p = 0,001$ oraz $p < 0,03$) [30].

Badanie TOSCA, do którego włączono 2216 pacjentów z 31 państw potwierdziło znaczenie regularnego wykonywania badań EEG zarówno we śnie jak i w czuwaniu, szczególnie w pierwszych miesiącach życia [31]. Umożliwia ono wczesne wykrywanie nieprawidłowych wyładowań elektrycznych prowadzących w kolejnych tygodniach do wystąpienia napadów klinicznych, co pozwala na prewencyjne włączenie wigabatryny. Obecnie zaleca się wykonywanie rutynowych badań EEG u bezobjawowych dzieci z TSC co sześć tygodni do ukończenia pierwszego roku życia, a następnie co trzy miesiące do ukończenia drugiego roku życia [15]. Wciąż poszukiwane są nowoczesne metody wczesnej diagnostyki, zapobiegania oraz leczenia pacjentów o dużym ryzyku wystąpienia DRE. Szczególnie interesujące wydają się inhibitory mTOR (mTORi). Mechanizm działania tych leków daje nadzieję na skuteczne leczenie przyczynowe, podczas gdy ASM przeważnie działają wyłącznie objawowo, zmniejszając nasilenie i częstość napadów [32].

Praca przeglądowa zatytułowana **„Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review”** podsumowuje aktualny stan wiedzy na temat czynników ryzyka związanych z rozwojem DRE u dzieci z TSC. Znajomość tych parametrów umożliwia identyfikację pacjentów wymagających dokładniejszej diagnostyki, oraz wczesnego włączania farmakoterapii. Ta grupa pacjentów może także odnieść największe korzyści z rozwoju nowoczesnych metod prewencji oraz leczenia padaczki, a w szczególności DRE.

4.3. Inhibitory mTOR w stwardnieniu guzowatym

Sirolimus oraz ewerolimus, leki należące do grupy mTORi są obecnie stosowane głównie w transplantologii jako leki immunosupresyjne [33]. Ich działanie na poziomie komórkowym polega na połączeniu z receptorem FKBP-12, co hamuje aktywność szlaku mTOR, zatem uniemożliwia tworzenie białek istotnych do przejścia do fazy S cyklu komórkowego [34,35]. W efekcie zahamowana zostaje proliferacja komórkowa, a tym samym tworzenie guzów w przebiegu TSC.

Ewerolimus posiada rejestrację Agencji Żywności i Leków (ang. *Food and Drug Administration*, FDA) i Europejskiej Agencji Leków (ang. *European Medicines Agency*, EMA) do leczenia guzów SEGA, AML, oraz u pacjentów z napadami częściowymi w przebiegu TSC [36–38]. Ponadto, może być stosowany poza zarejestrowanymi wskazaniami w celu zmniejszania CR [39,40]. W porównaniu z sirolimusem, ewerolimus jest lepiej wchłaniany, jego biodostępność przy podaniu doustnym jest wyższa, szybciej osiąga stabilne stężenie we krwi oraz jest szybciej wydalany po odstawieniu niż sirolimus [41].

Pomimo braku rejestracji sirolimusu do stosowania u pacjentów z TSC, w Polsce jest on często włączany u pacjentów pediatrycznych, głównie z uwagi na jego większą dostępność oraz niższą cenę. Wskazania refundacyjne ewerolimusu w Polsce obejmują wyłącznie pacjentów z SEGA w przebiegu TSC, AML u pacjentów powyżej 18 roku życia oraz dzieci powyżej drugiego roku życia, u których wystąpiła padaczka lekooporna. Sirolimus natomiast jest refundowany u wszystkich pacjentów z TSC, u których lekarz stwierdzi wskazania do zastosowania leku [42].

W wielośrodkowym randomizowanym badaniu EXIST-1 wykazano, że u 27 pacjentów (35%) wymiary guza SEGA zmniejszyły się o ponad 50%, w porównaniu do braku zmiany w grupie kontrolnej ($p < 0,0001$) [43]. W badaniu EXIST-2 stwierdzono zmniejszenie objętości AML o ponad połowę u 42% dorosłych pacjentów, którzy otrzymywali ewerolimus [44]. U pacjentów z grupy kontrolnej wymiary guza nie uległy zmianie ($p < 0,0001$). W obu badaniach obserwowano jednak odrost guzów po przerwaniu leczenia. Nieliczne publikacje opisują pozytywny wpływ ewerolimusu na zmniejszenie objętości guzów serca [45,46]. Skuteczność ta nie została jednak potwierdzona w większych badaniach klinicznych, a ze względu na charakterystykę CR i ich skłonność do samoistnego zaniku, mTORi nie zostały oficjalnie zarejestrowane w tym wskazaniu.

W prospektywnym randomizowanym badaniu klinicznym EXIST-3, w którym uczestniczyło 366 pacjentów powyżej drugiego roku życia, zaobserwowano znacząco lepszą długoterminową kontrolę padaczki u pacjentów, którzy otrzymywali ewerolimus. Częstość napadów była mniejsza w grupie pacjentów z wyższym stężeniem leku we krwi [47,48]. Ponad 50% zmniejszenie liczby napadów zostało osiągnięte u 15,1%, 28,2%, oraz 40% pacjentów, odpowiednio w grupie leczonej placebo, u pacjentów z niskim stężeniem ewerolimusu we krwi (3-7 ng/mL) oraz u pacjentów z wysokim stężeniem leku (9-15 ng/mL). Nie wiadomo, w jaki sposób zaprzestanie leczenia mTORi wpłynie na kontrolę

padaczki. W różnych modelach zwierzęcych opisywano przeciwpadaczkowy wpływ prewencyjnego leczenia inhibitorami mTOR [49–51]. Odstawienie leków powodowało wystąpienie napadów padaczkowych. Ponadto, zaobserwowano wspomniany wyżej odrost SEGA oraz AML po przerwaniu terapii ewerolimusem. Uznaje się zatem, że leczenie padaczki również powinno być kontynuowane przez wiele lat.

OUN człowieka jest najbardziej plastyczny oraz podatny na zmiany strukturalne i biochemiczne podczas pierwszych miesięcy życia, zatem ten okres jest kluczowy w prewencji epileptogenezy u pacjentów z TSC [52]. mTORi poprzez działanie bezpośrednio na szlak kinazy mTOR może go modyfikować, a tym samym zapobiegać powstawaniu padaczki [32]. Aktualny stan wiedzy na temat skuteczności mTORi w leczeniu i zapobieganiu padaczce opiera się przede wszystkim na publikacjach dotyczących stosowania ewerolimusu przez dorosłych oraz dzieci starsze, powyżej drugiego roku życia. Skuteczność sirolimusu w leczeniu padaczki u dzieci ze TSC nie została potwierdzona [50,51]. Dotychczasowe badania przeprowadzono na mało licznych grupach pacjentów, a ich wyniki nie są jednoznaczne. Obecnie prowadzone badanie ViRap ma na celu ocenę skuteczności, tolerancję i bezpieczeństwo prewencyjnego leczenia wigabatryną w porównaniu do sirolimusu u najmłodszych pacjentów w pierwszych miesiącach życia. Jak dotąd wyniki badania nie zostały opublikowane. W publikacji zatytułowanej **„Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under 2 Years of Age”** omówiono wpływ mTORi włączonych przed ukończeniem drugiego roku życia na przebieg padaczki u dzieci z TSC.

Potencjalne działania niepożądane (ang. *adverse effects*, AE) związane ze stosowaniem mTORi u dzieci poniżej drugiego roku życia wzbudzają zainteresowanie z uwagi na młody wiek pacjentów oraz konieczność wieloletniej kontynuacji leczenia. Randomizowane badania kliniczne EXIST-1 oraz EXIST-3 wykazały bezpieczeństwo stosowania ewerolimusu u małych dzieci [53–55]. Inne, mniejsze badania i doniesienia są zgodne z tymi wynikami [15,18,19]. Chociaż sirolimus jest stosowany częściej niż ewerolimus, jego skuteczność i bezpieczeństwo były oceniane jedynie w badaniach o niskim poziomie wiarygodności u starszych dzieci i dorosłych z TSC [47,50,51].

Zgodnie z literaturą, działania niepożądane mTORi są częste, jednak zazwyczaj o niewielkim stopniu nasilenia [37,56]. Niemal 70% pacjentów zgłasza nawracające zapalenia jamy ustnej, zazwyczaj w formie aft i owrzodzeń [57,58]. Inną, regularnie występującą grupę

działań niepożądanych stanowią infekcje górnych dróg oddechowych, a także biegunka [49,59]. Niekiedy infekcje u pacjentów leczonych sirolimusem oraz ewerolimusem stają się istotnym zagrożeniem z uwagi na immunosupresyjny charakter mTORi. U dwojga z czworga pacjentów, którzy zmarli podczas badania EXIST-3, przyczyną zgonu było zapalenie płuc oraz wstrząs septyczny [60]. Najczęstszymi nieprawidłowościami obecnymi w badaniach laboratoryjnych u pacjentów leczonych zarówno sirolimusem jak i ewerolimusem są anemia oraz zaburzenia lipidowe. Zaburzenia gospodarki lipidowej o największym nasileniu występują u pacjentów stosujących jednocześnie dietę ketogenną [56]. Zazwyczaj zmniejszenie dawki mTORi lub okresowe odstawienie leków wystarcza do ustąpienia AE.

Niewiele badań ocenia bezpieczeństwo mTORi w grupie dzieci poniżej drugiego roku życia. W większości są to pojedyncze opisy przypadków obejmujące przede wszystkim skutki uboczne ewerolimusu. W publikacji o tytule **„Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study”** przeanalizowano AE związane ze stosowaniem sirolimusu u pacjentów z TSC, u których lek ten włączono przed ukończeniem drugiego roku życia.

4.4. Uzasadnienie połączenia publikacji w cykl

Niniejszą rozprawę doktorską tworzy cykl trzech spójnych tematycznie publikacji, poruszających tematykę padaczki lekoopornej u pacjentów z TSC, a w szczególności czynników ryzyka jej wystąpienia, możliwości zapobiegania oraz leczenia. Cykl publikacji składa się z jednej pracy przeglądowej oraz dwóch prac oryginalnych.

W obu publikacjach oryginalnych przeanalizowano populację pacjentów z potwierdzoną diagnozą TSC, leczonych w latach 2008-2022 w Klinice Neurologii Dziecięcej Warszawskiego Uniwersytetu Medycznego oraz Klinice Neurologii i Epileptologii w Instytucie „Pomnik-Centrum Zdrowia Dziecka” w Warszawie.

- Praca przeglądowa o tytule **„Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review”** jest przeglądem systematycznym literatury podsumowującym aktualny stan wiedzy w zakresie czynników ryzyka związanych z występowaniem DRE u pacjentów z TSC. W pracy przedstawiono parametry mające wpływ na lekooporność napadów oraz omówiono istotną rolę prewencji i wczesnej identyfikacji dzieci z dużym ryzykiem lekooporności. Publikacja stanowi wprowadzenie do dalszych prac w cyklu, jako że pacjenci z czynnikami ryzyka wystąpienia DRE mogą odnieść

największe korzyści ze stosowania nowoczesnych metod leczenia i zapobiegania lekooporności.

- W publikacji zatytułowanej **„Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study”** przedstawiono działania niepożądane związane ze stosowaniem sirolimusu u dzieci z TSC poniżej drugiego roku życia. W publikacji po raz pierwszy przeanalizowano skutki uboczne sirolimusu w tak licznej grupie najmłodszych dzieci z TSC. Z uwagi na prawdopodobną konieczność kontynuacji leczenia przez wiele lat, za istotne uznano określenie zagrożeń wynikających ze stosowania mTORi przez dzieci z TSC przed ukończeniem drugiego roku życia.
- W publikacji o tytule **„Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under 2 Years of Age”** omówiono wpływ mTORi na kontrolę padaczki u dzieci z TSC. Przeanalizowano zmianę częstości napadów w dwóch grupach. Pierwsza grupa pacjentów otrzymała mTORi przed wystąpieniem pierwszych napadów padaczkowych. Drugą grupę stanowili pacjenci, którzy zgłaszali napady padaczkowe w miesiącu poprzedzającym włączenie mTORi W publikacji tej po raz pierwszy przedstawiono skuteczność mTORi w leczeniu padaczki w tak licznej grupie dzieci z TSC poniżej drugiego roku życia.

Publikacje stanowiące cykl podsumowują aktualny stan wiedzy oraz uzupełniając się, poszerzają tematykę istotną zarówno dla pacjentów, jak i specjalistów - bezpieczeństwa i skuteczności nowych terapii DRE u najmłodszych pacjentów z TSC.

5. Założenia i cel pracy

W ostatnich latach przeprowadzono liczne badania na temat lekooporności padaczki u pacjentów z TSC. Wiadomo, że istnieją parametry zwiększające prawdopodobieństwo wystąpienia padaczki u dzieci z TSC, a także nasilenia napadów oraz rozwoju lekooporności. W niniejszej rozprawie zdecydowano się zebrać oraz podsumować wyniki z publikacji omawiających wspomniane czynniki ryzyka.

Pomimo znaczących sukcesów w poprawie kontroli napadów padaczkowych za pomocą wigabatryny oraz innych ASM, u części pacjentów napady stają się lekooporne [15,21]. Zalecane opcje terapeutyczne w DRE, takie jak leczenie chirurgiczne, dieta ketogenna czy kannabinoidy niekiedy okazują się nieskuteczne [23]. Z uwagi na częstość występowania DRE oraz jej negatywny wpływ na rozwój pacjentów z TSC, za istotne uznano poszukiwanie alternatywnych metod leczenia padaczki. Założono, że wyniki badań dotyczące pozytywnego wpływu ewerolimusu na kontrolę napadów padaczkowych u dzieci starszych mogą mieć przełożenie na skuteczność zarówno ewerolimusu jak i sirolimusu u najmłodszych pacjentów przed ukończeniem drugiego roku życia [47,48]. Kluczowe zagadnienie, szczególnie u małych dzieci, u których leczenie wymaga kontynuacji przez wiele lat, stanowi ocena AE leków. W tym celu przeanalizowano AE związane ze stosowaniem sirolimusu przez pacjentów z TSC, u których lek włączono przed ukończeniem drugiego roku życia.

Biorąc powyższe pod uwagę, celem niniejszej rozprawy było:

1. Ustalenie czynników ryzyka wystąpienia DRE u pacjentów z TSC oraz wskazanie, które z nich mogą być modyfikowane w celu zmniejszenia tego ryzyka.
2. Ocena wpływu mTORi, sirolimusu oraz ewerolimusu, włączonych u pacjentów poniżej drugiego roku życia, na przebieg padaczki. Uwzględniono zarówno dzieci, u których leczenie zostało zastosowane przed wystąpieniem pierwszych napadów padaczkowych, jak i u pacjentów z obecnymi napadami padaczkowymi, w tym DRE.
3. Ocena bezpieczeństwa stosowania sirolimusu u dzieci z TSC, u których lek został włączony przed ukończeniem drugiego roku życia. Przeanalizowano częstość występowania AE, ich rodzaj oraz nasilenie.

6. Kopie opublikowanych prac

6.1. Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review

Journal of *Clinical Medicine*

MDPI

Review

Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review

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Abstract: Background: Epilepsy affects 70–90% of patients with tuberous sclerosis complex (TSC). In one-third of them, the seizures become refractory to treatment. Drug-resistant epilepsy (DRE) carries a significant educational, social, cognitive, and economic burden. Therefore, determining risk factors that increase the odds of refractory seizures is needed. We reviewed current data on risk factors associated with DRE in patients with tuberous sclerosis. Methods: The review was performed according to the PRISMA guidelines. Embase, Cochrane Library, MEDLINE, and ClinicalTrial.gov databases were searched. Only full-text journal articles on patients with TSC which defined risk factors related to DRE were included. Results: Twenty articles were identified, with a cohort size between 6 and 1546. Seven studies were prospective. Three factors appear to significantly increase DRE risk: *TSC2* mutation, infantile spasms, and a high number of cortical tubers. Conclusions: A proper MRI and EEG monitoring, along with genetic testing, and close observation of individuals with early onset of seizures, allow identification of the patients at risk of DRE.

Keywords: tuberous sclerosis complex; drug resistant epilepsy; refractory seizures; risk factors

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1. Introduction

Approximately 70–90% of individuals with tuberous sclerosis complex (TSC) experience epilepsy, and in approximately one-third of them, the seizures become refractory to treatment [1,2]. Refractory seizures, also defined as drug-resistant epilepsy (DRE) are commonly defined as "the failure of adequate trials of two tolerated and appropriately chosen and used antepileptic drug (AED) schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [3]. It bears a significant educational, social, cognitive, and economic burden.

Tuberous sclerosis complex (TSC) is a genetic disease affecting 1:6000–1:13,000 newborns, caused by a mutation in one of two loci: *TSC1* located on chromosome 9q34.13) or *TSC2* (located on chromosome 16p13.3) [4]. More than 60% of the cases are sporadic de novo mutations, while the remaining 30% are transmitted in an autosomal dominant pattern [5]. *TSC1* and *TSC2* genes mutation inactivate the function of the proteins produced by those genes [6]. The process leads to the mammalian target of Rapamycin (mTOR) overactivation. As mTOR signaling is involved in regulation of many basic cellular processes e.g., proliferation and growth, the mutation causes excessive growth of multiple benign tumors, and alteration in the morphology and function of neurons [7,8].

The current guidelines on TSC diagnosis recommend both clinical and genetic diagnostic criteria, recently updated and published in October 2021 by Northrup et al. [9].

In TSC, the clinical seizures appear mostly between three and five months of age [10]. The first seizures are often subtle and therefore not noticed or confused with physiological behavior. Among patients with TSC, there is a high risk of developing infantile spasms [11]. Those factors negatively impact intellectual development of the individuals, and, according to most caregivers (76.5%) in the TOSCA study, also hinders their family life and social relations [12,13].

Current diagnostic methods such as prenatal echocardiography, brain magnetic resonance imaging (MRI), and EEG monitoring allow an early diagnosis before the onset of clinical seizures enabling early disease-modifying treatment [14]. As a consequence, preventive treatment is becoming more approved and often introduced in clinical centers [15]. Therefore, defining parameters associated with refractoriness, especially those which may be identified early, is necessary. It enables early recognition of patients at-risk, to whom preventive treatment is crucial to facilitate their development.

This article aimed to provide a systematic review of current data regarding risk factors and early predictors of refractory epilepsy in individuals with TSC. It focuses on diagnostic imaging, EEG monitoring, clinical diagnosis, genetic and molecular findings.

2. Materials and Methods

To accomplish this systematic review, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [16] were followed, during the design, search, and reporting stages.

2.1. Eligibility Criteria

For article selection, we applied the standard PICOS (Population, Intervention, Comparators, Outcome measure, and Study design) criteria. The main hypothesis is that there are risk factors associated with the development of drug-resistant epilepsy (DRE). The definition of DRE differed between studies and is discussed below.

(a) P (patients)—patients with TSC.
(b) I (intervention)—development of DRE.
(c) C (comparator)—we searched for studies comparing patients with refractory and non-refractory epilepsy.
(d) O (outcome)—an association between risk factors and DRE.
(e) S (study design)—only full-text, original studies published in English or Polish.

We included only full-text journal articles on patients with TSC assessed based on epilepsy status and response to treatment. Only studies published in English or Polish were included. The search was not limited to any publication date or status.

We excluded articles that did not fulfill the inclusion criteria, studies on animal models or tissues, or non-full-text articles (e.g., conferences' abstracts).

2.2. Information Sources

One of the researchers (DM) searched by electronic databases: MEDLINE, Embase, and Cochrane Library. We also performed a manual search in the references of previously included studies and review articles. Additionally, a search for ongoing or previous trials on the topic was performed on ClinicalTrials.gov. The search was done between 13 May 2021 and 31 May 2021.

2.3. Search Strategy

The following search phrases were used to perform the search: ("tuberous sclerosis") AND ((refractory) OR ("drug resistant") OR (drug-resistant)) AND ((epilepsy) OR (seizures)). In ClinicalTrials.gov search, done on 31 May 2021, we applied only "tuberous sclerosis" term.

2.4. Study Selection

The articles were reviewed upon eligibility assessment according to the three-phase procedure: (1) title, (2) abstract, and (3) full-text analysis. All three phases were performed independently by two authors (DM, MS). The authors evaluated the full text if an abstract met the inclusion criteria but provided insufficient information. The review results were evaluated between the reviewers. Any disagreement was resolved by the discussion and consensus.

2.5. Data Collection Process and Data Items

The two reviewers (DM and MS) individually performed screening and selection of data from the articles. The discussion and consensus resolved the differences between the reviewers. To minimize the risk of data duplication, we examined patients' characteristics and authors' names. In case of any concern related to the data duplication, the articles were again evaluated and compared by DM.

Data regarding title, author's name, year of publication, study type, sample size and characteristics, inclusion and exclusion criteria, refractory epilepsy definition, risk factors associated with drug resistance, p values for each factor if available were retrieved.

2.6. Assessing the Risk of Bias in Individual Studies

To assess the risk of bias, we performed Cochrane risk of bias tool [17] for randomized trials and the Newcastle–Ottawa Scale (NOS) [18] for nonrandomized studies.

NOS scale assigns 0 or 1 point for each answer, in three groups of criteria: (1) Selection: 4 questions, a maximum of 4 stars, including (a) representativeness of the exposed cohort, (b) selection of the non-exposed cohort, (c) ascertainment of exposure, (d) if the outcome of interest was present or not at the start of the study; (2) Comparability of the cohorts on the basis of the study design or analysis: 2 questions, a maximum of 2 stars; (3) Outcome: 3 questions, a maximum of 3 stars, including (a) outcome assessment, (b) whether the follow-up duration was long enough for the outcomes to occur, (c) adequacy of follow-up of cohorts [18]. The studies were grouped based on the score; 9–7, 6–4, and 3–0 stars were defined as low, moderate, and high risk of bias, respectively.

The Cochrane risk of bias assesses the risk of bias in five domains. Domain 1 is the randomization process; domain two is a deviation from the intended interventions; domain 3—missing outcome data; domain 4—measurement of the outcome; and 5—selection of the reported result. In each domain, response options are given to a few signaling questions, and then the risk-of-bias judgment is performed [17]. Based on those responses, the overall risk of bias is estimated as low, high, or some concerns.

The studies were evaluated independently by DM and MS. Disagreements were resolved by the third reviewer or discussion and consensus.

2.7. Summary Measures

The primary outcomes in this study were: the association between risk factors and the development of drug-resistant epilepsy. Due to the diversity between risk factors assessed in each study, we reported all the measured parameters. We set a p-value of 0.05 or less as statistically significant.

2.8. Data Analysis

Data are expressed as a qualitative description of statistically significant risk factors.

3. Results

3.1. Study Selection and Available Literature

The search of MEDLINE, Embase, Cochrane Library, and ClinicalTrials.gov provided a total of 1187 citations. Additional six citations were supplemented from the references' lists of included or review articles. 84 duplicates were excluded. In the first and second phases of screening 1059 articles were not included. During full text analysis, additional thirty-one reports were excluded as they either did not meet inclusion or met exclusion criteria (Figure 1). Overall, 19 reports were included in the systematic review [2,10,12,14,19–33]. The inclusion and exclusion criteria available for each article are included in Table 1.

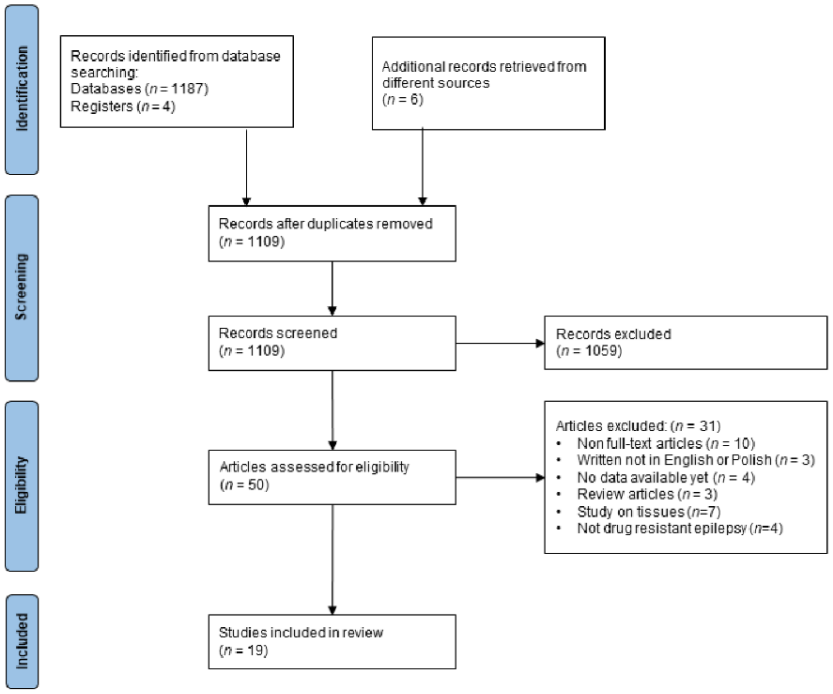

Figure 1. Flow diagram of the study selection.

3.2. Study Characteristics

Studies design: Six of the included studies were prospective, one of which was randomized, and two had randomized control and partially non-randomized open-label groups [24,28]. The remaining 13 studies were based on retrospective data.

Patients: The included studies involved patients with TSC. The number of patients included in each study varied between 6 and 1546 people.

Intervention: Each study compared patients with DRE and those responsive to therapy.

Primary and additional outcome: The included studies reported on the association between DRE and risk factors, and 18 out of the total 19 articles provided p value to assess statistical significance.

The definition of drug-resistant epilepsy differed among the studies, and therefore, each is included in Table 1.

3.3. Risk of Bias within Studies

Out of sixteen non-randomized studies assessed in the Newcastle–Ottawa Scale, 15 received a total score of either 7, 8, or 9 stars, placing among the low biased group. A study by Monteiro et al. received 5 stars (3 in the selection category, 0 in the comparability, and 2 in the exposure) [27]. Therefore, this article qualified as a moderate risk of bias.

All the randomized studies were assessed as a low overall risk of bias [24,28,33].

The rating of the risk of bias is included in Table 2.

3.4. Results of Included Studies

Data extracted from each study are presented in Table 1.

Table 1. Characteristics of the cohorts in the included studies.

Author, Year	DRE [1] Definition	Participants Included	Inclusion and Exclusion Criteria	Factors Associated with DRE	Factors Not Associated with DRE
Benova et al., 2018 [22]	Authors did not provide DRE definition. However, the following variables were considered markers of DRE: 1. number of AED [2] used 2. number of AED at the end of follow-up 3. the absence of seizure-free status at the end of follow up	22	Inclusion: pre/perinatal diagnosis of cardiac rhabdomyomas	Higher number of areas with FCD-like [3] features (uncorrected $p < 0.0001$, FDR [4] < 0.01) ID [5] (uncorrected $p < 0.001$, FDR < 0.05) *TSC2* (uncorrected $p < 0.01$, FDR < 0.05)	-
Chu-Shore et al., 2009 [2]	uncontrolled seizures after more than three AED (not including treatment for infantile spasms)	173 (2 months to 73 years, median 13 years)	-	At least one cyst-like cortical tuber ($p = 0.0007$) FCD	-
Chu-Shore et al., 2010 [23]	uncontrolled seizures after at least three first-line AED trials	291	-	Infantile spasms ($p < 0.0001$)	*TSC2* vs. *TSC1*, *TSC2* vs. NMI [6] ($p = 0.169$)
Hulshof et al., 2021 [14]	ILAE, 2010 [7], at 2 years	41	Inclusion: Fetal MRI of sufficient quality and available neurologic outcome data at the age of 2 years Exclusion: Epilepsy surgery before the age of 2 years.	-	Fetal (sub)cortical lesion sum score—4.89 vs. 4.41 in DRE and non-refractory epilepsy, respectively ($p = 0.62$)

Table 1. *Cont.*

Author, Year	DRE [1] Definition	Participants Included	Inclusion and Exclusion Criteria	Factors Associated with DRE	Factors Not Associated with DRE
Jeong et al., 2017 [12]	ILAE, 2010	1546 (9.6 to 25.5 years, median 16.0 years) 21.4%—*TSC1* 67.9%—*TSC2* 10.7%—NMI	Exclusion: if date fields were missing and age of onset and symptom duration could not be calculated	Onset of focal seizures prior to 1 year of age ($p < 0.001$) *TSC2* (*TSC2* vs. *TSC1* ($p < 0.001$)) Infantile spasms ($p < 0.001$) Drug-resistant infantile spasms ($p < 0.001$) ASD [8] ($p < 0.001$) Mild to moderate intellectual disability - ID ($p < 0.001$) and severe to profound ID ($p < 0.001$) ADHD [9] ($p < 0.001$) Anxiety ($p = 0.02$) Periungual fibromas ($p = 0.02$)—lower odds of DRE	Male vs. female ($p = 0.94$) Race ($p = 0.40$) *TSC2* vs. NMI ($p = 0.84$) *TSC1* vs. NMI ($p = 0.12$) Duration of infantile spasms ($p = 0.90$) Depression ($p = 0.08$) SEGA [10], SEN [11], cortical tubers, cerebral white matter migration lines Anxiety after adjusting for TSC mutation ($p = 0.69$)
Jozwiak et al., 2011 [30]	two or more seizures per month despite the use of two or more AED	45—total 35—standard treatment (AEDs within a week after the onset of seizures), 14—preventive treatment (AEDs within a week after appearance of active epileptic discharges on consecutive EEG, but before clinical seizures)	Inclusion: Diagnosis of TSC until the end of second month of life, follow-up till the end of 24 month of life Exclusion: children presenting with seizures	Standard treatment vs. preventive treatment ($p = 0.021$)	-
Jóźwiak et al., 2019 [29]	two or more seizures a month despite the use of two or more antiepileptic therapies, including AEDs, ketogenic diet, vagus nerve stimulation, and epilepsy surgery	39—total 25—standard treatment (vigabatrin within a week after first clinical seizures), 14-preventive treatment (vigabatrin introduced within a week after epileptiform discharges, before clinical seizure).	Inclusion: Diagnosis of TSC until the end of second month of life, follow-up till the end of 24 month of life Exclusion: children presenting with seizures	-	Standard treatment vs. preventive treatment ($p = 0.5$)

Table 1. *Cont.*

Author, Year	DRE [1] Definition	Participants Included	Inclusion and Exclusion Criteria	Factors Associated with DRE	Factors Not Associated with DRE
Kotulska et al., 2014 [10]	ILAE, 2010	21	Inclusion: Epilepsy onset within 4 weeks of life.	Presence of FCD	-
Kotulska et al., 2021 [24]	ILAE, 2010	94 (both groups underwent careful EEG surveillance)	Inclusion: TSC diagnosis within first 4 months of life, no history of clinical seizures or epileptiform abnormalities in EEG.	Lower odds of DRE if preventive treatment ($p = 0.047$)	-
Mert et al., 2019 [26]	seizures once a month or more for at least 1 year, while using at least two AED at the appropriate dose	83	Inclusion: At least 1 year follow-up.	Seizures in the neonatal period Age of onset of seizure less than 2 years of age ASD Status epilepticus Infantile spasms Generalization of EEG finding Tuber count of more than 3 ($p < 0.001$) IQ < 70	Sex Consanguinity Family history of TSC Attention-deficit and hyperactivity disorder SEN SEGA White matter dysplasia ($p > 0.05$)
Monteiro et al., 2014 [27]	ILAE, 2010	35	-	*TSC2* mutation	-
Ogórek et al., 2020 [28]	ILAE, 2010	94	Inclusion: Age ≤ 4 months, no prior seizures, no clinical seizures on baseline video EEG Exclusion: any condition considered by the investigator to hinder participation in the study or affect primary outcome.	*TSC2* (*TSC2* vs. *TSC1* mutation ($p = 0.0245$))	-

Table 1. *Cont.*

Author, Year	DRE [1] Definition	Participants Included	Inclusion and Exclusion Criteria	Factors Associated with DRE	Factors Not Associated with DRE
Peron et al., 2018 [19]	-	240	Inclusion: 0–80 years of age, conventional molecular analysis available for both *TSC1* and *TSC2*, complete clinical and imaging data available and updated to the latest follow-up encounter. Exclusion: (1) Possible clinical diagnosis or (2) Insufficient clinical records.	-	*TSC1* vs. NMI ($p = 1$) *TSC2* vs. NMI ($p = 0.7$)
Savini et al., 2020 [25]	-	6	-	ID Pathogenic variants in the GAP domain of *TSC2* (no *p*-value, just case reports)	-
de Ridder et al., 2021 [33]	ILAE, 2010	83—total 51—standard (S; clinical and EEG follow-up and start of vigabatrin after seizure onset) 23—preventive (P; follow-up and introduction of vigabatrin once EEG criteria met—focal IED for >10% of the recording time, multifocal IED, generalized IED, or hypsarrhythmia—and before seizure onset)	-	S group: Younger age of first IED [12] on EEG ($p = 0.019$). Multifocal IED on the first EEG compared to focal IED (OR 4.4, 95% CI 1.1–16, $p = 0.026$).	S group: Younger age of first IED on EEG in a multivariable model ($p = 0.429$). Multifocal IED on the first EEG compared to focal IED in a multivariable model ($p = 0.058$). P group: None of the features of the first EEG with epileptiform discharges.

Table 1. *Cont.*

Author, Year	DRE [1] Definition	Participants Included	Inclusion and Exclusion Criteria	Factors Associated with DRE	Factors Not Associated with DRE
Vignoli et al., 2013 [21]	ILAE, 2010	160	Inclusion: At least 1 year follow-up	Cognitive impairment ($p < 0.05$) *TSC2* mutation More than 6 cortical tubers SEN or SEGA Lower educational level Psychiatric disorder Earlier mean age of epilepsy onset (3.3 vs. 5.3 years, $p > 0.05$) Status epilepticus ($p < 0.05$) Younger age at TSC diagnosis (7.6 vs. 13.2 years, $p < 0.05$)	Infantile spasms ($p > 0.05$) Epilepsy onset in the first year of life
Vignoli et al., 2021 [20]	ILAE, 2010	257 (>18 years old)	-	ID ($p < 0.001$) Psychiatric disorders ($p = 0.004$) No family history of TSC ($p = 0.010$) Younger age of seizure (6 vs. 27 months, $p = 0.001$) Higher rate of spasms (27.1% vs. 48.8%, $p = 0.007$) Less frequently focal epilepsy ($p = 0.029$) Lower level of education ($p = 0.002$)	Age Sex Mutation Tubers SEN
Winterkorn et al., 2007 [31]	one of the following criteria met: more than three AED, epilepsy surgery was performed, or one or more seizures per day continued despite therapy	208	-	Family history of TSC—lower odds of DRE ($p = 0.003$) low IQ/DQ ($p < 0.0005$)	-
Zhang et al., 2018 [32]	ILAE, 2010	108 (3 months to 10 years, mean 2.2 years, median 1.4 years)	Inclusion: Taking rapamycin > 1 year	Calcification in the cerebral parenchyma ($p < 0.006$)	Patient's age ($p = 0.745$) Seizure type ($p = 0.788$) Genetic mutation ($p = 0.204$) Family history ($p = 0.927$)

[1] DRE—Drug-resistant epilepsy, [2] AED—antiepileptic drugs, [3] FCD—Focal cortical dysplasia, [4] FDR—False Discovery Rate correction from univariate tests, [5] ID—Intellectual disability, [6] NMI—No mutation identified, [7] "Drug-resistant epilepsy is defined as failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom". In [3] [8] ASD—Autism spectrum disoder, [9] ADHD—Attention deficit hyperactivity disorder, [10] SEGA—Subependymal giant cell astrocytomas, [11] SEN—Subependymal nodules, [12] IED—ictal epileptiform discharges.

Table 2. Methodic assessment of the included studies.

Author, Year	Study Design	Risk of Bias Assessment				
		The Newcastle–Ottawa Scale				The Cochrane Tool
		Selection (0–3)	Comparability (0–2)	Outcome (0–3)	Total (Risk of Bias)	Risk of Bias
Benova et al., 2018 [22]	prospective	4	2	2	8 (Low)	
Chu-Shore et al., 2009 [2]	retrospective, comparative	4	2	3	9 (Low)	
Chu-Shore et al., 2010 [23]	retrospective comparative	4	2	3	9 (Low)	
Hulshof et al., 2021 [14]	retrospective cohort	4	2	3	9 (Low)	
Jeong et al., 2017 [12]	retrospective, multicenter, from TSC Natural History Database Project	4	2	3	9 (Low)	
Jozwiak et al., 2011 [30]	prospective, nonrandomized clinical trial	4	2	3	9 (Low)	
Jóźwiak et al., 2019 [29]	prospective, nonrandomized clinical trial	3	2	3	8 (Low)	
Kotulska et al., 2014 [10]	retrospective	4	2	3	9 (Low)	
Kotulska et al., 2021 [24]	multicenter, prospective, randomized clinical trial and partially open-label	-	-	-	-	Low
Mert et al., 2019 [26]	retrospective	4	2	3	9 (Low)	
Monteiro et al., 2014 [27]	retrospective	3	0	2	5 (Moderate)	
Ogórek et al., 2020 [28]	randomised control and non-randomised open-label	-	-	-	-	Low
Peron et al., 2018 [19]	retrospective	4	2	3	9 (Low)	
Savini et al., 2020 [25]	retrospective	3	1	3	7 (Low)	
de Ridder et al., 2021 [33]	multicenter, prospective, randomized	-	-	-	-	Low
Vignoli et al., 2013 [21]	retrospective	4	2	3	9 (Low)	
Vignoli et al., 2021 [20]	retrospective	4	2	3	9 (Low)	
Winterkorn et al., 2007 [31]	retrospective	4	2	3	9 (Low)	
Zhang et al., 2018 [32]	retrospective	4	2	3	9 (Low)	

3.4.1. Definition of Drug-Resistant Epilepsy (DRE)

Ten studies (52.6%) used the exact ILAE 2010 definition of drug-resistant epilepsy: "a failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [3]. Of those, Hulshof et al. specified the threshold age for control as two years [14]. Other studies applied other various definitions of DRE. In four studies, authors did not provide a definition. Detailed criteria for DRE used in particular studies are included in Table 1.

3.4.2. Association of Genetic Mutation and DRE

Ten studies (52.6%) investigated a relationship between genetic mutations and DRE [12,19–23,25,27,28,32]. Six (60%, 6/10) found statistically significant correlation between *TSC2* and DRE. *TSC2* mutation as a risk factor was declared: in two studies when *TSC2* vs. *TSC1* mutations were compared [12,28] and in three studies without specifying the control. Savini et al. performed the study on six patients, and therefore did not estimate the *p* value [25]. Yet, they found a particular mutation variant associated with DRE: in the GAP domain of *TSC2*.

Two studies did not find statistical significance when *TSC1* vs. NMI and *TSC2* vs. NMI were compared [19], one of the articles previously mentioned as the article which observed *TSC2* vs. *TSC1* mutation [12]. The study by Chu-Shore et al., when compared *TSC2* vs. NMI and *TSC2* vs. *TSC1*, did not list it as a risk factor [23]. The last two studies did not find any association between known TSC mutations and DRE.

The impact of family history of TSC on DRE presence was reported in four studies. One study found it to be associated with a lower frequency of refractory seizures [31], and the other that the lack of family history of TSC was linked with DRE [20]. The studies by Mert et al. and Zhang et al., did not encounter statistical significance [26,32].

3.4.3. Type and Time of Seizures and DRE

Three out of four studies (75%) that focused on the history of infantile spasms identified it as statistically significantly associated with DRE. One of them determined drug-resistant infantile spasm as a more potent risk factor. Yet, the duration of infantile spasms does not appear to have an impact [12].

The epilepsy onset age is suggestive of being a parameter related to DRE. One article described the threshold age for the onset of focal seizures before 1 year of age [12], the other one below two years of age [26], and two more did not specify the age [20,21]. The second study also found a stronger association with DRE if the seizures were present in the neonatal period.

In two articles, a history of status epilepticus was associated with DRE risk [21,26].

3.4.4. Psychiatric Disorders and the Risk of DRE

In six studies, more severe cognitive impairment defined as mild to severe intellectual disability was associated with DRE. Lower educational level was observed to be related with refractoriness in two articles [20,21].

Two studies determined autism spectrum disorder as associated with DRE. One of them declared that attention deficit hyperactivity disorder (ADHD) is related to a higher risk of DRE and, on the contrary, anxiety lowers the risk [12]. At the same time, the other one did not find any association with ADHD [26]. Two studies observed a relationship between psychiatric disorders and DRE [20,21].

3.4.5. MRI/CT Changes and DRE

Zhang et al. determined calcification in cerebral parenchyma as a statistically significant ($p = 0.006$) risk factor for DRE [32].

The cortical tubers are known to cause seizures in TSC patients [34]. Six out of eight (75%) studies that analyzed MRI findings determined focal cortical dysplasia (FCD) as

associated with DRE. Four of those defined FCD as cortical tubers and ascertained the threshold number of tubers: seven and more cortical tubers [21], four and more [26], "at least one cyst-like cortical tuber" [2]. The last one compared the number of tubers 4.89 vs. 4.41 in DRE and non-refractory epilepsy, respectively, though the difference was not statistically significant [14].

One out of four articles that searched for the association between a presence of subependymal nodules (SEN) or subependymal giant cell astrocytoma (SEGA) and DRE found them to raise the risk of statistical significance.

Two articles analyzed white matter dysplasia and migration lines and did not find any association with DRE [12,26].

3.4.6. EEG Findings and DRE

Mert et al. observed an increased risk of DRE if EEG discharges became generalized [26].

Younger age of first ictal epileptiform discharges (IED) and multifocal IED on the first EEG are associated with refractoriness in patients treated according to the standard protocol, according to de Ridder et al. [33]. The difference was statistically significant only in a univariable model. When put in a multivariable model, or if the patients were preventatively treated, the risk was not statistically significant.

3.4.7. Treatment and the Risk of DRE

Three studies examined whether preventive treatment could be a predictor associated with reduced risk of DRE [24,29,30]. Two of them found a statistically significant difference between the groups [24,30].

4. Discussion

The present study aimed to summarize current knowledge on risk factors associated with DRE in patients with TSC. The results were classified into six categories: genetic mutation, time and type of seizures, psychiatric disorders, MRI/CT changes, EEG findings, and treatment protocol. Identifying the parameters related to increased risk of refractoriness, especially those which may be defined early, is crucial. Those factors related to the increased risk of refractoriness have the potential as indicators in finding patients at risk, who are most likely to benefit from early disease-modifying treatment.

Only ten articles had the same standardized DRE definition, based on ILAE, 2010 consensus. Jozwiak et al. in 2019 included in antiepileptic therapies: pharmacotherapy, VNS implantation, ketogenic diet, and epilepsy surgery. Other studies defined the highest acceptable number of seizures in a specific time. Five studies included in this review did not provide any definition of DRE. To minimize the impact of these discrepancies, we decided to include the information on the DRE definition in Table 1.

TSC2 mutation has been widely described as related to worse clinical outcomes, and the recent EPISTOP study confirmed the previous assumptions [35–41]. The results of our study also reflect this association, as most of the authors found a correlation between *TSC2* mutation and increased risk of DRE. This pathogenic variant remains a strong risk factor of DRE when compared with *TSC1* and NMI (60%, 6/10). However, some studies pointed out that the gene mutation might have no impact on seizure's refractoriness [12,19,20,23,32]. It is possible that the risk of DRE may more depend on the particular type of mutation. However, we did not find such detailed analysis of the association between the type of gene mutation and DRE. Although *TSC2* mutation is not always associated with DRE, patients with a mutation in this gene should be considered as having a higher risk of worsening the clinical course of TSC, including the risk of DRE.

A recent study by Liu et al. described a relationship between *TSC1* truncating mutation and intractability. However, it was performed on tissues from TSC patients operated on due to DRE, and we did not include the results in the review [42].

Family history of TSC lowers DRE risk; the difference may be explained by more attentive caregivers trained to early recognize alarming symptoms. Our results are consistent

with literature, where family *TSC2* cases tend to be described as less severe than de novo mutations [39]. Importantly, familial cases of TSC are more common to be caused by *TSC1* mutations, which is known to be less severe [39,43].

Regarding epilepsy, most of the studies that investigated infantile spasms found its relationship with DRE. However, there was some strong contra indicatory evidence from Vignoli et al. [21]. West syndrome's role in developing DRE may be explained by the early onset of seizures and difficulties in diagnosis and introducing proper treatment. Interestingly, the duration of infantile spasms appears not associated with DRE in the study by Jeong et al. [12]. Yet, the authors suggest the results were based on incomplete data, which impaired the correct analysis of this variable.

Younger age of onset of IED and clinical seizures seem to increase the odds of DRE [9,44]. The age threshold remains undefined, with some limiting it to two years, others one year of age or even the neonatal period [12,20,21,26]. Our results are reflected in the other studies' findings, according to which IED presence in most children is a sign of epileptogenesis and a predictor for refractory epilepsy [33,45,46]. Therefore, frequent EEG monitoring of children with TSC before clinical seizures to introduce preventive treatment is currently recommended [9,24,25,47,48]. Many centers already implement the early EEG and preventive treatment in TSC patients, based on the results of clinical trials performed in the last decade and European recommendations [48,49]. According to Słowińska et al., half of the treatment centers (31/60, 51.7%), introduce treatment based on EEG findings prior to clinical seizure onset [48,49].

Some studies included in this review also showed that early or even preventive antiseizure treatment of patients with TSC may reduce the risk of DRE [24,30,50]. Therefore, early TSC diagnosis and proper education for TSC patients' custodians become crucial in early and effective treatment. According to the EPISTOP study, preventive treatment is related to a significant reduction of DRE risk compared to the introduction of treatment after clinical seizures (28% vs. 64%, respectively) [24]. Currently, European recommendations advocate the introduction of preventive antiseizure treatment in children within 24 months of life if ictal discharges occur on EEG, with or without clinical manifestation [47]. On the other hand, recently updated international recommendations also notice potential benefits of preventive treatment; however, the consensus committee determined that additional evidence is needed before preventative treatment with vigabatrin can be recommended for all infants with TSC universally [9]. A recent questionnaire study showed that preventive approach is becoming more and more widely implemented in clinical practice [48].

Interestingly, de Ridder et al. found that once preventive treatment is implemented, none of the factors which had increased the risk of DRE (younger age of the first IED on EEG and multifocal IED on the first EEG) remained significant [33]. EEG abnormalities have been recently considered as a biomarker of epileptogenesis in infants with TSC [45,46]. In our review, Mert et al. observed an increased risk of DRE in case of generalized discharges on EEG [26]. EEG is a non-invasive and, in many centers, easily available study. Therefore, it may be used as a valuable for the early detection of patients with increased risk of DRE. Regular EEG studies within first 24 months of life in patients with TSC are currently recommended by international and European recommendations [9,47].

Primary evidence from the recent EXIST-3 trial on everolimus confirms that early introduction of this mTOR inhibitor decreases DRE risk [51,52]. In addition, some former smaller studies demonstrated its positive effect on cardiac rhabdomyomas, SEGA size, and epilepsy in TSC patients, including as an adjunctive treatment for DRE [51,53–55].

Intellectual disability is linked with DRE, yet it appears to be the result, not the cause of DRE [56–58]. Early-onset of severe epilepsy is known to hinder the intellectual development of the patients and lead to lowered IQ [44,59–61]. The results of our review also reflect this association, as Goh et al. found a relationship between intellectual disability and infantile spasms, while according to Winterkorn et al., the cognitive outcome is related to DRE and *TSC2* mutation [31,62]. Age of onset, *TSC2* mutation, and infantile spasms are

independently related to DRE, as described in our article. Therefore, we may assume that refractory seizures impair the cognitive and intellectual development of TSC patients.

The relationship between other psychiatric disorders, including ASD and ADHD, and DRE remains unclear. Specchio et al. suggested both ASD and DRE be driven by FCD [63]. Other studies found that the early onset of seizures and infantile spasms to be the causes of autism development [60,64,65]. It appears that the same factors cause ASD and DRE or that ASD takes origin in DRE. Interestingly, anxiety and depression continue without any association with refractoriness.

MRI or CT imaging findings appear to be related to DRE. Sixty-six percent of the studies which investigated FCD found it to increase the risk of DRE. Though the difference is not statistically significant in one of them, we included the results due to its specific limitations, such as fetal MRI quality and the study's retrospective design [14]. A study on resected cortical tubers and perituberal cortex by Ruppe et al. has demonstrated the epileptogenic potential of both [66]. The abnormalities in the proximity of ventricles, such as SEN and SEGA, and white matter disruption seem unrelated to epileptogenesis.

The relationship between sleep—its quality, duration, any disturbances, and seizures, is well-known in many epileptic syndromes [67–69]. Few studies indicated the increased risk of sleep disturbances in patients with TSC [70,71]. However, none of the analyzed articles discussed the influence of sleep on DRE in individuals with TSC. As sleep disturbances present a potentially modifiable factor it seems to be reasonable to include them onto the list of possible risk factors of DRE in TSC in future studies.

The general characteristics of the patients, such as sex, age, and race, show no association with refractoriness in the included studies [12,20,26,32]. The male to female ratio appears to be maintained at an equal level. Age is a risk factor only in specific circumstances, e.g., the onset of seizures or the onset of IED on EEG. Both instances were discussed above.

Limitations of the Study

Limitations of the search and selection: The search of the articles—was conducted only by one reviewer. The risk of bias may be higher than if the search was performed by two reviewers separately.

We included only articles published in English or Polish. Therefore, some articles written in other languages may have been omitted.

Due to differences in DRE definitions, neither comparison between the studies nor a metaanalysis were performed.

The limitation at the outcome level: Most of the included studies were retrospective. In one study, only six patients were included. Therefore, the risk of bias is higher compared to randomized and prospective studies. Limitations of the presented systemic review: The main limitation of this systematic review is a low number of high-quality data from randomized studies. Moreover, studies differed, i.e., in terms of the applied definition of DRE. Therefore, due to the risk of bias, no metanalysis or comparison between the studies was conducted.

5. Conclusions

Most studies observed an association between DRE and three main parameters: *TSC2* mutation, infantile spasms, and the number of cortical tubers. According to the authors, epileptiform discharges on EEG and early onset of seizures, especially before one year of age, also increase the risk of refractoriness of the seizures. The majority of the risk factors is unmodifiable, yet regular EEG monitoring and proper education of the caregivers was observed to reduce the risk of refractoriness.

Psychiatric disorders, such as ADHD, ASD, and cognitive impairment appear to be the consequence rather than the cause of DRE.

Importantly, all three studies, which focused on preventive treatment, observed lowered DRE risk if the treatment was introduced before clinical seizures.

This study summarizes current knowledge on risk factors related to increased risk of DRE in individuals with TSC. The results facilitate identifying patients with the highest odds of developing refractory seizures. In those individuals, an introduction of treatment before clinical seizures may contribute to their developmental improvement.

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6.2. Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study

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Article

Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age—A Bicenter Retrospective Study

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Abstract: Background: mTOR inhibitors are a novel pharmacotherapy recommended for subependymal giant astrocytomas, refractory epilepsy, and the treatment of the other clinical manifestations of tuberous sclerosis complex (TSC). Clinical trials on everolimus proved it to be effective and safe in children. Despite its common use in clinical practice, the research on sirolimus is limited. This study is the first to determine and assess the severity of the adverse effects (AEs) of sirolimus administered to children with TSC under two years of age. Methods: We performed a bicenter retrospective data analysis of medical records of individuals with TSC who initiated therapy with sirolimus under the age of two. Results: Twenty-one patients were included in the study. At least one AE was reported in all participants. The most prevalent AEs were anemia, thrombocytosis, and hyperlipidemia. Infections and mouth ulcerations, often reported in the studies on older patients, were infrequent and of mild or moderate grade. Conclusions: Adverse effects associated with sirolimus use in infants and young children with TSC are frequent yet not life- or health-threatening. Further multicenter prospective clinical trials should determine the long-term safety of sirolimus.

Keywords: tuberous sclerosis complex; infant; mTOR inhibitor; sirolimus; adverse effect; safety

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1. Introduction

Tuberous sclerosis complex (TSC) is a genetic disorder caused by a heterozygous mutation in either of the genes *TSC1* at 9q34 (encoding hamartin) or *TSC2* at 16p13.3 (encoding tuberin), affecting one in 6000 live births [1]. Hamartin and tuberin inhibit the mechanistic target of rapamycin (mTOR), a kinase that regulates protein and lipid synthesis. The mutation in the *TSC1* and *TSC2* genes leads to the overactivation of the mTOR. Its selective activation results in a kinase signaling cascade that increases cell proliferation and growth; hence, multiple benign tumors are formed in different organs [2,3]. Among the most common clinical manifestations of TSC, patients report epilepsy, cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas (SEGA) in the central nervous system, cardiac rhabdomyomas (CRs), as well as renal involvement, such as angiomyolipomas (AML) and renal cysts [4]. Despite their benign character, TSC-associated tumors often become symptomatic and require adequate management. SEGA leads to obstructive hydrocephalus when the tumor grows in the proximity of the interventricular foramen. CRs may block the ventricular flow or cause arrhythmias.

Epilepsy affects approximately 80% of patients with TSC, and in up to 30% of them, seizures become refractory to treatment [2,5]. The earlier appearance of the seizures correlates with worse neurological outcomes and refractoriness, despite adequate antiseizure treatment, even before the onset of seizures [6,7].

Novel pharmacotherapy targeted at the metabolic pathway to inhibit the mTOR kinase has recently been approved in many countries, including EU members, US, and Japan [8]. The mTOR inhibitors changed our view on the management of TSC-associated tumors. Everolimus is approved for treating SEGA, lymphangioleiomyomatosis (LAM), and partial-onset seizures [9–11]. As an off-label treatment, it has successfully been used to reduce CRs in children [12,13]. Sirolimus is approved only as LAM therapy, and its efficacy in reducing the frequency and severity of epileptic seizures or treating SEGA or CRs is still being researched. Preclinical studies suggest the mTOR inhibitor's therapeutic role in autism spectrum disorder, a common psychiatric disorder associated with TSC [14,15].

Randomized clinical trials on everolimus have shown its safety profile in patients with TSC [10,16–18]. Despite the lack of research with a similar level of evidence, sirolimus is more often used in clinical practice due to its accessibility. Reports on sirolimus-related adverse effects (AEs), especially in infants and children under two years of age, are limited. These individuals are potential beneficiaries of early antiseizure treatment and SEGA or CRs reduction. Therefore, it raises the question about the safety of sirolimus use in this group of patients.

This study aims to address the gaps in mTOR inhibitor therapy research. We assess the safety of sirolimus in young patients with TSC under the age of two years.

2. Materials and Methods

2.1. Study Design and Data Collection

The present study is a bicenter retrospective data review of children with TSC treated with mTOR inhibitors between 2014 and 2022.

The data was collected from two hospitals in Warsaw, Poland: the Department of Neurology and Epileptology, The Children's Memorial Health Institute, and the Department of Child Neurology, the Medical University of Warsaw. We searched the database with the alphanumeric coding of TSC in the ICD-10 list: Q85.1. The data was extracted from patients' medical records in paper or electronic health records and put into a standardized spreadsheet. The data collection process ended on 30 June 2022.

The inclusion criteria were:

1. Clinical or genetic diagnosis of TSC;
2. Treatment with oral sirolimus before the age of two years;
3. A follow-up at least three months after the initiation of treatment.

The patients were excluded if mTOR inhibitors were introduced after two years of age, the patient was not followed for at least three months, or the applied mTOR inhibitor was everolimus.

The recovered baseline data included:

1. Genetic analysis results: *TSC1* or *TSC2* gene mutation;
2. Sex;
3. Age at the initiation of mTOR inhibitor treatment;
4. Age at the onset of epileptic seizures;
5. Reason for mTOR inhibitor treatment;
6. Whether antiepileptic drugs (ASMs) were included;
7. If the ASM treatment was preventive.

We stored the results of laboratory blood tests, the incidence of infections or mouth ulcerations, sirolimus dose, sirolimus blood concentrations, the patient's body mass, and the ASMs used. For each patient, the data was analyzed twice, depending on the following:

1. Age: from birth to 6 months of age, 6–12 months, 12–24 months, and 24–36 months of age.
2. Treatment duration (months).

Anemia was defined as either hemoglobin levels below the lower limit of normal (LLN) or red blood cell (RBC) count below the LLN. Hyperlipidemia was determined in case of the elevated blood level of at least one of the below: cholesterol, low-density lipoprotein cholesterol (LDL-C), or triglycerides. Thrombocytosis was considered "mild" for a platelet

count (PLT) between 450,000 and 700,00/µL and "moderate" up to 900,000/µL [19]. All the laboratory's norms for blood tests were adjusted for age.

2.2. Study Outcomes

The primary outcome of this study was to assess the safety profile of sirolimus in young children with TSC under the age of two. The data collected at the onset of treatment and then three, six, twelve, and twenty-four months after the initiation focused on the known AEs caused by mTOR inhibitors. Additionally, repetitive alterations in the laboratory results and general outcomes were recorded.

The AEs were graded based on their severity according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0 [20]:

1. Grade 1: Mild
2. Grade 2: Moderate
3. Grade 3: Severe, of medical significance
4. Grade 4: Life-threatening consequences
5. Grade 5: Death related to AE

The interpretation of the laboratory test results was based on the norms provided by the laboratories and adjusted for age.

2.3. Statistical Analysis

Continuous variables were calculated; central tendency was reported as mean and median while the variation as standard deviation (SD) and minimum and maximum values, respectively. Quantitative variables were used for sirolimus serum levels calculation. Each AE was assigned a qualitative value (present or not present), and the results were reported as the count and frequency. Analyses were conducted using Statistica 13.3. for Windows.

3. Results

Twenty-five patients at both clinical centers started mTOR inhibitor therapy under two years of age. Twenty-one were treated with sirolimus and four with everolimus. One patient began the treatment with sirolimus. After three months, the pharmacotherapy was changed to everolimus due to their inclusion in the national program of everolimus treatment for patients with SEGA not qualified for surgical treatment.

We included all 21 patients treated with sirolimus in this study. Seventeen out of twenty-one patients were treated in the Department of Neurology and Epileptology, The Children's Memorial Health Institute in Warsaw (17/21, 80.9%). All individuals began pharmacotherapy in the years 2014–2022.

Eight patients were male (8/21, 38.10%), and thirteen were female (13/21, 61.90%). The median age at the onset of sirolimus therapy was 104 days (range 3–656 days). Genetic analysis was performed on 15 patients (15/21, 71.4%). In all 15 patients, a mutation in the *TSC2* gene was found. In addition, three of them carried an additional mutation in the *PKD1* gene (Table 1).

3.1. Reasons for mTOR Inhibitor Treatment

The treatment with mTOR inhibitors was initiated due to SEGA in twelve patients (12/21, 57.1%), drug-resistant epilepsy in seven (7/21, 33.3%), rhabdomyomas causing arrhythmia or obstruction of blood flow in five (5/21, 28.6%), and renal angiomyolipomas and retinal hamartomas in one each (1/21, 4.8%). Six patients presented with more than one reason for mTOR inhibitor introduction (6/21, 28.6%) (Table 1).

Table 1. Patients' characteristics.

Variable	Value (%)
	N = 21
Sex	
Female	13 (61.9)
Male	8 (38.1)
TSC mutation	
TSC1	0 (0)
TSC2	15 (71.4)
PKD1	3 (14.3)
Not studied	6 (28.6)
Reason for mTORi [1] treatment	
Cardiac rhabdomyomas	5 (28.6)
SEGA	12 (57.1)
Renal AML	1 (4.8)
Retinal hamartomas	1 (4.8)
Epilepsy	7 (33.3)
Age at the initiation of mTORi treatment (days)	
Mean (SD)	211.9 (233.2)
Median [Min, Max]	104 [3, 656]
mTORi treatment follow-up duration (months)	
Mean (SD)	16 (8.7)
Median [Min, Max]	22 [3, 24]
Antiepileptic treatment	
Yes	19 (90.5)
No	2 (9.5)
Number of ASM [2]	
Mean (SD)	2.0 (1.1)
Median [Min, Max]	2.0 [0, 3.0]
Preventive antiepileptic treatment	
Yes	7 (33.3)
No	14 (66.7)

[1] mTORi—mTOR inhibitor. [2] ASM—antiseizure medication.

3.2. Sirolimus Dosing

In all patients, sirolimus was administered once a day, orally, in the form of a solution of concentration 1 mg/mL. The protocol for mTOR inhibitor initial dose was 0.5 mg/m^2/day and varied between 0.01–0.07 mg/kg/day. The dosing regimens were then adjusted according to the sirolimus plasma levels. The targeted levels of sirolimus ranged between 3–4 ng/mL. The median plasma levels of sirolimus throughout the study were 4.34 ng/mL (0.79–13.89 ng/mL).

3.3. Data Collection

Data was extracted from medical records collected during the routine check-ups performed at the treatment initiation and three, six, twelve, and twenty-four months after pharmacotherapy initiation.

The clinical tests included laboratory testing, such as complete blood count, lipid profile, liver transaminases, ALT, AST, and creatinine. In some patients, fibrinogen, D-dimers, bilirubin, and gamma-glutamyl transferase (GGTP) were also analyzed. In addition, physical examination, echocardiography, and neuroimaging were performed.

3.4. Long-Term mTOR Inhibitor Continuation

Eighteen patients (18/21, 85.71%) continued pharmacotherapy until the end of the follow-up. Nine of them were observed for two years, and nine continued the treatment until the end of data collection, yet the observation period was shorter than two years. Three individuals discontinued the treatment before the end of the follow-up (3/21, 14.29%). The median follow-up period was 12 months.

The reasons for withdrawal were an unsatisfactory response to the treatment; four patients were qualified for neurosurgery, and one began the treatment with cannabidiol. None of the individuals discontinued the treatment due to the AE.

3.5. Additional Therapies

During the follow-up, nineteen patients (19/21, 90.5%) received antiseizure treatment. The median number of ASM was two. All of the patients who received ASM were treated with vigabatrin (19/19, 100%), ten with valproic acid (10/19, 52.6%), five with carbamazepine (5/19, 26.3%), five with levetiracetam (5/19, 26.3%), two with clobazam (2/19, 10.5%), and two with topiramate (2/19, 10.5%). Two children received adrenocorticotropic hormone (ACTH), and two patients were on the ketogenic diet.

3.6. The Safety Profile of Sirolimus Therapy

At least one adverse effect was reported in all patients (21/21, 100.0%) (Table 2). The number of tested patients varied for each AE, which is specified in the corresponding column in Table 2.

Table 2. Sirolimus adverse effects depending on age.

Adverse Effect | Age	0–6 Months	6–12 Months	12–24 Months	24–36 Months
	N [1] (%)	N (%)	N (%)	N (%)
Hyperlipidemia	10/12 (83.33)	11/13 (84.62)	13/13 (100)	5/8 (62.5)
Hypercholesterolemia	8/12 (66.67)	5/12 (41.67)	8/13 (61.54)	3/8 (37.5)
Elevated LDL	5/11 (45.45)	5/12 (41.67)	7/13 (53.85)	2/7 (28.57)
Hypertrigliceridemia	7/12 (58.33)	8/12 (66.67)	10/13 (76.92)	3/7 (42.86)
Anemia	11/12 (91.67)	8/14 (57.14)	9/14 (64.29)	5/10 (50)
Thrombocytosis	7/12 (58.33)	5/14 (35.71)	11/14 (78.57)	7/10 (70)
Neutropenia	5/12 (41.67)	3/14 (21.43)	5/14 (35.71)	1/10 (10)
Elevated D-dimers	2/2 (100)	0/1 (0)	1/2 (50)	0/0 (0)
Elevated bilirubin	3/7 (42.86)	0/4 (0)	0/4 (0)	1/6 (16.67)
Low ALT	4/12 (33.33)	4/13 (30.77)	6/12 (50)	4/7 (57.14)
Elevated AST	2/12 (16.67)	1/13 (7.69)	4/12 (25)	2/7 (28.57)
Elevated fibrinogen	0/6 (0)	1/2 (50)	1/3 (33.33)	0/1 (0)
Infections	2/12 (16.67)	3/13 (23.08)	5/13 (38.46)	5/10 (50)
Mouth ulcers	2/11 (18.18)	0/14 (0)	0/13 (0)	1/10 (10)

[1] N—number of patients who reported the adverse effect divided by the number of patients who were tested for the adverse effect.

Twelve individuals began the treatment between birth and six months of age. In this group, the most common AE was anemia in eleven (11/12, 91.67%), hyperlipidemia in ten

(10/12, 83.33%), with the most frequent being hypertriglyceridemia in seven (7/12, 58.33%), and elevated PLT in seven (7/12, 58.33%). One patient had low PLT (1/12, 8.33%).

Fourteen patients continued or started treatment with sirolimus between six and twelve months of age. The most common side effects were anemia in eight (8/14. 57.14%), hyperlipidemia in eleven (11/13, 84.62%), and elevated PLT in five (5/14, 35.71%).

Fourteen patients were on treatment between one and two years of age. Nine of them had anemia (9/14, 64.29%), thirteen had hyperlipidemia (13/13, 100.0%), and eleven had elevated PLT (11/14, 78.57%). Two patients with hyperlipidemia were on the ketogenic diet at that time.

The data was available for ten patients two years and older. The most common AE was elevated PLT in seven (7/10, 70.0%), anemia in five (5/10, 50.0%), and hyperlipidemia in five (5/8, 62.5%).

D-dimers and fibrinogen were rarely tested despite high PLT.

Throughout the follow-up, infections were reported in 16.67% up to 50% of the patients, most common in individuals two years and older. On the other hand, neutropenia was reported in 10% up to 41.67% of the patients, and it was most prevalent in patients under six months of age. One individual required hospitalization several times. One patient's parents noticed a pattern between higher serum sirolimus levels and the frequency of infections.

Three cases of mouth ulcerations were reported, two in patients younger than six months (2/11, 18.18%) and one in a patient older than two years (1/10, 10.0%).

The majority of adverse effects were grade 1 and 2 according to the CTCAE. All patients with anemia reported hemoglobin levels between 8.5 g/dL and the LLN for the respective age group.

Elevated PLT in most participants was between 450 and 600/µL. One child reported a PLT count of 745/µL.

None of the participants in the study had to be hospitalized due to hyperlipidemia. In most patients with elevated LDL-C, the levels were between 130 mg/dL and 280 mg/dL. Hypertriglyceridemia was reported to be between 150 mg/dL and 300 mg/dL. One patient had a triglyceride level of 750 mg/dL. The individual was on the ketogenic diet at that time; a modification of proportions led to the normalization of lipid levels. The levels exceeded 1000 mg/dL in none of the participants.

One patient was hospitalized two times due to infections: varicella and not identified bacterial infection treated with antibiotics. Both times the patient presented with fever and epileptic seizures. No AEs grade 4 or 5 were reported.

Table 3 and Figures 1–3 show the three most common AEs: anemia, thrombocytosis, and hyperlipidemia, depending on the duration of sirolimus administration. The figures depict all patients who reported any of those AEs: blue-coded individuals with a history of laboratory alternation prior to sirolimus treatment and orange-coded patients with no record of such disorder.

The prevalence of anemia, thrombocytosis, and hyperlipidemia was first calculated for all patients who had the factor tested. Then the frequency was estimated among the patients with normal hemoglobin and RBC, PLT, or serum lipids prior to the sirolimus administration.

Anemia was reported in 40–70% of individuals during the follow-up. This AE was observed for the first time in the first three months of pharmacotherapy in more than half of the patients.

Thrombocytosis was reported for the first time in almost 40% of the patients in the first three months after starting the treatment. After six months of pharmacotherapy, in this group, the frequency of elevated PLT rose to 70%; it remained elevated in over two-thirds of the patients until the end of the follow-up.

In the first three months of treatment, lipid blood levels were elevated in more than 60% of the patients. This number dropped to 40% in the following three months. Regardless of the group size, hyperlipidemia remained present in 40–60% of individuals during the follow-up.

Table 3. Anemia, thrombocytosis and hyperlipidemia as an adverse effect depending on the duration of treatment.

	Anemia		Elevated Platelet Count		Hyperlipidemia	
Duration of the Treatment (Months)	**All Patients**	**No History of Anemia Prior to Sirolimus**	**All Patients**	**No History of Elevated PLT [1] Prior to Sirolimus**	**All Patients**	**No History of Hyperlipidemia Prior to Sirolimus**
	N [2] (%)	**N (%)**	N (%)	**N (%)**	**N (%)**	**N (%)**
0	10/21 (47.62)	0/11 (0)	7/21 (33.33)	0/14 (0)	7/18 (38.89)	0/11 (0)
0–3	14/20 (70)	6/11 (54.55)	10/20 (50)	5/13 (38.46)	11/18 (61.11)	7/11 (63.64)
3–6	7/19 (36.84)	5/10 (50)	8/20 (42.11)	4/11 (36.36)	7/16 (43.75)	4/10 (40)
6–9	8/14 (57.14)	4/8 (50)	8/14 (57.14)	5/7 (71.43)	7/12 (58.33)	4/7 (57.14)
9–12	6/10 (60)	4/6 (66.67)	4/11 (36.36)	4/6 (66.67)	5/9 (55.56)	2/5 (40)
12–18	7/10 (70)	4/5 (80)	6/10 (60)	3/3 (100)	7/11 (63.64)	4/6 (66.67)
18–24	4/9 (44.44)	2/5 (40)	5/9 (55.56)	3/4 (75)	6/8 (75)	4/6 (66.67)
24–36	5/8 (62.50)	2/4 (50)	4/7 (57.14)	3/3 (100)	4/7 (57.14)	2/4 (50)

[1] PLT—platelet count. [2] N—number of patients who reported the adverse effect divided by the number of patients who were tested for the adverse effect.

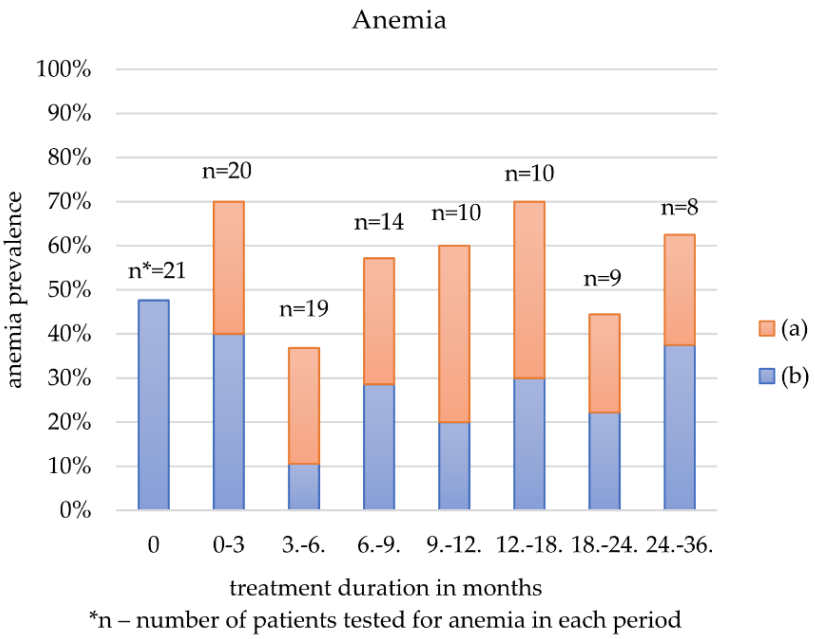

Figure 1. Anemia according to the treatment duration in patients with: (a) no history of anemia prior to sirolimus treatment, (b) a history of anemia prior to sirolimus treatment.

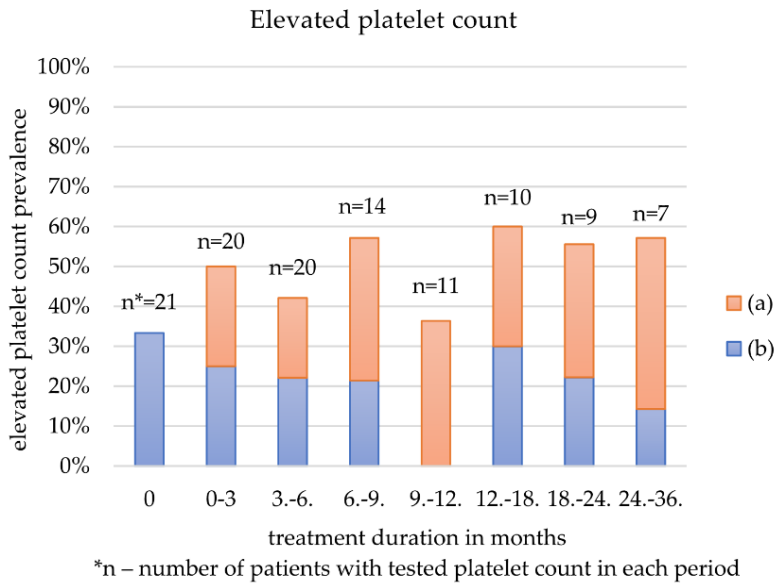

Figure 2. Elevated platelet count depending on the treatment duration in patients with: (a) platelet count within normal ranges prior to sirolimus treatment, (b) thrombocytosis prior to sirolimus treatment.

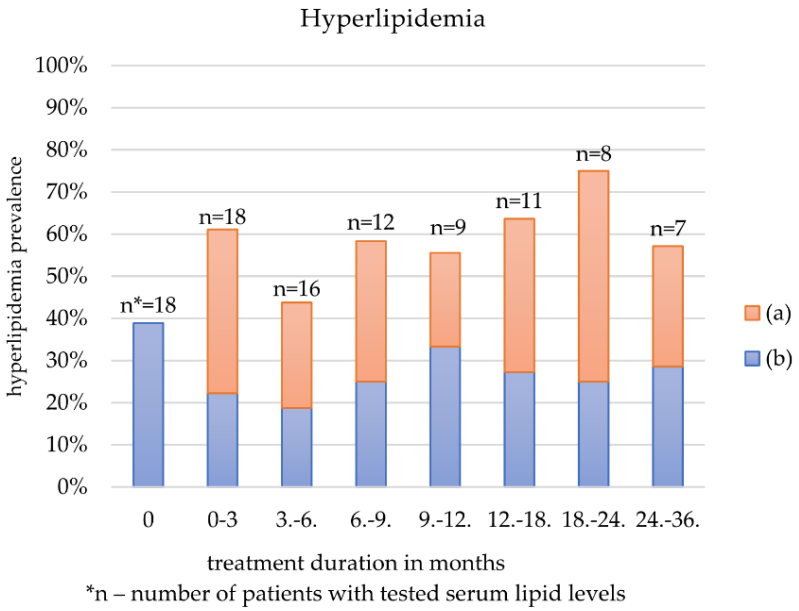

Figure 3. Hyperlipidemia depending on the treatment duration in patients with: (a) normal lipid blood levels prior to sirolimus treatment, (b) hyperlipidemia prior to sirolimus treatment.

4. Discussion

Randomized clinical trials, EXIST-1, and EXIST-3 proved everolimus as effective and safe in young children [16,21,22]. Other studies and reports are consistent with those findings [9–11]. Although sirolimus is used more often than everolimus, its efficacy and

safety have only been assessed in studies with a smaller level of evidence in older children and adults with TSC [23–27]. This is the first study to report AEs caused by sirolimus use in the youngest patients under the age of two.

The AEs were observed in all participants during the follow-up, yet they tended not to be severe and did not lead to pharmacotherapy discontinuation. Clinical trials on sirolimus used in older patients with TSC also found it to cause low-grade AEs, grade 1 or 2 [10,16,28]. Children under three years of age treated with everolimus during the EXIST study reported AEs of severity grade 1 or 2 as well. Only one patient from this group withdrew from the treatment due to an AE [16].

In research studies on sirolimus, the target serum levels range between 4–20 ng/mL, typically 5–15 ng/mL [9,26,28,29]. In this study, blood trough levels remained in the lower spectrum of those ranges, which may contribute to the lower severity of the AEs.

All the included participants started the treatment with mTOR inhibitors due to SEGA, CRs, or refractory seizures, which are the most common causes according to Krueger et al. and Saffari et al. [10,17,28]. In every individual with genetic testing performed, a *TSC2* mutation was found, which is known to correlate with more severe TSC symptoms [30]. As a result of the severity of the disease, six individuals were treated with mTOR inhibitors due to more than one cause.

Hyperlipidemia, anemia, and thrombocytosis were the most frequently reported AEs during the follow-up, regardless of age or the duration of the treatment.

4.1. Hyperlipidemia

The majority of participants reported mild or moderate hyperlipidemia, especially hypertriglyceridemia, during the follow-up. It is consistent with previous clinical trials, which found mTOR inhibitors lead to elevated, yet not life-threatening, serum lipid levels [4,12,13,16]. Hyperlipidemia caused by mTOR inhibitors is repeatable, reversible, and dose-dependent [31]. As we did not follow the patient's lipid levels after therapy discontinuation, the reversibility of hyperlipidemia was not assessed.

Two patients with the highest blood lipid levels were on a ketogenic diet at that time, also known to cause hyperlipidemia [32]. In those children, the lipid-to-nonlipid ratio was modified, as according to Fang et al., diet modification should improve the laboratory results [33]. After the adjustment, the lipid levels lowered; however, they remained elevated compared to the normal ranges.

4.2. Anemia

Anemia is one of the most often reported sirolimus-induced AEs [10,25,26]. In this study, anemia was the most frequent among the youngest individuals between birth and six months of age. Sirolimus affects iron homeostasis, leading to mild microcytic anemia [34]. However, in this specific group of patients, anemia may have been partially due to physiological anemia, which occurs in all infants between ten and twelve weeks of age, being the most severe in preterm infants [35]. Table 3 represents the data not biased by the physiological anemia, as it is based on the duration of treatment, not the age. This data demonstrates that anemia is frequently reported regardless of the age of the participants.

According to the literature, mTOR inhibitor discontinuation due to anemia is rare [25,26]. During the follow-up in this study, no radical interventions were undertaken. As the AE was of grade 1 or 2 in most cases, iron supplementation and dietary modifications were recommended.

4.3. Thrombocytosis

Sirolimus use in human trials often leads to thrombocytopenia [24,25,36–38]. According to Busca et al., low platelet count correlates significantly with sirolimus through concentrations, yet they tend to be much higher than the concentrations maintained in our research [39]. In this study, only one patient reported thrombocytopenia.

Elevated PLT was frequent in all age groups, yet in none of the individuals did it lead to any further clinical consequences, nor did it require intervention. No literature on the sirolimus impact on PLT elevation was found. Interestingly, due to its immunosuppressive effect, sirolimus can be applied as rescue therapy for thrombocytopenia in primary antiphospholipid syndrome [40].

4.4. Infections

Sirolimus belongs to a class of immunosuppressants and has proven its efficacy in solid organ transplantation. Its immunosuppressive effect may increase the susceptibility to infections, in particular pneumonitis or stomatitis [41–44]. Despite being reported frequently by patients treated with sirolimus, infections tend to be mild and often do not lead to hospitalization [17,23,28]. During the follow-up, one individual had to be hospitalized due to an infection to provide constant surveillance. No emergency interventions were undertaken. According to Krueger et al. and Guemes et al., infections often lead to resignation from the treatment, yet in our study, none of the participants withdrew due to this cause [27,28].

Stomatitis or mouth ulcerations are likely dose-dependent and often reported in older children, requiring short-term or complete treatment withdrawal [10,16,18]. During the follow-up, they were reported sporadically and, being of low severity grade, required only sirolimus discontinuation for a few days.

4.5. Discontinuation

Few interventions were needed to minimalize the AEs caused by sirolimus use. In most cases, a dose adjustment or temporary discontinuation had to be undertaken. Sirolimus dosing alternations may cause imprecise serum level calculations and require further modifications to the dosing scheme.

The typical drop-out rate in the studies on mTOR inhibitors in patients with TSC is 0–5% [17,45,46]. In our study, almost 15% of the participants resigned from pharmacotherapy with sirolimus. However, an AE was not the reason for the discontinuation in any of them. Most patients resigned due to unsatisfactory results and treatment alternatives available.

5. Limitations of the Study

The study was a retrospective data collection, which may be subject to patient, parent, and physician recall bias and differences in the history-taking methodology. The relatively small number of participants assessed in this analysis can lead to conclusions that should not be applied to all patients.

6. Conclusions

The analysis is the first to assess the safety of sirolimus in infants and young children under two years of age. In the EU and the US, mTOR inhibitors are currently recommended as a treatment for distinct TSC clinical manifestations. Clinical trials have proved everolimus' efficacy and safety, yet the research on sirolimus is limited. The innovative character of this study is emphasized by the size of the cohort, which included all patients from two clinical centers specializing in child neurology in Poland.

Adverse effects associated with sirolimus use in infants and young children with TSC are common yet not life- or health-threatening. The most frequent AEs in this group of patients are anemia, hyperlipidemia, and thrombocytosis. Mouth ulcerations or stomatitis are not common, and infections tend to be mild. Sirolimus appears to be safe and well tolerated in young patients with TSC.

Further prospective studies are recommended to support the findings.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and the Bioethics Committee of Medical University of Warsaw was informed of the study (AKBE/101/2021, 14 June 2022). Ethical approval was waived for this study due to study characteristics.

Informed Consent Statement: Patient consent was waived due to retrospective of the study, minimal risk and data anonymization.

Data Availability Statement: The data generated and/or analysed in this study are available from the corresponding author upon reasonable request.

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

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6.3. Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under 2 Years of Age

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ORIGINAL RESEARCH

Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under 2 Years of Age

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ABSTRACT

Introduction: Mechanistic target of rapamycin (mTOR) inhibitors sirolimus and everolimus are an effective therapy for subependymal giant cell astrocytomas, cardiac rhabdomyomas, renal angiomyolipomas, and lymphangioleiomyomatosis associated with tuberous sclerosis complex (TSC). Everolimus was recently approved in the EU and the USA for the treatment of refractory focal-onset seizures. Despite frequent use of mTOR inhibitors, there are only a few studies on their effect on epilepsy control in children under 2 years of age. This study aims to assess the effect of adjunctive mTOR inhibitor treatment on seizure frequency in this age group.

Methods: We performed retrospective data analysis of medical records of patients with TSC who initiated sirolimus or everolimus under the age of 2 years. Participants' antiseizure medication was adjusted according to their epilepsy control independently from mTOR inhibitor administration. The data was assessed separately for patients treated with mTOR inhibitors before and after the onset of seizures. We also compared the treatment group with a matched control group. The follow-up duration was up to 24 months.

Results: Twenty-one patients with TSC from two clinical centers were included in the study. Nine participants had no history of seizures before mTOR inhibitor initiation. Twelve reported active epilepsy in the month prior to treatment initiation. Most patients treated preventively with mTOR inhibitors did not report active epilepsy at the end of their follow-up. In the second group, the mean frequency of seizures decreased with time. According to the comparative analysis, seizure control was better in the groups treated with mTOR inhibitors.

Conclusion: Patients with TSC treated with mTOR inhibitors demonstrated better seizure control than individuals without this treatment. Adjunctive pharmacotherapy with mTOR inhibitors appears to have a beneficial effect on epilepsy outcome in young children. Further prospective clinical trials should be conducted

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to determine the efficacy of mTOR inhibitors on epilepsy in patients with TSC under the age of 2 years.

Keywords: Children; Epilepsy; Everolimus; mTOR inhibitors; Seizures; Sirolimus; Tuberous sclerosis complex

Key Summary Points

mTOR inhibitors are a novel pharmacotherapy alternative for treating TSC-associated subependymal giant cell astrocytomas, renal angiomyolipomas, focal-onset epilepsy, cardiac rhabdomyomas, and lymphangioleiomyomatosis. However, there is limited research on their impact on seizure control in patients under the age of 2 years.

In this study, we aimed to assess the effect of mTOR inhibitors on epilepsy control in young children when administered preventively and when added to the current antiseizure medication.

Patients treated with mTOR inhibitors demonstrated better seizure control when compared with the patients in the control group.

INTRODUCTION

Mechanistic target of rapamycin (mTOR) pathway plays a specific role in epileptogenesis in several neurological disorders, belonging to the group of "mTORopathies", including focal cortical dysplasia, tuberous sclerosis complex (TSC), and hemimegalencephaly [1–3]. TSC is an autosomal dominant neurocutaneous disorder resulting from a mutation in *TSC1* or *TSC2* genes. Under normal conditions, their protein products, hamartin and tuberin, inhibit mTOR function. mTOR is a protein kinase that regulates cellular growth, metabolism, and differentiation [4, 5]. The mutation leads to disinhibition and overactivation of the mTOR pathway and, thus, multiple benign tumor formation in various organs.

Results of several clinical trials demonstrated that mTOR inhibitors (mTORi) sirolimus and everolimus are effective in the treatment of TSC-associated subependymal giant cell astrocytomas (SEGA), cardiac rhabdomyomas (CR), renal angiomyolipomas (AML), and lymphangioleiomyomatosis (LAM) [6, 7]. mTOR inhibitors were recently approved in the EU, USA, and Japan [8]. Besides focal-onset seizures, everolimus is currently approved for treating SEGA and LAM and used as an off-label treatment for CR reduction [9–13]. Sirolimus is approved only as LAM therapy in patients with TSC, while its impact on seizure frequency is not yet determined.

Up to 80–90% of patients with TSC report epilepsy, and almost 80% of them experience the onset of seizures under 2 years of age [14]. The direct mechanism of epileptogenesis in TSC is still unknown. mTOR mutations may impact synaptic plasticity mechanisms, while molecular changes in protein expression may increase neuronal excitability, leading to seizures [15–18].

Epilepsy control depends widely on the timing of the treatment introduction, with the best outcome when initiated prior to or within a week from clinical seizure onset [19–22]. Vigabatrin is recommended as the first-line treatment for infantile spasms in the USA and all seizures under the age of 1 year in the EU in patients with TSC [23]. It increases GABA (γ-aminobutyric acid) levels and, thus, has a different mechanism of action from mTOR inhibitors. Preclinical animal studies demonstrated the antiepileptogenic effect of mTOR inhibitor treatment [24, 25]. The synergistic action of those drugs may strengthen their preventive and disease-modifying function.

One-third of epileptic seizures in children with TSC become refractory to treatment, and it correlates with the early age at the first clinical seizure, a mutation in the *TSC2* gene, and the number of cortical tubers [26].

Adjunctive treatment with sirolimus or everolimus in the youngest patients with drug-resistant seizures could improve their epilepsy control, as was reported by a few studies [9, 27].

However, analyses were conducted only on a small number of patients. Prospective clinical trials are ongoing in the EU and USA, but no results have been published yet. This is the largest study so far to assess the effect of adjunctive mTOR inhibitor treatment in seizure control in patients with TSC under the age of 2 years.

METHODS

Study Design

We performed a retrospective analysis of medical records of patients with TSC born between 2008 and 2022. The data was derived from the medical history of individuals treated with mTOR inhibitors for SEGA, CR, renal AML, and epilepsy in the two clinical centers in Warsaw, Poland: the Department of Neurology and Epileptology, The Children's Memorial Health Institute, and the Department of Pediatric Neurology, the Medical University of Warsaw in Warsaw, Poland. The ICD coding number for TSC, Q85.1, was applied for the database search. The data was extracted and transferred into a spreadsheet. The data collection process was performed until 30 June 2022.

The inclusion criteria were:

1. Genetic or clinical diagnosis of TSC
2. Oral pharmacotherapy with an mTORi, sirolimus or everolimus, initiated before the age of 2 years
3. Active epilepsy in the month prior to mTORi introduction or no history of epilepsy
4. At least 3 months of follow-up after mTORi pharmacotherapy commencement

Patients' characteristics collected from the medical records included gene mutation and sex. We also retrieved information on the age at the first epileptic seizure and whether epilepsy was active the month before mTORi treatment introduction.

The details of mTORi and antiseizure medication (ASM) used during the follow-up were collected.

We identified patients with refractory epilepsy, also known as drug-resistant epilepsy. It was defined according to the International League Against Epilepsy (ILAE) definition, "the failure of adequate trials of two tolerated and appropriately chosen and used antepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom" [28].

Regarding the patient's medical history, the following information from the follow-up visits was collected: the number of seizures per week, sirolimus and everolimus dose, their blood concentration, and ASM used at the time. For each patient, the data was collected from the visit at the beginning of the treatment with mTOR inhibitor, 3 months later, 6 months after the initiation of the treatment, and 1 year and 2 years after starting pharmacotherapy (V0, V3, V6, V12, and V24, respectively).

After data collection, the patients were divided into two groups:

G0 individuals with no history of epileptic seizures prior to mTOR inhibitor initiation
G1 individuals with epileptic seizures prior to mTOR inhibitor initiation

Each patient was then matched 1:1 with the control group. The control group was recruited from a database of patients with TSC from the same two clinical centers but not treated with mTOR inhibitors.

The study was conducted according to the guidelines of the Declaration of Helsinki 1964, and the Bioethics Committee of the Medical University of Warsaw was informed of the study. Ethical approval was waived for this study as a result of the study characteristics. Patient consent was waived because of the retrospective nature of the study, minimal risk, and data anonymization.

Propensity Score Matching

The matching of treated patients with the control group used a propensity score matching (PSM) method based on propensity score (PS), defined as the probability of assigning an individual due to a set of specified variables, to minimize differences between the individuals being compared. Patients were matched on the

basis of the covariates: gene mutation, sex, and age at the onset of pharmacotherapy, ASM, or mTOR inhibitor, depending on which was first.

Patients from the G0 group were matched with individuals treated preventively with vigabatrin. In the G0 group, the age at the onset of pharmacotherapy was set at the initiation of the mTORi treatment, while for their control group it was set as the age at the initiation of vigabatrin. The aim was to compare the effect of preventive treatment with an mTOR inhibitor and vigabatrin.

Participants from the G1 group were matched with patients treated conventionally with ASM after the initiation of seizures. In the G1 group and their control group, the age at the onset of pharmacotherapy was set at the first ASM administration.

Detailed information on the statistical analysis applied in the PSM is available in the supplementary materials.

The V0 visit in the control group for the G0 was set at the age of the first preventive ASM administration. The V0 date in the G1 control group was set at the age of the V0 visit of their matched pair from the G1 group. The data on the seizure frequency was recovered from the control group at the V0 visit and after 3, 6, 12, and 24 months (V3, V6, V12, V24).

Statistical Analysis

The statistical analysis is divided into two parts:

1. Baseline analysis—characteristics of the treated patients; statistical analysis, and graphical analysis of the course of seizures in the group treated with mTOR inhibitors.
2. Comparative analysis—statistical analysis comparing the epilepsy control between the treatment and control groups. It was performed separately for the G0 group and their control, the G1 group and their control, and all treated patients collectively compared with both control groups.

For quantitative variables, minimum and maximum values, mean, standard deviation, median, and interquartile range were assessed. For qualitative variables, the number and frequency of occurrence were described. For numerical variables on consecutive visits, visits were treated as dependent variables. Appropriate statistical tests were used to assess the significance of the impact of individual variables between groups or visits. More detailed information on the statistical tests applied in the analysis is available in the supplementary materials.

Differences were considered statistically significant if $p \leq 0.05$.

The change in seizure frequency between visits was compared to the baseline (V0). The differences were also described in percentages: relative and absolute changes in the number of seizures between the visits.

Categories were assigned for relative change calculations and are described in Table 1. The formula to calculate the change was:

$$\text{Seizure change} = \frac{-(y-x)}{x} \times 100$$

where y is the number of seizures at a consecutive visit; x is the number of seizures at the first visit (V0)

In the G0 group, the patients with stabilization were categorized as “No seizures”, while those who reported epileptic seizures as “Seizures”.

The analysis was conducted with R programming language, version 4.1.1 (2021-08-10).

Table 1 Relative seizure frequency change: category description

Change (%)	Category description
$\geq$ 75.00%	Significant improvement
30.00% to 74.99%	Improvement
0.00% to 29.99%	Stabilization
− 25.00% to − 0.01%	No improvement
< − 25.01%	Deterioration

RESULTS

Study Population

Twenty-one patients born between 2008 and 2022 were included in this study. Nine patients (9/21, 42.86%) started treatment with an mTORi before the first epileptic seizures and were included in the G0 group. Twelve patients began the mTORi treatment after the onset of epilepsy and were included in the G1 group (12/21, 57.14%). Thirteen patients were female (13/21, 61.90%), and eight were male (8/21, 38.10%). All included individuals had a *TSC2* mutation, and three had an additional mutation in the *PKD1* gene.

SEGA was the reason for treatment initiation in 13 patients (13/21, 61.90%), refractory epilepsy in seven (7/21, 33.33%), CR in seven (7/21, 33.33%), and renal AML in one (1/21, 4.76%). Eight patients received mTORi as a result of more than one cause (8/21, 38.10%). Ten children (10/12, 83.33%) had epilepsy refractory to treatment when the mTORi treatment was initiated. One patient from the G0 group (1/9, 11.10%) developed drug-resistant epilepsy during the follow-up.

The median age at the mTORi treatment initiation was 20 days in the G0 group, and the median age at the onset of seizures was 122 days. In the G1 group, the median age at the mTOR inhibitor introduction was 444 days, while epilepsy was first reported at 90 days.

Patients' baseline characteristics are reported in Table 2.

Dosing

Eighteen patients received sirolimus (18/21, 85.71%) and three everolimus (3/21, 14.29%). All patients treated with everolimus were in the G1 group. Patients received mTOR inhibitors orally in the form of a solution.

The initial dosing of sirolimus was 0.5 mg/m^2 per day, while that of everolimus was 5 mg/m^2 per day. During the follow-up, doses were adjusted on the basis of the mTORi blood levels and the severity and frequency of reported adverse effects. During the follow-up, the median blood levels were 3.72 ng/mL (range 1.72–16.10) for sirolimus and 4.96 ng/mL (range 1.9–6.6) for everolimus.

Treatment Discontinuation

Three patients (3/9, 33.33%) in the G0 group completed the 2-year follow-up. The rest, six individuals (6/9, 66.67%), continued the mTORi treatment until the end of data collection, although their follow-up was shorter than 24 months. None of the patients withdrew from the treatment during the follow-up. The median follow-up duration in this group was 183 days.

Seven patients (7/12, 58.33%) in the G1 group completed the 2-year follow-up. Two individuals continued the mTORi treatment until the end of data collection (2/12, 16.67%), yet their follow-up was shorter than 2 years. Three patients with large dysplastic lesions in the brain discontinued pharmacotherapy because of unsatisfactory results (3/12, 25.00%), one after 6 months of treatment and two after 12 months. All qualified for neurosurgery. The median follow-up duration in the G1 group was 732 days.

ASM Modification During Follow-up

During the follow-up, 19 patients (19/21, 90.48%) received antiseizure treatment.

In the G0 group, two patients did not receive any ASM (2/9, 22.22%). Seven were administered vigabatrin during the follow-up because of the onset of seizures or EEG abnormalities; two required additional valproic acid and levetiracetam. The mean number of ASM was 1.

In the G1 group, all patients were treated with vigabatrin and had antiseizure pharmacotherapy modified during the follow-up. All received more than one ASM, including valproic acid, carbamazepine, levetiracetam, clobazam, and topiramate. Two children received adrenocorticotropic hormone (ACTH), and two patients were on the ketogenic diet. The mean number of ASMs was 2.5.

Table 2 Characteristics of the patients in the G0 and G1 groups

Variable	G0 (*N* = 9)	G1 (*N* = 12)	Total (*N* = 21)
Sex			
Female	6 (66.70%)	7 (58.30%)	13 (61.90%)
Male	3 (33.30%)	5 (41.70%)	8 (38.10%)
Family history of TSC			
Yes	0 (0.00%)	1 (11.10%)	1 (7.10%)
No	5 (100.00%)	8 (88.90%)	13 (92.90%)
No information	4	3	7
Gene mutation			
TSC1	0 (0.00%)	0 (0.00%)	0 (0.00%)
TSC2	9 (100.00%)	12 (100.00%)	21 (100.00%)
PKD1	0 (0.00%)	3 (25.00%)	3 (14.29%)
Reason for mTORi treatment			
SEGA	6 (66.70%)	7 (58.30%)	13 (61.90%)
Cardiac rhabdomyoma	4 (44.40%)	3 (25.00%)	7 (33.33%)
Renal AML	0 (0.00%)	1 (8.30%)	1 (4.76%)
Epilepsy	0 (0.00%)	7 (58.30%)	7 (33.33%)
Epileptic seizures during follow-up			
Yes	7 (77.78%)	12 (100.00%)	19 (90.48%)
No	2 (22.22%)	0 (0.00%)	2 (9.52%)
Age at onset of seizures (days)			
Median [min, max]	122.00 [40, 304]	90.00 [1, 145]	93.00 [1, 304]
Mean (SD)	142.43 (86.28)	79.50 (43.16)	102.68 (67.77)
Age at mTORi initiation (days)			
Median [min, max]	20.00 [3, 264]	444.00 [52, 656]	146.00 [3, 656]
Mean (SD)	49.56 (83.35)	413.50 (223.27)	257.52 (253.49)
mTORi			
Sirolimus	9 (100.00%)	9 (75.00%)	18 (85.70%)
Everolimus	0 (0.00%)	3 (25.00%)	3 (14.30%)
Blood concentration (ng/mL), median [min, max]			
Sirolimus	3.72 [1.71, 16.10]	3.59 [1.99, 13.89]	3.72 [1.71, 16.10]
Everolimus	N/A	4.96 [1.9, 6.6]	4.96 [1.9, 6.6]
Follow-up duration (days)			
Median [min, max]	183 [91.50, 732.00]	732 [91.50, 732.00]	549 [91.50, 732.00]

Table 2 continued

Variable	G0 (*N* = 9)	G1 (*N* = 12)	Total (*N* = 21)
Mean (SD)	376.17 (276.19)	571.88 (247.14)	488.00 (271.95)
Refractory epilepsy			
Yes	1 (11.11%)	10 (83.33%)	11 (52.38%)
No	6 (66.67%)	2 (16.67%)	8 (38.10%)
No epileptic seizures	2 (22.22%)	0 (0.00%)	2 (9.52%)

mTORi mTOR inhibitor, *SEGA* subependymal giant cell astrocytoma, *ASM* antiseizure medication, *N/A* not applicable

Table 3 Seizure occurrence in the G0 group, patients who initiated mTOR inhibitor treatment before the onset of seizures

	V3 (*N* = 9)	V6 (*N* = 8)	V12 (*N* = 4)	V24 (*N* = 3)	*p* value
Change					0.456
No seizures	4 (44.4%)	5 (62.5%)	3 (75.0%)	3 (100.0%)	
Seizures	5 (55.6%)	3 (37.5%)	1 (25.0%)	0 (0.0%)	

V0 baseline visit, *V3* visit 3 months after treatment initiation, *V6* visit 6 months after treatment initiation, *V12* visit 12 months after treatment initiation, *V24* visit 24 months after treatment initiation

Adverse Effects of mTORi

All included patients (21/21, 100.00%) reported at least one adverse effect (AE). Adverse events were of mild and moderate severity, grade 1 and 2, according to Common Terminology Criteria for Adverse Events v5.0 [29]. None of the participants discontinued the mTOR inhibitor treatment because of an AE. The interpretation of the blood test results was based on the norms provided by the laboratories after adjusting for age.

The most common adverse effects in patients treated with sirolimus were hyperlipidemia in 16 (16/18, 88.89%), anemia in 11 (11/18, 61.11%), and elevated platelet count in 10 (10/18, 55.56%).

All patients treated with everolimus reported mouth ulcerations (3/3, 100%), hyperlipidemia was found in two (2/3, 66.67%), and anemia in two (2/3, 100%). Mouth ulcerations required temporary treatment discontinuation.

None of the patients reported hemoglobin levels below 8.0 g/dL, nor did they require transfusion. Most patients who reported thrombocytosis reported a platelet count between 450,000 and 700,000/μL, considered "mild"; one patient had a platelet count of 750,000/μL [29].

None of the participants had to be hospitalized because of hyperlipidemia, and it was managed with dietary modifications. Two patients with the highest hyperlipidemia levels were on a ketogenic diet, and the blood test results normalized after the adjustment of the lipid-to-nonlipid ratio.

Effect on Epilepsy Control

G0 Group: Patients with mTORi Introduced Before Onset of Seizures

Nine patients started pharmacotherapy with mTOR inhibitors with no prior history of epilepsy (9/21, 42.86%). Five individuals had their first seizure in the first 3 months after treatment initiation; in three, it was a one-time event, and no other seizures appeared until the end of the

Table 4 Mean seizure frequency per week in the G1 group: patients who started mTOR inhibitor treatment after the onset of seizures

	Number of seizures per week		Visit comparison	*p* value
	Median [min, max]	Mean (SD)		
V0 (*N* = 12)	21.00 [0.25, 140.00]	37.10 (44.48)	V0–V0	N/A
V3 (*N* = 11)	21.00 [0.00, 105.00]	26.45 (33.72)	V3–V0	0.407
V6 (*N* = 10)	7.00 [0.00, 105.00]	24.60 (35.96)	V6–V0	0.683
V12 (*N* = 9)	0.40 [0.00, 98.00]	17.49 (31.76)	V12–V0	0.173
V24 (*N* = 6)	3.00 [0.00, 17.00]	5.00 (6.69)	V24–V0	0.031

V0 baseline visit, *V3* visit 3 months after treatment initiation, *V6* visit 6 months after treatment initiation, *V12* visit 12 months after treatment initiation, *V24* visit 24 months after treatment initiation, *p* value estimated for the differences between the visit and V0, *N/A* not applicable

follow-up. Two patients had their first epileptic seizure between 3 and 6 months after the initiation of the mTORi, one of them as a one-time event. One patient had a follow-up of less than 6 months. At V3, they developed seizures yet did not discontinue the treatment.

The detailed estimated frequency of epileptic seizures per week during the follow-up in this group is presented in supplementary Table S1.

Five patients were seizure-free 6 months after starting the treatment (5/8, 62.50%). At 24 months, all patients who reached that observation time did not have seizures (3/3, 100.00%). No seizures were reported in seven patients in total at the end of their follow-up (7/9, 77.78%) (Table 3).

G1 Group: Patients with mTORi Introduced After Onset of Seizures

Twelve patients reported active epilepsy the month before the initiation of mTOR inhibitor treatment (12/21, 57.14%). The estimated frequency of epileptic seizures per week during the follow-up in this group is presented in Table 4. The statistical significance was calculated for differences between the seizure frequency at the particular visit and the baseline (V0).

The mean number of seizures per week reduced during the follow-up. Improvement in epilepsy control between the baseline visit (V0) and 24 months later (V24) was statistically significant. The number of patients remaining in the study and the standard deviation decreased. The data on seizure frequency at V24 were not available for one patient, although they did not discontinue the treatment for more than 24 months.

Relative increase was used to characterize the treatment outcome in this group (supplementary material Table S2). At 24 months, six patients were followed up; a significant improvement was achieved in five (5/6, 83.33%) and improvement in one (1/6, 16.67%), compared with V0. Four patients were seizure-free at the end of the follow-up. One patient with the shortest follow-up of less than 6 months presented a minimal initial response to the treatment and a decrease in seizure frequency by 25%.

Absolute changes in the number of seizures between each visit and the baseline are presented in supplementary materials Figs. S2–S5.

Propensity Score Matching

A total of 77 and 28 patients were included in the initial database before PSM for the control group for patients from G0 and G1 treatment groups, respectively.

Detailed results of PSM are available in the supplementary materials. A summary of patients' characteristics after matching is presented in Table S3.

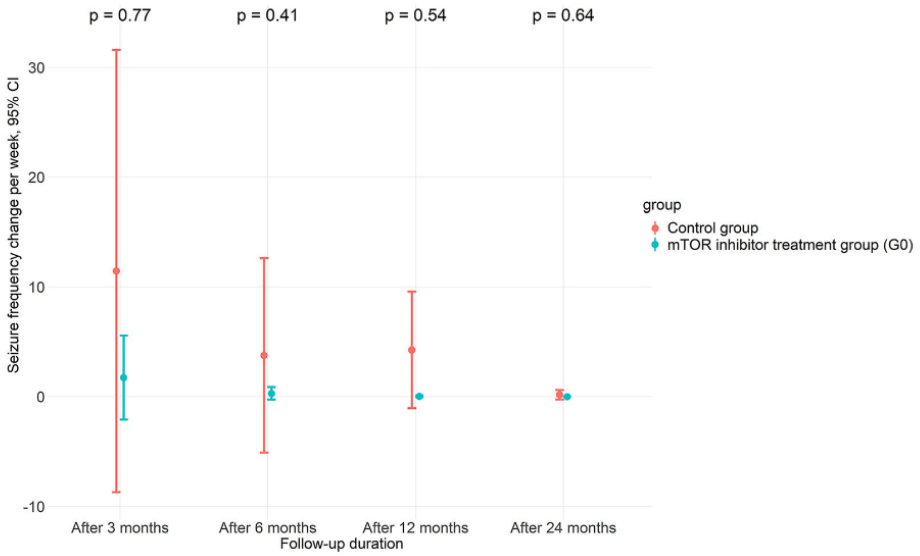

Fig. 1 Seizure frequency change compared to the baseline (V0). Mean change in the number of seizures was calculated for patients from the G0 group, with mTOR inhibitor treatment initiated before the onset of seizures (blue), and their control group (red)

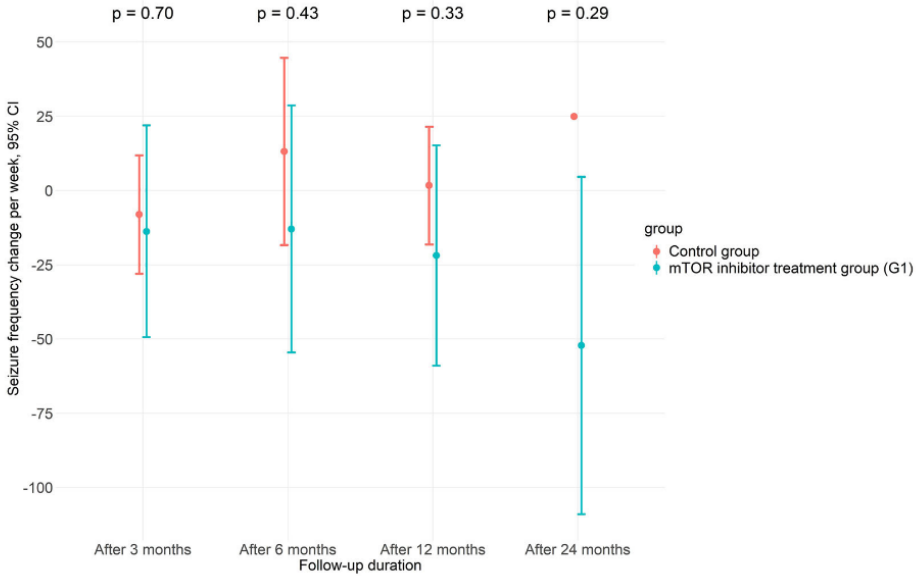

Fig. 2 Seizure frequency change compared to the baseline (V0). Mean change in the number of seizures was calculated for patients from the G1 group, with mTOR inhibitor treatment initiated after the onset of seizures (blue), and their control group (red)

The groups were not completely balanced, yet the matching could be performed. After analysis, nine patients from the control group were matched with the G0 group, and 12 were matched with the G1 group. All patients from the G0 control group were treated preventively with vigabatrin, and their V0 visit was set at the age of the vigabatrin treatment initiation. All patients from the G1 control group had active epilepsy, and their V0 visit was set at the age of the V0 visit of their respective matched pair from the G1 mTORi treatment group.

All patients from the G0 control group received vigabatrin preventively, before the onset of seizures. During the follow-up, one patient required two additional ASMs, levetiracetam and topiramate, and four patients were administered valproic acid because of insufficient seizure control. The mean number of ASMs was 1.67.

Patients from the G1 control group received vigabatrin, valproic acid, levetiracetam, lamotrigine, carbamazepine, and clobazam. The mean number of ASMs was 2.42. One patient underwent surgical removal of cortical dysplasia after the follow-up period, and one was on a ketogenic diet.

Comparative Analysis

Three comparative analyses were performed: the G0 treatment group compared with their control group, the G1 treatment group compared with their control group, and all patients treated with mTOR inhibitors (G0 and G1) compared with the whole control group.

The mean number of seizures increased in both the G0 and their control groups compared to no active epilepsy at the baseline. The increment was more noticeable in the control group, especially 3 months after the initiation of the treatment. At 24 months, both groups reached stabilization (Fig. 1).

In the G1 mTORi treatment group, the reduction in the number of seizures was more noticeable than in the control group. The mTOR inhibitor in the treatment group was administered as an adjunctive treatment to the ASM, while the control group received only ASMs (Fig. 2).

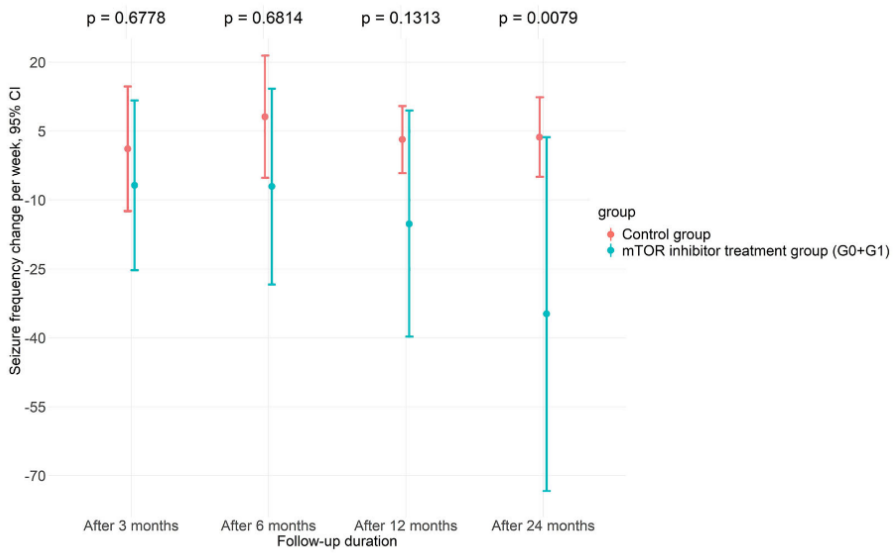

Fig. 3 Seizure frequency change compared to the baseline (V0). Mean change in the number of seizures was calculated for all patients with mTOR inhibitor treatment (G0 and G1, blue) and their control group (red)

The whole mTORi treatment group (G0 and G1) was compared with the control group. The seizure frequency reduction was greater in the treatment than in the control group. The difference was statistically significant at 24 months of follow-up (Fig. 3).

The detailed data on the changes in all three versions is presented in supplementary material Tables S4–S6.

DISCUSSION

Randomized clinical trial EXIST-3 reported that everolimus reduced seizure frequency in patients with TSC and refractory epilepsy [30–32]. Adjunctive treatment with everolimus improved long-term epilepsy control in children under 3 years of age, both in low- and high-exposure groups.

Our study reports the results of the largest research so far, assessing the effect of two mTOR inhibitors, sirolimus and everolimus, in epilepsy treatment in children under 2 years of age.

Various animal models described an antiepileptogenic effect of preventive treatment with mTOR inhibitors on epilepsy development [25, 33, 34]. We collected data from nine patients with no history of seizures prior to sirolimus initiation, the G0 group. In most of them, seizures appeared during follow-up as one-time events, and the patients did not develop active epilepsy. The appearance of seizures could overlap with the typical age at the onset of epilepsy in patients with TSC, as more than 60% of them experience the first epileptic seizure in the first year of life [35]. The study by Domańska-Pakieła et al. reported that 70% of patients with normal EEG in the first 2 months of life develop EEG abnormalities in the next few months [36].

The G1 treatment group, to whom the mTOR inhibitors were administered after the onset of seizures, reported a decrease in seizure frequency in all visits compared with the baseline. The difference was statistically significant at 24 months from the mTORi initiation. The positive impact of everolimus on seizure reduction in similarly young patients with TSC under 2 years of age was reported in a smaller retrospective study by Saffari et al. [9]. Everolimus was effective as adjunctive therapy in two out of four patients with refractory epilepsy. Kotulska et al. reported improved seizure control in three out of five patients with drug-resistant epilepsy who received everolimus as a result of SEGA [27].

Most patients in this study received sirolimus, a more common and available pharmacotherapy than everolimus, although according to the literature, its efficacy in epilepsy control remains inconclusive [37–39]. Research studies on mTOR inhibitors often set the target blood levels of everolimus and sirolimus at 5–15 mg/mL and 4–20 ng/mL, respectively [10]. In our study, the median serum levels of both medications were at the lower threshold. The antiseizure effect could probably be more significant if the blood levels were greater. Similar observations were described in the EXIST-3 reports, where the group with lower exposure to everolimus (3–7 ng/mL) had less significant improvement in epilepsy control than the high-exposure group (9–15 ng/mL) [40].

Once seizures develop, vigabatrin is a treatment of choice in infantile spasms and refractory focal-onset seizures in Europe and the USA [23]. Other conventional ASMs are considered second-line therapy, and in our study, ASMs and their dosing were modified independently from the mTOR inhibitor treatment during the follow-up. The modifications, such as increasing the dose or changing to another ASM were decided on the basis of epilepsy control. It may have impacted the significance of the seizure frequency reduction and led to an overestimation of the role of mTOR inhibitors.

However, according to the comparative analysis, seizure control improved in the group treated additionally with mTOR inhibitors. This study aimed to determine the effect of mTORi added to currently recommended pharmacotherapy. The mean number of ASM and the type of ASMs used in the treatment and control groups were similar. All patients were treated in the two clinical centers, so we assume that the antiseizure pharmacotherapy approach was similar. Therefore we may assess the positive

mTORi effect on seizure control as an added disease-modifying treatment.

In all patients included in our study, *TSC2* gene mutation, most commonly associated with worse clinical outcomes and a higher prevalence of refractoriness, was found [26, 41]. Dabora et al., in a study on 224 patients, found that seizures are significantly more common in patients with *TSC2* than *TSC1* mutation (98% vs. 86%, $p = 0.02$) [42].

Early administration of mTORi could delay the onset of seizures and reduce their severity, as suggested by the results of our study. The risk of refractoriness correlates with the age at the first epileptic seizure [26]. Intellectual impairment, learning difficulties, and disturbances in social development also appear to be related to earlier epilepsy onset [43]. Therefore, it is possible that delaying the onset of epilepsy may contribute to overall better control in the future.

Those findings are important to patients with *TSC2* mutation, as epilepsy is more likely to become refractory to treatment if seizures appear early, in the neonatal period, often in individuals with perinatal complications and SEGA [44]. Most patients in our study were administered mTOR inhibitors as a result of SEGA, CR, and epilepsy. According to Krueger et al. and Saffari et al., those are the most common reasons for mTORi treatment introduction [6, 9]. In the last few years, clinical trials have determined a positive effect of mTORi on SEGA size reduction, and currently everolimus is recommended for use in this indication. Therefore, owing to the established role of mTORi in SEGA size change, it was not included as an outcome parameter in this study. mTORi discontinuation may lead to SEGA recurrence, especially if the treatment duration is relatively short [45]. Therefore, most patients who begin the treatment continue it for many years. Epilepsy control after sirolimus and everolimus discontinuation has not been determined yet. Most patients in this study continued the treatment at the end of data collection, so seizure recurrence assessment in a long-term follow-up after mTORi cessation was impossible. The long-term effect of mTORi on epilepsy control after their discontinuation should be addressed in future studies.

Safety is one of the main concerns regarding mTOR inhibitor administration to young children and infants. All participants in our study reported adverse effects, yet of mild or moderate severity. Likewise, the results from EXIST-1 and EXIST-3 clinical trials demonstrated everolimus safety in children under 3 years of age [46, 47]. The adverse effects were more common and significant in the high-dose group, although they remained relatively safe and reversible. Various reports also described sirolimus safety, mostly in older children and adults with TSC [48–50]. According to the studies, sirolimus adverse effects are also frequent yet not severe [9, 51]. Both sirolimus and everolimus appear to be safe in young children as well as adults with TSC.

The main limitation of this study is its retrospective character, which may hinder the objectivity and availability of all data. The anamnesis technique and accuracy differed among the physicians. The seizure types and epileptiform discharge types could not be determined for some patients in the treatment and control groups because of missing data in the medical records. PSM aims to assemble two groups with similar characteristics with equal mean baseline data. As the groups are analyzed as a whole, not individually, the analysis was mainly balanced despite the lack of those parameters.

Secondly, the small cohort size, divided into smaller subgroups, could impede the statistical significance of the analysis. One participant from each group, G0, and G1, had a follow-up shorter than half a year, yet they did not withdraw from the treatment until the end of data collection. All information on the effect of mTORi in epilepsy control was considered relevant as few patients start mTORi treatment at such a young age. Therefore incomplete follow-up data were not excluded.

During the follow-up, the ASMs were adjusted according to the epilepsy control and thus could impact the significance of mTOR inhibitors in seizure reduction.

Despite these limitations, our study assesses the effect of mTOR inhibitor treatment on seizure control in infants and young children.

CONCLUSIONS

mTOR inhibitors are a novel promising treatment for TSC-related symptoms and conditions, including epilepsy. Young children and infants could benefit from sirolimus and everolimus use, which leads to a suppression of epileptic activity and a lower risk of refractoriness.

Adjunctive treatment with mTOR inhibitors appears to improve seizure control when administered preventively or after the onset of seizures. Further prospective clinical studies on the efficacy of mTOR inhibitors on epilepsy in young children with TSC should be performed.

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Compliance with Ethics Guidelines. The study was conducted according to the guidelines of the Declaration of Helsinki 1964, and the Bioethics Committee of the Medical University of Warsaw was informed of the study. Ethical approval was waived for this study as a result of the study characteristics. Patient consent was waived because of the retrospective nature of the study, minimal risk, and data anonymization.

Data Availability. The data sets generated during and/or analyzed during the current study are included as supplementary information files or are available from the corresponding author on reasonable request.

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“Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under 2 Years of Age” - materiały uzupełniające

Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under Two Years of Age

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STATISTICAL ANALYSIS

Appropriate statistical tests were used to assess the significance of the impact of individual variables between arms: Kruskal-Wallis rank sum test, Fisher's Exact test, Pearson's Chi-squared test, paired Wilcoxon test.

To compare the number of seizures between visits, the tests for normality of distribution (Shapiro-Wilk test) and homogeneity of variance (Levene test) were tested first. If the assumptions were met, the paired t-test was used for comparison. Otherwise, the Wilcoxon signed-rank test was used.

The non-parametric Wilcoxon test for independent trials was used to compare the changes in the number of seizures in relation to the baseline visit (V0) between the control and mTOR inhibitor-treated groups. The results of the Wilcoxon test are presented in the comparative analysis section.

For all analysis type I error was assumed to be 5%. Differences between the groups were considered statistically significant if $p \leq 0.05$. If there were too few observations in the analysis, the p-value was not provided.

RESULTS

The G0 Group - mTOR Inhibitors Introduced Before the Onset of Seizures

The statistical significance was estimated for differences between the seizure frequency at the particular visit and the baseline (V0). Therefore p-value was not calculated for V0.

Table S1 Mean seizure frequency per week in the G0 group - patients who started mTOR inhibitor treatment before the onset of seizures.

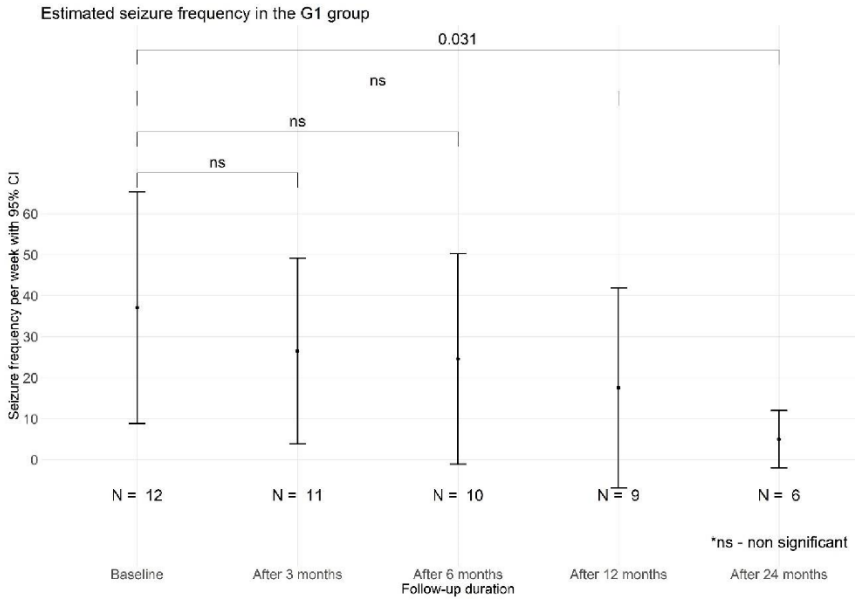
	Number of seizures per week			
	Median [min, max]	**Mean (SD)**	**Visit comparison**	***p* value**
V0 (*N* = 9)	0.00 [0.00, 0.00]	0.00 (0.00)	V0-V0	N/A
V3 (*N* = 9)	0.14 [0.00, 15.00]	1.73 (4.98)	V3-V0	0.048
V6 (*N* = 8)	0.00 [0.00, 2.00]	0.30 (0.69)	V6-V0	0.181
V12 (*N* = 4)	0.00 [0.00, 0.10]	0.02 (0.05)	V12-V0	1.000
V24 (*N* = 3)	0.00 [0.00, 0.00]	0.00 (0.00)	V24-V0	1.000

V0 baseline visit, *V3* visit 3 months after treatment initiation, *V6* visit 6 months after treatment initiation, *V12* visit 12 months after treatment initiation, *V24* visit 24 months after treatment initiation, *p* value estimated for the differences between the visit and V0, *N/A* not applicable

The G1 Group – mTOR Inhibitors Introduced After the Onset of Seizures

Figure S1 is a visual representation of the data from Table 3 from the Article.

Fig. S1 Estimated frequency of epileptic seizures per week during the follow-up in the G1 group, patients treated with mTOR inhibitor after the onset of seizures.



The categories for relative change are assigned based on the Table 1 from the Article.

Table S2 Seizure frequency relative increase compared to the baseline (V0) in the G1 group, patients who initiated mTOR inhibitor treatment after the onset of seizures.

	V3 (*N* = 11)	**V6 (*N* = 10)**	**V12 (*N* = 9)**	**V24 (*N* = 6)**	***p* value**
Change					0.401
Significant improvement	4 (36.4%)	4 (40.0%)	4 (44.4%)	5 (83.3%)	
Improvement	1 (9.1%)	2 (20.0%)	2 (22.2%)	1 (16.7%)	
Stabilization	3 (27.3%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	
Deterioration	3 (27.3%)	4 (40.0%)	2 (22.2%)	0 (0.0%)	

V0 baseline visit, *V3* visit 3 months after treatment initiation, *V6* visit 6 months after treatment initiation, *V12* visit 12 months after treatment initiation, *V24* visit 24 months after treatment initiation

Absolute changes in the number of seizures between each visit and the baseline are presented in Figures S1-S4. Improvement in epilepsy control is reported as seizure frequency reduction, a negative change, while the numbers above 0 describe the deterioration in seizure control.

Fig. S2 Absolute seizure frequency change in the G1 group, V3 compared to the baseline

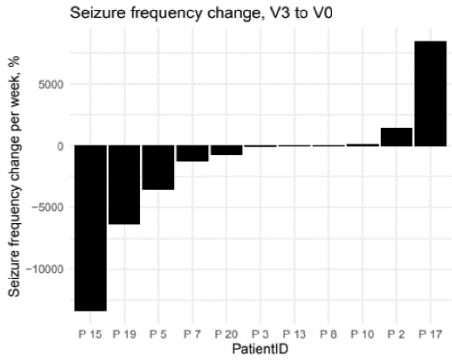


Fig. S3 Absolute seizure frequency change in the G1 group, V6 compared to the baseline

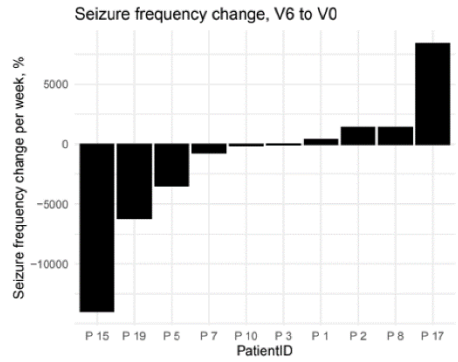


Fig. S4 Absolute seizure frequency change in the G1 group, V12 compared to the baseline

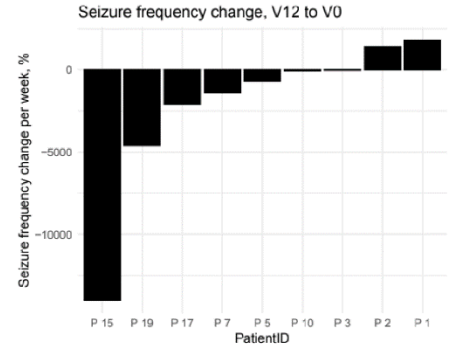
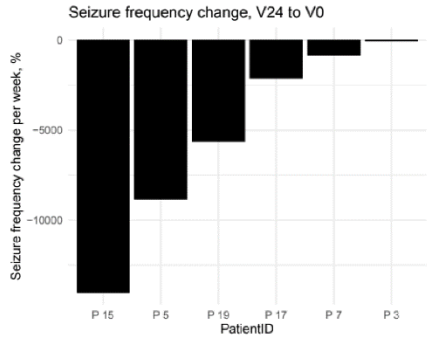


Fig. S5 Absolute seizure frequency change in the G1 group, V24 compared to the baseline



PROPENSITY SCORE MATCHING

Logistic regression was used to match patients by propensity score matching (PSM). The rating was based on the propensity index. Patients were matched in a 1: 1 ratio using the k-nearest neighbors method based on selected covariates. Cook's distance was calculated to identify outliers. Standardized mean difference and variance ratio were assessed to balance the probability. Appropriate descriptive statistics were used for the evaluation. For quantitative variables, mean, median, standard deviation, and interquartile range were assessed. For qualitative variables, number and frequency were used.

Fisher's exact test was used to evaluate categorical variables. For continuous variables the non-parametric test of the appropriate analysis of variance - the Kruskal-Wallis test was applied. Differences were considered statistically significant if $p \leq 0.05$.

PSM Results

The standardized mean difference test was not met for all variables, yet the variance ratio was calculated. Although the groups were not completely balanced, the matching could be performed.

PSM for the G0 Group, Treated with mTOR Inhibitors Before the Onset of Seizures

The threshold for outliers was set at 0.0465116, which means that observation "7" affected the model significantly. Observation "7" was a patient from the treatment group, which could not be removed.

Fig. S6 Cook's distance for the G0 group and their control

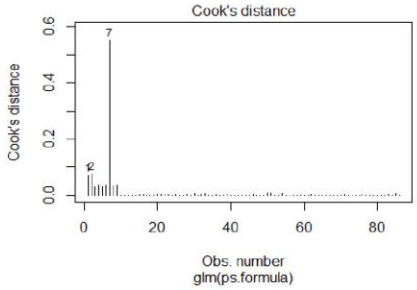


Fig. S7 Standardized mean difference plot in the G0 group and their control group

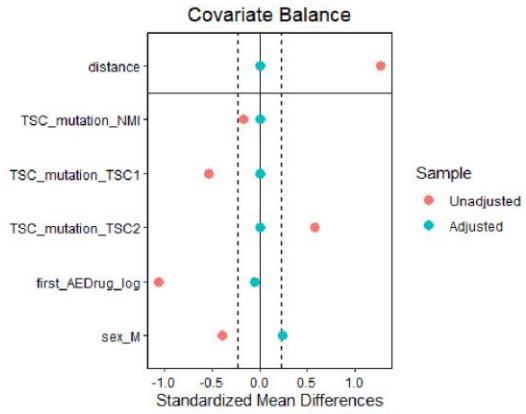
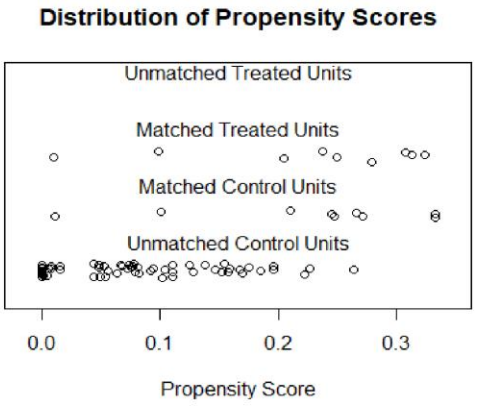


Fig. S8 Distribution of propensity scores for matched and unmatched individuals in the G0 group and their respective control



In both groups, the assumptions about the normality of distributions were not met. The differences between the treatment and the control groups were not statistically significant.

PSM for the G1 Group, Treated with mTOR Inhibitors After the Onset of Seizures

The threshold for outliers was set at 0.097561, which means that observation "3" and "29" affected the model significantly. Observation "3" was a patient from the treatment group, which we could not remove; observation "29" was a control patient, then removed from the analysis.

Fig. S9 Cook's distance before outliers' removal in the G1 group and their control group

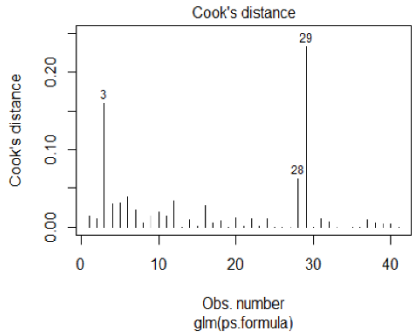


Fig. S10 Cook's distance after outliers' removal in the G1 group and their control group

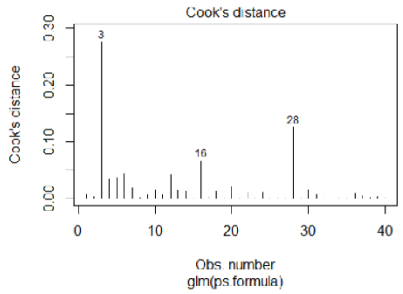


Fig. S11 Standardized mean difference plot in the G1 group and their control group

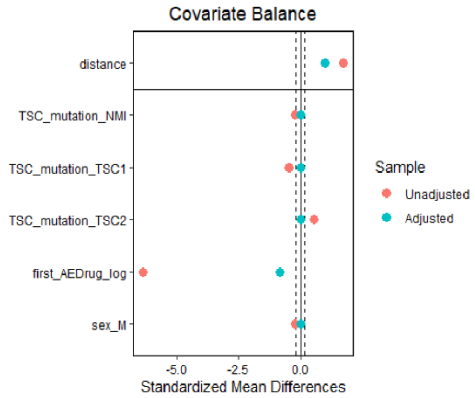


Fig. S12 Distribution of propensity scores for matched and unmatched individuals in the G1 group and their control group

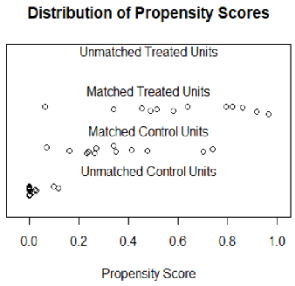


Table S3 Summary of data after propensity score matching

Variables	mTORi treatment before the onset of seizures			mTORi treatment after the onset of seizures			Total		
	G0, treatment (*N* = 9)	G0, control (*N* = 9)	*p* value	G1, treatment (*N* = 12)	G1, control (*N* = 12)	*p* value	G0+G1, treatment (*N* = 21)	G0+G1, control (*N* = 21)	*p* value
TSC mutation			1.000			1.000			1.000
TSC2	9 (100.00%)	9 (100.00%)		12 (100.00%)	12 (100.00%)		21 (100.00%)	21 (100.00%)	
Age at the mTORi initiation (days)			0.825			0.010			0.148
Mean (SD)	49.56 (83.35)	54.22 (83.58)		78.83 (44.96)	117.33 (28.05)		66.29 (64.12)	90.29 (65.20)	
Median	20.00	33.00		86.50	120.50		56.00	109.00	
[min, max]	[3.00, 264.00]	[0.00, 255.00]		[5.00, 175.00]	[65.00, 168.00]		[3.00, 264.00]	[0.00, 255.00]	
Sex			0.599			1.000			0.747
Female	6 (66.67%)	7 (77.78%)		7 (58.33%)	7 (58.33%)		13 (61.90%)	14 (66.67%)	
Male	3 (33.33%)	2 (22.22%)		5 (41.67%)	4 (41.67%)		8 (38.10%)	7 (33.33%)	

mTORi mTOR inhibitor

COMPARATIVE ANALYSIS

Due to the differences in the sample size in the compared groups, the non-parametric Wilcoxon test, which bases on the median values, was used to assess the significance of changes in the number of seizures between the groups.

Table S4 The seizure frequency per week and number of patients in each follow-up visit in the G0 treatment group and their control.

Visit comparison	G0 treatment group		G0 control group		*p* value
	Mean seizure frequency change (SD)	*N*	Mean seizure frequency change (SD)	*N*	
V3 to V0	1.73 (4.98)	9	11.4 (26.2)	9	0.77
V6 to V0	0.30 (0.69)	8	3.8 (10.6)	8	0.41
V12 to V0	0.03 (0.05)	4	4.2 (6.4)	8	0.54
V24 to V0	0.00 (0.00)	3	0.2 (0.4)	6	0.64

Table S5 The seizure frequency per week and number of patients in each follow-up visit in the G1 treatment group and their control.

Visit comparison	G1 treatment group		G1 control group		*p* value
	Mean seizure frequency change (SD)	*N*	Mean seizure frequency change (SD)	*N*	
V3 to V0	-14.00 (53.00)	11	-8.00 (28.00)	10	0.70
V6 to V0	-13.00 (58.00)	10	13.00 (34.00)	7	0.43
V12 to V0	-22.00 (48.00)	9	2.00 (19.00)	6	0.33
V24 to V0	-52.00 (54.00)	6	25.00 (N/A)	1	0.29

Table S6 The seizure frequency per week and number of patients in each follow-up visit in the G0 and G1 treatment group and their control.

Visit comparison	mTORi treatment group		Control group		*p* value
	Mean seizure frequency change (SD)	*N*	Mean seizure frequency change (SD)	*N*	
V3 to V0	-7.00 (39.00)	20	1.00 (28.00)	19	0.6778
V6 to V0	-7.00 (43.00)	18	8.00 (24.00)	15	0.6814
V12 to V0	-15.00 (41.00)	13	3.00 (13.00)	14	0.1313
V24 to V0	-35.00 (50.00)	9	4.00 (9.00)	7	0.0079

7. Podsumowanie i wnioski

Przegląd systematyczny piśmiennictwa na temat czynników ryzyka wystąpienia DRE u pacjentów z TSC posłużył do przygotowania pracy przeglądowej. Wstępnie włączono 1109 publikacji, z których ostatecznie przeanalizowano 19 artykułów naukowych. W artykule podsumowano parametry zwiększające ryzyko lekooporności. Publikacje przeważnie opisywały mutację w genie *TSC2*, obecność napadów zgięciowych oraz dużą liczbę guzków korowych. Zaobserwowano, że wczesny wiek wystąpienia napadów również predysponuje do gorszej kontroli padaczki. Na podstawie analizy publikacji wykazano, że większość czynników ryzyka jest niemodyfikowalna, jednak regularne monitorowanie EEG i odpowiednia edukacja opiekunów obniżają ryzyko DRE. Publikacja **„Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review”** podsumowuje obecny stan wiedzy na temat parametrów zwiększających ryzyko lekooporności u pacjentów z TSC, uwzględniając najnowsze wyniki badań. Praca ta jest najbardziej aktualnym przeglądem piśmiennictwa o tej tematyce.

Obie prace oryginalne powstały na podstawie danych medycznych pacjentów z dwóch ośrodków klinicznych. Łącznie do bazy danych włączono 529 pacjentów z potwierdzoną diagnozą TSC lub jej podejrzeniem. Pacjenci z Oddziału Neurologii i Epileptologii Instytutu „Pomnik-Centrum Zdrowia Dziecka” stanowili znaczącą większość, łącznie 458 osób. W Klinice Neurologii Dziecięcej Warszawskiego Uniwersytetu Medycznego leczonych było 71 pacjentów włączonych do bazy.

Po wstępnej analizie bazy danych oraz literatury na temat DRE u pacjentów z TSC, uszczegółowiono kryteria włączenia do badania. Z uwagi na pozytywne doniesienia z ostatnich lat dotyczące roli ewerolimusu w leczeniu DRE, zdecydowano się ocenić skuteczność dwóch mTORi – sirolimusu i ewerolimusu – oraz objawy uboczne związane z ich stosowaniem u najmłodszych dzieci z TSC [47,55,61]. Znacząca część pacjentów z obu ośrodków otrzymywała sirolimus ze względu na jego większą dostępność oraz mniej restrykcyjne kryteria refundacji, a tym samym niższe koszty leczenia. Ze względu na znaczenie wczesnego leczenia padaczki i jej prewencji u najmłodszych pacjentów, zdecydowano się włączyć do analiz wyłącznie pacjentów, u których mTORi zastosowano przed ukończeniem drugiego roku życia. Działanie ewerolimusu u pacjentów w tej grupie wiekowej jak dotąd omawiane było w formie opisów przypadków, jednak nie przeprowadzono większych badań randomizowanych. Wiedza na temat wpływu sirolimusu na

przebieg padaczki oraz AE związane z jego stosowaniem również jest ograniczona. Postanowiono zatem ocenić również działania niepożądane sirolimusu występujące u pacjentów, u których lek ten jest włączany w pierwszych latach życia.

Po uwzględnieniu powyższych kryteriów, ostatecznie do analiz włączono dwadzieścioro czworo pacjentów. Troje otrzymało ewerolimus (3/24, 12,5%), a dwadzieścioro jeden sirolimus (21/24, 87,5%). Wykazano, że u wszystkich pacjentów leczonych sirolimusem wystąpiły AE, najczęściej: anemia, zaburzenia gospodarki lipidowej oraz nadpłytkowość, obecne u ponad połowy pacjentów we wszystkich grupach wiekowych. Pacjenci z najwyższymi stężeniami lipidów we krwi stosowali jednocześnie dietę ketogenną, a modyfikacja żywienia pozwoliła na poprawę tych parametrów. Analogicznie do wniosków z innych badań omawiających skutki uboczne mTORi, zaobserwowane AE były o niewielkim nasileniu, stopnia 1 oraz stopnia 2 według powszechnych kryteriów terminologii dla zdarzeń niepożądanych (ang. *Common Terminology Criteria for Adverse Events,* CTCAE) [37,56,62]. Pacjenci nie wymagali istotnych interwencji, wdrażania dodatkowego leczenia ani hospitalizacji. U niektórych konieczne było kilkudniowe przerwanie leczenia mTORi.

Zgodnie z literaturą, afty oraz infekcje są jednymi z najczęstszych AE występujących u pacjentów stosujących mTORi [56,58,63]. W niniejszym badaniu były one jednak zgłaszane stosunkowo rzadko. Jeden pacjent był hospitalizowany w przebiegu infekcji górnych dróg oddechowych w celu obserwacji ewentualnych powikłań i zaostrzeń związanych z jednoczesnym stosowaniem mTORi. Pacjent nie wymagał interwencji podczas pobytu w szpitalu. W publikacji zatytułowanej **„Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age - A Bicenter Retrospective Study”** wykazano, że działania niepożądane związane z przyjmowaniem sirolimusu są częste, jednak nie stanowią istotnego zagrożenia zdrowia i życia u dzieci z TSC poniżej drugiego roku życia.

Celem drugiej części analiz była ocena skuteczności sirolimusu oraz ewerolimusu w leczeniu padaczki u dzieci z TSC. Wyniki zostały przedstawione w publikacji o tytule **„Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Comples Under 2 Years of Age”**. Pacjentów włączonych do badania podzielono na dwie grupy: dziewięcioro leczonych prewencyjnie (9/21, 42,86%), u których mTORi zostały włączone przed pierwszymi napadami oraz dwanaścioro (12/21, 57,14%) z napadami padaczkowymi w miesiącu poprzedzającym zastosowanie mTORi. Troje pacjentów

leczonych sirolimusem nie było analizowanych w tej części badania, z uwagi na ostatni napad padaczkowy zarejestrowany ponad miesiąc przed rozpoczęciem leczenia. Pacjenci otrzymywali leczenie przeciwpadaczkowe niezależnie od mTORi, a ASM były modyfikowane w zależności od kontroli napadów padaczkowych.

Wykazano, że u niemal 80% dzieci, które otrzymały mTORi przed wystąpieniem napadów padaczkowych, napady zostały zaobserwowane w kolejnych miesiącach. Były to jednak napady o niewielkim nasileniu, a większość z nich wystąpiła jednorazowo. Znaczna część pacjentów leczonych prewencyjnie (7/9, 77,78%) nie prezentowała napadów padaczkowych w momencie zakończenia obserwacji. Powyższe analizy jako pierwsze przedstawiają wpływ prewencyjnego włączenia mTORi na rozwój padaczki oraz jej dalszą kontrolę u pacjentów z TSC.

W grupie pacjentów, u których występowały napady padaczkowe w miesiącu poprzedzającym włączenie mTORi, zmniejszenie średniej liczby napadów było istotne statystycznie ($p = 0,031$). Podczas włączania leczenia, u większości pacjentów (10/12, 83,33%) padaczka była lekooporna. Wyniki analiz sugerują, że dołączenie mTORi, zarówno sirolimusu jak i ewerolimusu, poprawia kontrolę padaczki, a w szczególności DRE, u najmłodszych dzieci z TSC.

Do analizy porównawczej włączono grupę kontrolną pacjentów dobranych przy użyciu techniki *propensity score matching* na podstawie zmiennych wpływających na ryzyko wystąpienia padaczki, nasilenie napadów oraz rozwój lekooporności. Metoda ta umożliwiła dobór grupy kontrolnej podobnej do naszej grupy badanej tak, aby były one możliwie zrównoważone pod względem wybranych zmiennych [64]. Spadek średniej liczby napadów w grupie kontrolnej był mniejszy niż w grupach leczonych mTORi. Wykazano, że przy porównaniu wszystkich pacjentów leczonych mTORi z całą grupą kontrolą, różnica w zmianie częstości napadów w 24 miesiącu obserwacji była istotna statystycznie ($p = 0,0079$). Włączenie mTORi przed ukończeniem drugiego roku życia niezależnie od stosowanego leczenia przeciwpadaczkowego wydaje się poprawiać kontrolę padaczki u dzieci z TSC.

Wysokie ryzyko DRE, a w rezultacie zaburzenia rozwoju intelektualnego oraz emocjonalno-społecznego, stwierdzane są u pacjentów, u których napady pojawiły się w pierwszych miesiącach życia. Opóźnienie wystąpienia padaczki, zmniejszenie częstotliwości i nasilenia napadów może poprawić długoterminową kontrolę napadów oraz rozwój dzieci.

Stąd też niezwykle ważna jest ocena bezpieczeństwa oraz skuteczności nowych leków, mTORi, właśnie w tej grupie pacjentów.

Przedstawione w publikacjach będących przedmiotem niniejszej rozprawy wyniki badań są pierwszymi omawiającymi skuteczność i bezpieczeństwo mTORi na tak licznej grupie pacjentów z TSC, u których leczenie włączono przed ukończeniem drugiego roku życia.

7.1. Wnioski

Na podstawie przeprowadzonych analiz można postawić następujące wnioski:

1. Istnieje wiele czynników ryzyka wystąpienia DRE u pacjentów z TSC. Wśród nich najbardziej istotne to: mutacja w genie *TSC2*, obecność napadów zgięciowych, duża liczba guzków korowych, oraz wczesny wiek wystąpienia napadów.
2. Większość parametrów predysponujących do wystąpienia DRE jest niemodyfikowalna, jednak przy zastosowaniu odpowiedniej diagnostyki i prewencji można zmniejszyć ryzyko lekooporności.
3. Stosowanie sirolimusu u dzieci z TSC przed ukończeniem drugiego roku życia wiąże się z częstym występowaniem działań niepożądanych o niewielkim lub średnim nasileniu, w szczególności: anemii, zaburzeń gospodarki lipidowej oraz nadpłytkowości. AE nie zagrażają życiu i zdrowiu pacjentów, ani nie skutkują koniecznością istotnych interwencji.
4. Pomimo licznych doniesień z literatury na temat zwiększonej częstości aft oraz infekcji o dużym nasileniu u dzieci leczonych mTORi, nie wydają się one istotnym AE w analizowanej grupie pacjentów.
5. Włączenie inhibitorów mTOR, sirolimus oraz ewerolimusu, u pacjentów z TSC przed ukończeniem drugiego roku życia, poprawia kontrolę napadów padaczkowych.
6. Prospektywne badania kliniczne powinny zostać przeprowadzone w celu potwierdzenia wniosków z niniejszych badań.

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9. Opinia Komisji Bioetycznej

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Warszawa, dnia 06.03 2023

AKBE/ 49 / 2023

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OŚWIADCZENIE

Niniejszym oświadczam, że Komisja Bioetyczna przy Warszawskim Uniwersytecie Medycznym w dniu 06 marca 2023 r. przyjęła do wiadomości informację na temat badania pt. "Ocena czynników prognostycznych oraz nowoczesne terapie padaczki lekoopornej u chorych ze stwardnieniem guzowatym." Przedstawione badanie nie stanowi eksperymentu medycznego w rozumieniu art. 21ust.1 ustawy z dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (Dz.U. z 2018 r poz. 617) i nie wymaga uzyskania opinii Komisji Bioetycznej przy Warszawskim Uniwersytecie Medycznym, o której mowa w art. 29 ust.1 ww. ustawy.

Przewodnicząca Komisji Bioetycznej

Prof. dr hab. n. med. Magdalena Kuźma –Kozakiewicz

10. Oświadczenia współautorów prac

Warszawa, 5.04.2023
(miejscowość, data)

prof. dr hab. n. med. Sergiusz Jóźwiak
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
nadzór merytoryczny, krytyczna korekta manuskryptu, akceptacja wersji ostatecznej publikacji.
Mój udział procentowy w przygotowaniu publikacji określam jako 10%.
Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 75%,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji i planu badania, przegląd literatury, analizę i interpretację danych, opracowanie wyników, przygotowanie manuskryptu oraz jego edycję.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*
Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.
(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

5.04.2023
(miejscowość, data)

prof. dr hab. n. med. Sergiusz Jóźwiak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie koncepcji i projektu badania, nadzór merytoryczny, krytyczna korekta manuskryptu, akceptacja wersji ostatecznej publikacji.

Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 80%,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji i metodologii badania, analizę historii chorób i dokumentacji medycznej pacjentów, stworzenie bazy danych pacjentów, analizę i interpretację danych, opracowanie wyników, napisanie manuskryptu oraz jego edycję.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.
(imię i nazwisko kandydata do stopnia)

S. Jóźwiak
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

5.04.2023
(miejscowość, data)

prof. dr hab. n. med. Sergiusz Jóźwiak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under Two Years of Age" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie koncepcji i projektu badania, nadzór merytoryczny, krytyczna korekta manuskryptu, akceptacja wersji ostatecznej publikacji.

Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji i metodologii badania, analizę historii chorób i dokumentacji medycznej pacjentów, stworzenie bazy danych pacjentów, analizę i interpretację danych, opracowanie wyników, napisanie manuskryptu oraz jego edycję.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.
(imię i nazwisko kandydata do stopnia)

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, 05.04.2023
(miejscowość, data)

prof. dr hab. n. med. Katarzyna Kotulska-Jóźwiak
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Safety of Sirolimus in Patients with Tuberous Sclerosis Complex under Two Years of Age-A Bicenter Retrospective Study" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie koncepcji badania, nadzór merytoryczny, akceptacja wersji ostatecznej publikacji.

Mój udział procentowy w przygotowaniu publikacji określam jako 10%.

Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 80%,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji i planu badania, metodykę, zebranie historii chorób i dokumentacji medycznej pacjentów, stworzenie bazy danych pacjentów, analizę i interpretację danych, opracowanie wyników, pisanie manuskryptu oraz jego edycję.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.

(imię i nazwisko kandydata do stopnia)

.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

05.04.2023
(miejscowość, data)

prof. dr hab. n. med. Katarzyna Kotulska-Jóźwiak
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under Two Years of Age" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

opracowanie koncepcji badania, nadzór merytoryczny, akceptacja wersji ostatecznej publikacji.

Mój udział procentowy w przygotowaniu publikacji określam jako 5%.

Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 70%,

(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji i metodologii badania, analizę historii chorób i dokumentacji medycznej pacjentów, stworzenie bazy danych pacjentów, analizę i interpretację danych, opracowanie wyników, napisanie manuskryptu oraz jego edycję.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.

(imię i nazwisko kandydata do stopnia)

Prof. dr hab. n. med. Katarzyna Kotulska-Jóźwiak specjalista neurologii pediatrycznej

(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

.....
(miejscowość, data)

Monika Sugalska
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt.: „Risk Factors Associated with Refractory Epilepsy in Patients with Tuberous Sclerosis Complex: A Systematic Review" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:

współuczestnictwo w przygotowaniu metodyki oraz przeglądzie literatury, interpretacji danych oraz edycja manuskryptu artykułu.

Mój udział procentowy w przygotowaniu publikacji określam jako 15%.

Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 75%,
(imię i nazwisko kandydata do stopnia)

obejmował on: opracowanie koncepcji i planu badania, przegląd literatury, analizę i interpretację danych, opracowanie wyników, przygotowanie manuskryptu artykułu oraz jego edycję.

(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.
(imię i nazwisko kandydata do stopnia)

Monika Sugalska
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników

Warszawa, dn. 11.04.2023 r.
(miejscowość, data)

Aleksandra Duda
.....
(imię i nazwisko)

OŚWIADCZENIE

Jako współautor pracy pt. „Effect of mTOR Inhibitors in Epilepsy Treatment in Children with Tuberous Sclerosis Complex Under Two Years of Age" oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji stanowi:
opracowanie metodologii badania, przeprowadzenie analiz statystycznych, oraz opracowanie wyników
Mój udział procentowy w przygotowaniu publikacji określam jako 15%.
Wkład Dominiki Śmiałek w powstawanie publikacji określam jako 70%,
(imię i nazwisko kandydata do stopnia)
obejmował on: opracowanie koncepcji i metodologii badania, analizę historii chorób i dokumentacji medycznej pacjentów, stworzenie bazy danych pacjentów, analizę i interpretację danych, opracowanie wyników, napisanie manuskryptu oraz jego edycję.
(merytoryczny opis wkładu kandydata do stopnia w powstanie publikacji)*

Jednocześnie wyrażam zgodę na wykorzystanie w/w pracy jako część rozprawy doktorskiej lek. Dominiki Śmiałek.
(imię i nazwisko kandydata do stopnia)

Duda Aleksandra
.....
(podpis oświadczającego)

*w szczególności udziału w przygotowaniu koncepcji, metodyki, wykonaniu badań, interpretacji wyników