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# „Ocena skuteczności leczenia immunosupresyjnego orbitopatii tarczycowej w zależności od wcześniejszego leczenia choroby Gravesa- poszukiwanie czynników ryzyka niekorzystnego przebiegu choroby. Wykorzystanie badań elektrofizjologicznych i OCT do oceny orbitopatii tarczycowej”

*Effectiveness of immunosuppressive treatment of Graves' Orbitopathy against earlier treatment modalities in Graves' disease – determination of unfavorable disease course risk factors. Electrophysiological and OCT tests in Graves' Orbitopathy evaluation.*

*Praca doktorska*

Promotor: dr hab n. med. Agata Bałdys- Waligórska

Pracę wykonano w:

1. Oddziale Klinicznym Okulistyki i Onkologii Okulistycznej Szpitala Uniwersyteckiego w Krakowie

Kierownik jednostki: Prof. dr hab. n. med. Bożena Romanowska-Dixon

2. Oddziale Klinicznym Endokrynologii Szpitala Uniwersyteckiego w Krakowie

Kierownik jednostki: Prof. dr hab. n. med. Alicja Hubalewska-Dydejczyk

Kraków, rok 2023

*Dysertację doktorską dedykuję mojej rodzinie, która mobilizowała mnie i wspierała na każdym kroku powstawania tej pracy.*

*Pragnę też podziękować Pani Promotor dr hab. n. med. Agacie Bałdys-Waligórskiej, która wprowadziła mnie w świat nauki i intensywnie uczestniczyła w powstawaniu i redagowaniu pracy.*

*Składam również wyrazy wdzięczności Pani Prof. dr hab. n. med. Bożenie Romanowskiej-Dixon, Kierownikowi Katedry i Kliniki Okulistyki UJ CM, w której mam przyjemność pracować od przeszło 10 lat, za opiekę od początku mojej okulistycznej drogi, za cenne rady oraz wsparcie na co dzień w pracy zawodowej i naukowej.*

# Spis treści:

# 1 Wykaz publikacji stanowiących rozprawę doktorską.

**Jagiełło-Korzeniowska A**, Sokołowski A, Krzentowska-Korek A, Miklaszewska G, Bałdys-Waligórska A. The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease. *Endokrynol Pol. 2016;67(6):554-561. doi: 10.5603/EP.2016.0073.*

**Jagiełło-Korzeniowska A**, Sokołowski A, Hubalewska-Dydejczyk A, Romanowska-Dixon B, Bałdys-Waligórska A. Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy. *Ophthatherapy. 2022;9(2):98-105. https://doi.org/10.24292/01.OT.190522*

**Jagiełło-Korzeniowska A**, Bałdys-Waligórska A, Hubalewska-Dydejczyk A, Romanowska-Dixon B. Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy. *J Clin Med. 2022 Jul 15;11(14):4095. doi: 10.3390/jcm11144095.*

# 2 Streszczenie

*Cele pracy:*

1. Ocena skuteczności leczenia immunosupresyjnego orbitopatii tarczycowej u pacjentów, którzy byli wcześniej leczeni lekami tyreostatycznymi lub przebyli terapię radiojodem.
2. Poszukiwanie czynników wpływających na aktywność i stopień zaawansowania orbitopatii tarczycowej a także czynników ryzyka niezadowalającej odpowiedzi na leczenie glikokortykosteroidami i radioterapią oczodołów.
3. Ocena funkcji i morfologii przedniego odcinka drogi wzrokowej u pacjentów z orbitopatią tarczycową przy użyciu badań elektrofizjologicznych oraz badania optycznej koherentnej tomografii (OCT) a także poszukiwanie parametrów, które mogłyby być użyteczne w wykrywaniu wczesnego uszkodzenia nerwu wzrokowego.

*Materiał i Metodyka:*

W części pierwszej wzięło udział 214 pacjentów z zaostrzeniem orbitopatii tarczycowej. Pacjenci zostali podzieleni na dwie grupy: grupę ATD leczoną lekami tyreostatycznymi, składającą się ze 168 pacjentów oraz grupę 131-I po przebytej terapii radiojodem, składającą się z 46 pacjentów. Wszyscy pacjenci byli leczeni dożylnymi pulsami methylprednisolonu do łącznej dawki 8,0 g z następową radioterapią oczodołów (20 Gy w 10 frakcjach). Indeksy CAS (Clinical Activity Score) i IO (Index of Orbitopathy) oraz stężenia TSH, fT4 oraz TRAb były oceniane przed leczeniem, a także 1,6 i 12 miesięcy po leczeniu.
W części drugiej wzięło udział 47 pacjentów z orbitopatią tarczycową. Pacjenci zostali podzieleni na trzy grupy w zależności od stopnia zaawansowania choroby: Grupa 1 z łagodną orbitopatią, Grupa 2 z umiarkowaną i ciężką orbitopatią oraz Grupa 3 z orbitopatią zagrażającą widzeniu. Porównano wzrokowe potencjały wywołane stymulowane wzorcem (pVEP), wzrokowe potencjały wywołane stymulowane błyskiem (fVEP), elektroretinogram stymulowany wzorcem (pERG) oraz wyniki badania OCT pomiędzy grupami.

*Wyniki i wnioski:*

1. Skuteczność leczenia orbitopatii tarczycowej w obu grupach była taka sama bez względu na rodzaj wcześniejszego leczenia choroby Gravesa-Basedowa (leki tyreostatyczne lub leczenie radiojodem)
2. Wyższe stężenie TRAb oraz starszy wiek zwiększają ryzyko aktywnej orbitopatii tarczycowej. Wyższe stężenie TRAb oraz bardziej aktywna choroba zwiększają ryzyko bardziej zaawansowanej orbitopatii tarczycowej. Z kolei pacjenci z bardziej zaawansowaną chorobą mają większe ryzyko niezadowalającej odpowiedzi na leczenie immunosupresyjne.
3. Pacjenci z neuropatią nerwu wzrokowego (Grupa 3) różnią się od pozostałych pacjentów (Grupa 1 i 2) w zakresie większości odpowiedzi elektrofizjologicznych i parametrów OCT. Z kolei pacjenci z umiarkowaną i ciężką orbitopatią tarczycową (Grupa 2) mają zmiany w zakresie załamka p100 wzrokowych potencjałów wywołanych i w zakresie kompleksu komórek zwojowych w badaniu OCT w porównaniu do pacjentów z łagodną orbitopatią (Grupa 1). Badania elektrofizjologiczne jak i badanie OCT mogą być pomocne w postawieniu diagnozy neuropatii nerwu wzrokowego, ale i w monitorowaniu pacjentów z mniej zaawansowaną chorobą do wykrywania subklinicznych zmian w zakresie funkcjonowania i morfologii nerwu wzrokowego.

# 3 Summary

*Aims of study*

1. To evaluate the efficacy of immunosuppressive treatment of Graves' Orbitopathy (GO) in patients who had earlier been treated with anti-thyroid drugs or who had undergone radioiodine therapy.

2. To determine factors affecting the activity and severity of Graves' Orbitopathy and to identify predictive factors of poor response to glucocorticoid treatment followed by orbital irradiation.

3. To perform functional and structural evaluation of the anterior visual pathway in patients with Graves' Orbitopathy (GO) using electrophysiological tests and OCT and to identify potential parameters that could be useful in detecting early optic nerve damage.

*Material and methods*

In publications 1 and 2, a cohort of 214 patients with exacerbation of GO were studied. Patients were divided into two groups: the ATD group - treated with anti-thyroid drugs consisting of 168 patients, and the 131-I group - after radioiodine therapy consisting of 46 patients. All patients were treated with methylprednisolone intravenous (iv) pulses (total dose 8.0 g) followed by orbital irradiation (20 Gy in 10 fractions). CAS (Clinical Activity Score) and IO (Index of Orbitopathy) indices, TSH, fT4, and TRAb levels were evaluated prior to, and 1, 6, and 12 months after treatment.

In publication 3, a cohort of 47 GO patients were enrolled in the study and divided into three groups, depending on their disease severity: Group 1 with mild GO, Group 2 with moderate-to- severe GO, and Group 3 with sight-threatening GO (DON-Dysthyroid Optic Neuropathy). Pattern visual evoked potential (pVEP), flash visual evoked potential (fVEP), pattern electroretinogram (pERG), and optical coherence tomography (OCT) findings were compared between these groups.

*Results and conclusions*

1. Treatment of Graves' Orbitopathy was equally effective in both groups, independently of earlier Graves' disease treatment (antithyroid drugs or radioiodine).
2. Higher TRAb levels and more advanced age increase the risk of active Graves' Orbitopathy. Higher TRAb levels and more active disease increase the risk of more severe Graves' Orbitopathy. Patients with more severe disease have a higher risk of poor response to immunosuppressive treatment.
3. Patients with Dysthyroid Optic Neuropathy (Group 3) differ from other patients (Group 1 and 2) in terms of electrophysiological responses and OCT parameters. Patients with moderate-to-severe Graves' Orbitopathy (Group 2) show differences in the P100 component of pVEP and ganglion cell complex in OCT, compared to patients with mild orbitopathy (Group 1). Electrophysiological examinations as well as OCT may be of use in DON diagnosis, but also in monitoring patients with less severe GO, to detect subclinical changes in optic nerve function and morphology.

# 4 Wprowadzenie

Orbitopatia tarczycowa jest najczęstszą pozatarczycową manifestacją choroby Gravesa-Basedowa i dotyczy około 25% pacjentów z tym schorzeniem tarczycy [1]. W wyniku produkcji przeciwciał przeciwko receptorowi TSH (TRAb), dochodzi do jego nadmiernej aktywacji nie tylko w komórkach tarczycy, ale i w fibroblastach i adipocytach obecnych w oczodole. Prowadzi to do wzmożonej adipogenezy i produkcji glikozaminoglikanów, rozwoju przewlekłego stanu zapalnego, obrzęku, a następnie włóknienia tkanek oczodołu [2]. Do czynników ryzyka rozwoju orbitopatii tarczycowej należy zarówno nadczynność jak i niedoczynność tarczycy [3]. Również rodzaj leczenia choroby Gravesa - Basedowa może wpływać na częstość zachorowania i na przebieg orbitopatii tarczycowej [4]. Czynnikiem ryzyka rozwoju orbitopatii tarczycowej może być np. przebyta terapia radiojodem (131-I) [5]. Sugeruje się, że w wyniku zniszczenia komórek tarczycy przez 131-I dochodzi do nagłego uwolnienia antygenów tarczycowych, co prowadzi do wzmożonej produkcji TRAb. Uważa się, że wysokie stężenie TRAb w surowicy krwi utrzymuje się dużo dłużej u pacjentów po leczeniu radiojodem niż u pacjentów leczonych lekami tyreostatycznymi, co może prowadzić do rozwoju orbitopatii tarczycowej, bądź też zaostrzenia obecnych już objawów ocznych [6, 7]. Interesujące, z punktu widzenia klinicznego, jest ustalenie czy sposób leczenia choroby Gravesa-Basedowa (leki tyreostatyczne vs radiojod) wpływa na przebieg choroby i skuteczność leczenia orbitopatii.

Pacjenci z orbitopatią tarczycową są oceniani pod względem aktywności i stopnia zaawansowania choroby. Do oceny aktywności stosowana jest skala CAS (Clinical Activity Score), natomiast do oceny stopnia zaawansowania używa się klasyfikcji NONSPECS (NO signs or symptoms; Only signs; Soft tissue involvement; Proptosis; Extraocular muscle involvement; Corneal involvement; Sight loss) [8] lub klasyfikacji opracowanej przez EUGOGO (European Group on Graves' Orbitopathy) [9]. Stopień zaawansowania choroby oceniony w skali NONSPECS może być zapisywany jako indeks orbitopatii (IO).

Mimo, że zachorowanie na orbitopatię tarczycową zwłaszcza na postać umiarkowaną i ciężką lub zagrażającą widzeniu wykazuje w Europie tendencję spadkową [1] to jednak leczenie tej choroby nadal pozostaje niedoskonałe [10]. U części pacjentów mimo zastosowanego leczenia nie udaje się powstrzymać postępu choroby. Są to tzw. „non-

responders", u których odpowiedź na leczenie immunosupresyjne jest niezadowalająca [11].

U niektórych pacjentów szybki postęp choroby może doprowadzić do uszkodzenia nerwu wzrokowego poprzez bezpośredni ucisk lub też w mechanizmie niedokrwiennym [12]. Neuropatia nerwu wzrokowego (DON-Dysthyroid Optic Neuropathy) dotyczy 5-8% pacjentów z orbitopatią tarczycową i może mieć podstępny przebieg. Wymaga pilnego podjęcia leczenia, gdyż może doprowadzić do trwałej utraty widzenia [13,14]. Postawienie diagnozy DON na wczesnym etapie może być utrudnione, dlatego wykorzystanie badań elektrofizjologicznych takich jak wzrokowe potencjały wywołane stymulowane wzorcem (pVEP- pattern visual evoked potential) lub błyskiem (fVEP-flash visual evoked potential) oraz elektroretinogram stymulowany wzorcem (pERG-pattern electroretinogram), które umożliwiają ocenę funkcji nerwu wzrokowego i komórek zwojowych, może być pomocne w monitorowaniu pacjentów z orbitopatią tarczycową [15-21]. Oprócz oceny funkcji przydatna może być ocena morfologii tarczy nerwu wzrokowego i komórek zwojowych przy użyciu optycznej koherentnej tomografii (OCT-Optical Coherence Tomography). Jest to badanie obrazowe umożliwiające ocenę parametrów morfometrycznych tarczy nerwu wzrokowego, kompleksu komórek zwojowych w rejonie plamkowym (GCC-ganglion cell complex) oraz warstwy włókien nerwowych okołotarczowych (RNFL-retinal nerve fiber layer) [22-24].

# 5 Cele pracy

Praca składa się z trzech publikacji.

*Publikacja pierwsza*

Celem pracy było ustalenie czy sposób leczenia choroby Gravesa-Basedowa wpływa na przebieg i leczenie orbitopatii tarczycowej. Jak na leczenie immunosupresyjne i radioterapię oczodołów odpowiadają pacjenci leczeni doustnymi lekami tyreostatycznymi (grupa ATD) w porównaniu do pacjentów leczonych radiojodem (grupa 131-I)?

Czy istnieje zależność pomiędzy skutecznością leczenia, a stężeniem przeciwciał przeciwko receptorowi TSH (TRAb)?

*Publikacja druga*

Celem pracy było poszukiwanie czynników, które mają wpływ na aktywność oraz stopień zaawansowania orbitopatii tarczycowej oraz czynników ryzyka niezadowalającej odpowiedzi na leczenie immunosupresyjne glikokortykosteroidami i radioterapią oczodołów.

*Publikacja trzecia*

Celem pracy była równoczesna ocena funkcji i morfologii przedniego odcinka drogi wzrokowej u pacjentów z orbitopatią tarczycową przy użyciu badań elektrofizjologicznych (pVEP, fVEP, pERG) oraz badania OCT tarcz. Wyniki powyższych badań porównano u pacjentów z różnym stopniem zaawansowania choroby i poszukiwano parametrów, które mogłyby być użyteczne w wykrywaniu wczesnego uszkodzenie nerwu wzrokowego.

# 6 Materiał i metodyka

*Publikacja pierwsza i druga*

W badaniu wzięło udział 214 pacjentów z chorobą Graves-Basedowa, leczonych w Oddziale Klinicznym Endokrynologii Szpitala Uniwersyteckiego z powodu orbitopatii tarczycowej. Okres obserwacji pacjentów wynosił 12 miesięcy po zakończeniu leczenia. Przed przyjęciem do oddziału pacjenci byli leczeni lekami tyreostatycznymi lub otrzymali leczenie radiojodem z powodu współistniejącej nadczynności tarczycy. Pacjenci zostali podzieleni na 2 grupy: grupę ATD (anti-thyroid drugs) leczoną lekami tyreostatycznymi oraz grupę 131-I leczoną radiojodem. Grupa ATD liczyła 168 pacjentów; 119 kobiet i 49 mężczyzn. Grupa 131-I liczyła 46 pacjentów; 37 kobiet i 9 mężczyzn. Wszyscy pacjenci byli badani przez tego samego okulistę w Klinice Okulistyki i Onkologii Okulistycznej Szpitala Uniwersyteckiego. Aktywność choroby była oceniana przy użyciu 7 – punktowej skali CAS (Clinical Activity Score). 15-stopniowa skala NOSPECS wyrażona w postaci Indeksu Orbitopatii (IO) była używana do oceny stopnia zaawansowania choroby. Po osiągnięciu eutyreozy pacjenci byli leczeni dożylnymi pulsami methylprednisolonu w schemacie 1,0 g przez dwa kolejne dni tygodnia do łącznej dawki 8,0 g. Miesiąc po zakończonej sterydoterapii pacjenci byli poddawani radioterapii oczodołów do sumarycznej dawki 20 Gy podawanej w 10 frakcjach w Zakładzie Radioterapii Dzieci i Dorosłych Uniwersyteckiego Szpitala Dziecięcego w Krakowie. Pacjenci, którzy wyrazili zgodę na udział w badaniu byli konsultowani przed rozpoczęciem leczenia oraz 1, 6, i 12 miesięcy po leczeniu. Każdemu pacjentowi wykonano następujące badania laboratoryjne: poziom hormonu tyreotropowego (TSH), tyroksyny (fT4) i przeciwciał przeciwko receptorowi TSH (TRAb) w surowicy krwi oraz badanie okulistyczne z oceną CAS i IO. Grupy ATD i 131-I zostały dokładnie opisane i porównane w pierwszej publikacji. Porównano odpowiedź na leczenie dożylnymi pulsami methylprednisolonu i radioterapią oczodołów w obu grupach. W publikacji drugiej poszukiwano czynników, które mają wpływ na aktywność i stopień zaawansowania choroby. Analizując zależność pomiędzy CAS i IO, podzielono pacjentów na 3 podgrupy w zależności od stopnia zaawansowania choroby (IO 1-2- łagodna orbitopatia, IO 3-8-umiarkowana orbitopatia, IO 9-15-ciężka orbitopatia) i zbadano przynależność tych pacjentów do podgrup CAS≤ 3 (z nieaktywną

GO) i CAS>3 (z aktywną GO). Pacjenci, których reakcja na leczenie dożylnymi pulsami methylprednisolonu i radioterapię oczodołów była niewystarczająca i wymagali dodatkowego leczenia doustnymi glikokortykosteroidami zostali uznani za pacjentów „non-responder". Poszukiwano czynników ryzyka niezadawalającej odpowiedzi na leczenie.

*Publikacja trzecia*

W badaniu wzięło udział 47 pacjentów z orbitopatią tarczycową, w tym 13 mężczyzn i 34 kobiety. Do badania nie byli włączani pacjenci z krótkowzrocznością większą niż -6,0D, z podwyższonym ciśnieniem wewnątrzgałkowym, z chorobami neurologicznymi, z cukrzycą powikłaną polineuropatią lub retinopatią cukrzycową oraz z innymi chorobami, które mogłyby mieć wpływ na wyniki badań elektrofizjologicznych oraz OCT tarcz. Pacjenci mieli wykonywane pełne badanie okulistyczne w Klinice Okulistyki i Onkologii Okulistycznej Szpitala Uniwersyteckiego w Krakowie. Badanie było przeprowadzane zawsze przez tego samego okulistę i zawierało badanie w lampie szczelinowej, badanie dna oka, tonometrię, ocenę widzenia barw na tablicach Ishihary, exoftalmometrię, badanie ruchomości gałek ocznych na ekranie Hessa oraz kinetyczną perymetrię Goldmanna. Stopień zaawansowania choroby był oceniany przy użyciu klasyfikacji EUGOGO (European Group on Graves' Orbitopathy). Pacjenci zostali podzieleni na trzy grupy w zależności od stopnia zaawansowania choroby: Grupę 1 z łagodną orbitopatią tarczycową (16 pacjentów), Grupę 2 z umiarkowaną i ciężką orbitopatią (23 pacjentów), oraz Grupę 3 z ciężką orbitopatią zagrażającą widzeniu (8 pacjentów). Wszyscy pacjenci mieli wykonane następujące badania elektrofizjologiczne: wzrokowe potencjały wywołane stymulowane wzorcem (pVEP-pattern visual evoked potential) dla czterech różnej wielkości znaczków (0,4º, 0,9º, 1,5º, 2,5º), wzrokowe potencjały wywołane stymulowane błyskiem (fVEP-flash visual evoked potential) oraz elektroretinogram stymulowany wzorcem (pERG- pattern electroretinogram). Poza badaniami elektrofizjologicznymi pacjenci mieli wykonywaną optyczną koherentną tomografię (OCT-Optical Coherence Tomography) tylnego odcinka oka. Analizie podlegała warstwa okołotarczowych włókien nerwowych (RNFL- retinal nerve fiber layer) oraz kompleks komórek zwojowych ocenianych w rejonie plamkowym (GCC-ganglion cell complex). Analizowano również parametry FLV (focal loss volume) (%) i GLV (global loss volume) (%), wyrażające objętość utraconego GCC w rejonie plamkowym. Porównano wyniki badań

elektrofizjologicznych i wyniki badania OCT u pacjentów bez ewidentnych cech neuropatii nerwu wzrokowego (Grupa 1 i Grupa 2) z wynikami pacjentów z potwierdzoną klinicznie neuropatią nerwu wzrokowego (DON- Dysthyroid Optic Neuropathy) (Grupa 3). Następnie porównano te same parametry u pacjentów z łagodną orbitopatią tarczycową (Grupa 1) i u pacjentów z umiarkowaną i ciężką orbitopatią (Grupa 2).

# 7 Podsumowanie wyników i wnioski

*Publikacja pierwsza*

Porównanie grupy ATD leczonej lekami tyreostatycznymi i grupy 131-I leczonej radiojodem wykazało, że grupy przed leczeniem nie różniły się istotnie statystycznie pod względem płci, wieku, czasu trwania orbitopatii tarczycowej, stopnia zaawansowania choroby (IO-0), aktywności choroby (CAS-0), a także stężenia fT4.

Czas trwania leczenia nadczynności tarczycy był znacznie dłuższy w grupie 131-I a poziom TSH przed leczeniem GO był znamiennie statystycznie wyższy niż u pacjentów leczonych lekami tyreostatycznymi. Mediana CAS-0 przed leczeniem nie różniła się w obu grupach i wynosiła 4. Miesiąc po leczeniu CAS-1 zmniejszyło się istotnie statystycznie w obu grupach w porównaniu do CAS-0, ale było istotnie wyższe w grupie 131-I. Mediana CAS-1 wynosiła odpowiednio 1 w grupie ATD i 2 w grupie 131-I.

Stężenia TRAb w grupie 131-I pozostawały zawsze powyżej normy i były znamiennie statystycznie wyższe niż w grupie ATD zarówno przed leczeniem jak i przez cały okres 12 miesięcy obserwacji. Mimo to, stopień zaawansowania choroby (IO) w obu grupach nie różnił się istotnie przez cały okres obserwacji. Mediana IO-0 przed leczeniem wynosiła 5 w obu grupach i uległa istotnej redukcji po 12 miesiącach (IO-12) do 2 w obu grupach, co oznacza, że wcześniejszy schemat leczenia choroby Gravesa-Basedowa nie miał wpływu na skuteczność leczenia orbitopatii tarczycowej, pomimo utrzymującego się wyższego stężenia TRAb w grupie 131-I.

*Publikacja druga*

Poszukując czynników, które mogłyby mieć wpływ na aktywność i stopień zaawansowania orbitopatii tarczycowej przeanalizowano: wiek, płeć, przebytą terapię radiojodem, stężenie w surowicy TSH, fT4 i TRAb przed leczeniem (TSH-0, FT4-0, TRAb-0). Przyjęto, że CAS $\geq$ 3 i IO$\geq$ 3 przemawia za aktywną postacią orbitopatii tarczycowej. Stwierdzono, że jedynymi czynnikami mającymi wpływ na aktywność choroby jest poziom TRAb przed leczeniem (TRAb-0) oraz wiek. Wzrost stężenia TRAB-0 o jedną jednostkę (U/L) w obu grupach analizowanych jednocześnie (grupie ATD i grupie 131-I) zwiększa ryzyko względne aktywnej orbitopatii tarczycowej o 4,7 %. Z kolei każdy rok dodany do wieku pacjenta zwiększa ryzyko względne aktywnej orbitopatii tarczycowej o 2,8 %. Przyjęto, że IO>5 reprezentuje umiarkowaną i ciężką

orbitopatię. W grupie 131-I stężenie TRAb miesiąc po leczeniu orbitopatii tarczycowej czyli TRAb-1 jest skorelowany z jednoczasowym IO-1 i jest dobrym predyktorem IO po 6 miesiącach. Stężenie TRAb -1 większe o jedną jednostkę U/L w pierwszym miesiącu po leczeniu orbitopatii tarczycowej oznacza o 5,6% większe ryzyko umiarkowanej lub ciężkiej orbitopatii tarczycowej w pierwszym miesiącu (IO-1>5) oraz o 8,7% większe ryzyko IO>5 po sześciu miesiącach (IO-6). Stwierdzono również, że stopień zaawansowania choroby wyrażony jako indeks orbitopatii (IO) zależy od stopnia aktywności choroby ocenianej w CAS. Wszyscy pacjenci z bardzo zaawansowaną orbitopatią tarczycową (IO 9-15) przed leczeniem mieli CAS-0 >3, natomiast pacjenci z łagodną orbitopatią (IO 1-2) mieli CAS-0≤ 3. Słaba odpowiedź na leczenie dotyczyła 28% pacjentów w grupie 131-I oraz 41% pacjentów z grupy ATD. Z analizowanych czynników w grupie pacjentów leczonych radiojodem tylko TSH-0 i IO-0 okazały się mieć wartość predykcyjną złej odpowiedzi na leczenie immunosupresyjne. W grupie pacjentów leczonych lekami tyreostatycznymi (ATD) tylko IO-0 okazał się mieć wartość predykcyjną. Każdy dodatkowy punkt w skali NOSPECS przed leczeniem zwiększał ryzyko bycia non-respoder o 30%. Teoretyczny rozkład logarytmiczno-normalny IO-0 w podgrupach pacjentów wymagających i niewymagających dodatkowego leczenia doustnymi glikokortykosteroidami po leczeniu dożylnymi pulsami methylprednisolonu wykazał, że punktem odcięcia dla pacjentów non-responder jest IO-0 >5. Oznacza to, że pacjenci z IO-0>5 mają większe ryzyko niezadawalającej odpowiedzi na leczenie.

Podsumowując, pacjenci z wyższymi stężeniami TRAb w surowicy mają wyższe ryzyko rozwoju aktywnej orbitopatii tarczycowej. Wysoki poziom TRAb zwiększa również ryzyko umiarkowanej i ciężkiej orbitopatii. Starsi pacjenci są obarczeni większym ryzykiem rozwoju aktywnej orbitopatii tarczycowej a większa aktywność z kolei niesie ze sobą ryzyko bardziej zaawansowanej choroby. Pacjenci z bardziej zaawansowaną chorobą mają większe ryzyko, że nie zareagują na leczenie dożylnymi pulsami methylprednisolonu i radioterapią oczodołów. Dlatego bardzo istotne jest uważne monitorowanie pacjentów z orbitopatią tarczycową i wczesne kierowanie ich do specjalistycznych ośrodków.

*Publikacja trzecia*

Porównanie wyników badań pacjentów bez klinicznych cech neuropatii nerwu wzrokowego (Grupa 1 i Grupa 2) z pacjentami z klinicznie potwierdzoną neuropatią (Grupa 3) wykazało, że pacjenci z DON mają wydłużone latencje załamków N75 oraz P100, a także obniżone amplitudy załamka P100 w badaniu wzrokowych potencjałów wywołanych stymulowanych wzorcem (pVEP). W badaniu wzrokowych potencjałów wywołanych stymulowanych błyskiem (fVEP) pacjenci z neuropatią nerwu wzrokowego prezentowali istotne statystycznie wydłużenie latencji załamka P2 bez istotnej różnicy w zakresie amplitudy. Grupa 3 charakteryzowała się również znamiennym statystycznie obniżeniem amplitud załamków N95 i P50, a także wydłużeniem latencji załamka P50 w elektroretinogramie stymulowanym wzorcem (pERG). W badaniu OCT tarcz u pacjentów z DON stwierdzono istotne statystycznie ścieńczenie kompleksu komórek zwojowych (GCC) w rejonie plamkowym, wzrost parametrów GLV i FLV, a także ścieńczenie warstwy włókien nerwowych (RNFL) w porównaniu do pacjentów bez klinicznych cech neuropatii.

W porównaniu do pacjentów z łagodną orbitopatią tarczycową (Grupa 1), pacjenci z umiarkowaną i ciężką orbitopatią tarczycową (Grupa 2) mieli obniżone amplitudy załamka P100, wydłużoną latencję załamka P100 obserwowaną jedynie dla wielkości znaczka 0,9 º oraz zmniejszoną średnią grubość kompleksu GCC i grubość GCC w górnym kwadrancie.

Amplitudy i latencje załamków P2, P50 i N95 nie różniły się pomiędzy grupami, podobnie jak grubość warstwy włókien nerwowych.

Neuropatia nerwu wzrokowego w przebiegu orbitopatii tarczycowej może mieć podstępny początek, dlatego ocena funkcji nerwu wzrokowego przy użyciu badań elektrofizjologicznych może być przydatna w postawieniu diagnozy DON. Wykonywanie zarówno badania pVEP jak i pERG umożliwia różnicowanie pomiędzy zmianami wynikającymi z uszkodzenia plamki a zmianami będącymi konsekwencją uszkodzenia komórek zwojowych. fVEP z kolei może być przydatny w postawieniu diagnozy DON u pacjentów z ciężkimi zaburzeniami powierzchni oka, ze słabą ostrością wzroku, z intensywnym łzawieniem lub dwojeniem, które uniemożliwiają uzyskanie wiarygodnej odpowiedzi na bodziec szachownicy. fVEP nie jest jednak wystarczająco czuły do wykrywania wczesnych, subklinicznych postaci DON. Bardziej

czułym badaniem jest pVEP. Znaczne obniżenie amplitud załamka P100 oraz subtelne wydłużenie latencji załamka P100 dla wielkości znaczka 0,9 º w grupie pacjentów z umiarkowaną i ciężką orbitopatią w porównaniu do grupy z łagodną orbitopatią może sugerować, że u tych pacjentów mimo braku klinicznych cech DON dochodzi do zaburzeń funkcjonalnych nerwu wzrokowego będących konsekwencją niedokrwienia lub zaburzeń transportu aksonalnego. Subtelne zmniejszenie średniej grubości GCC i grubości GCC w górnym kwadrancie w Grupie 2 w porównani do Grupy1 może jednak sugerować, że nie są to jedynie zmiany funkcjonalne, ale że u tych pacjentów dochodzi do powolnej utraty komórek zwojowych.

Podsumowując, odpowiedzi elektrofizjologiczne i parametry w OCT tarcz różnią się u pacjentów z różnym stopniem zaawansowania choroby, dlatego wprowadzenie regularnej oceny funkcjonalnej i morfologicznej nerwu wzrokowego u pacjentów z orbitopatią tarczycową mogłoby przynieść korzyść i przyczynić się do wykrywania subtelnych cech wczesnego uszkodzenia nerwu wzrokowego.

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# 9 Publikacje

## 9.1 *The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease*

PRACE ORYGINALNE/ORIGINAL PAPERS

Endokrynologia Polska
DOI: 10.5603/EP.2016.0073
Tom/Volume 67; Numer/Number 6/2016
ISSN 0423–104X

# The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease

## Wcześniejsze leczenie choroby Gravesa lekami tyreostatycznymi lub radiojodem nie wpływa na skuteczność leczenia immunosupresyjnego orbitopatii tarczycowej

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**Abstract**

**Introduction:** We studied the efficacy of immunosuppressive treatment of GO in a group of patients who had been treated with anti-thyroid drugs (the ATD group) and in another group that had undergone radioiodine therapy (the 131-I group).
**Material and methods:** A total of 214 patients with exacerbation of GO were studied; the ATD group consisting of 168 patients, and the 131-I group consisting of 46 patients. All patients were treated with methylprednisolone IV pulses (total dose 8.0 g) followed by orbital irradiation (20 Gy in 10 fractions). CAS and IO indices, TSH, fT4, and TRAb levels were evaluated prior to, and 1, 6, and 12 months after treatment.
**Results:** One month after treatment the CAS index decreased significantly in both groups, against values before treatment, $p < 0.05$. In the ATD group the median level of TRAb-0 before treatment was 5.6 IU/L (min = 0.1; max = 114.0), and 12 months later (TRAb-12) it was 1.4 IU/L (min = 0.1; max = 75.3) ($p < 0.05$). In the 131-I group the median level of TRAb-0 was 14.3 IU/L (min = 0.6; max = 90.0) *vs.* TRAb-12 of 3.65 IU/L (min = 0.1; max = 41.0) ($p < 0.05$). In the ATD group the median value of IO-0 before treatment was 5.0 (min = 1.0; max = 12.0) *vs.* IO-12 of 2.0 (min = 0.0; max = 8.0) ($p < 0.05$). In the 131-I group the median value of IO-0 was 5.0 (min = 2.0; max = 9.0) *vs.* IO-12 of 2.0 (min = 0.0; max = 6.0) ($p < 0.05$).
**Conclusions:** The severity of GO in the ATD and 131-I groups did not differ significantly over the course of observation despite differences noted in their TRAb levels. The efficacy of GO treatment did not differ between these groups. **(Endokrynol Pol 2016; 67 (6): 554–561)**

**Key words:** *Graves' orbitopathy, radioiodine therapy, intravenous steroids, orbital irradiation*

**Streszczenie**

**Wstęp:** Porównano skuteczność leczenia immunosupresyjnego orbitopatii tarczycowej (GO) u pacjentów leczonych wcześniej doustnymi lekami tyreostatycznymi (grupa ATD) oraz u pacjentów po leczeniu radiojodem (grupa 131-I).
**Materiał i metody:** Przebadano 214 pacjentów z zaostrzeniem GO. Grupa ATD składała się ze 168 pacjentów leczonych lekami tyreostatycznymi. Grupa 131-I składała się z 46 pacjentów leczonych radiojodem. Wszyscy pacjenci byli leczeni pulsami dożylnymi methylprednisolonu (łączna dawka 8,0 g), a następnie poddawani radioterapii oczodołów (20 Gy w 10 frakcjach). Indeksy CAS i IO, stężenia TSH, fT4 oraz TRAb oceniano przed leczeniem, a następnie 1, 6 i 12 miesięcy po leczeniu.
**Wyniki:** W obu grupach miesiąc po leczeniu indeks CAS istotnie zmniejszył się w porównaniu z wartościami wyjściowymi, $p < 0,05$. W grupie ATD mediana stężenia TRAb-0 wynosiła 5,6 U/l (min = 0,1; max = 114,0) przed leczeniem i 1,4 U/l (min = 0,1; max = 75,3) ($p < 0,05$) 12 miesięcy po leczeniu (TRAb-12). W grupie 131-I mediany stężenia TRAb-0 i TRAb-12 wynosiły odpowiednio 14,3 IU/L (min = 0,6; max = 90,0) i 3,65 IU/l (min = 0,1; max = 41,0) ($p < 0,05$). Mediana wartości indeksu IO przed leczeniem (IO-0) w grupie ATD wynosiła 5,0 (min = 1,0; max = 12,0), a po leczeniu (IO-12) 2,0 (min = 0,0; max = 8,0) ($p < 0,05$). W grupie 131-I mediany wartości IO-0 i IO-12 wynosiły odpowiednio 5,0 (min = 2,0; max = 9,0) i 2,0 (min = 0,0; max = 6,0) ($p < 0,05$).
**Wnioski:** Stopień zaawansowania GO u pacjentów w grupie ATD i 131-I nie różnił się znamiennie statystycznie przez cały okres obserwacji. Mimo że stężenia TRAb w grupie 131-I zawsze przekraczały górny zakres wartości prawidłowych i były wyższe niż w grupie ATD, skuteczność leczenia GO w obu grupach była taka sama. **(Endokrynol Pol 2016; 67 (6): 554–561)**

**Słowa kluczowe:** *orbitopatia tarczycowa; leczenie radiojodem; glikokortykosteroidy dożylne; radioterapia oczodołów*

*This study was supported by Statutory Grant of the Jagiellonian University Medical College No Wl/470/KL/L.*

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## Introduction

Graves' orbitopathy (GO) is a rare disease associated with autoimmune disorder — Graves' disease (GD), with a prevalence of about 25% in GD patients [1–3]. According to Tanda et al. [4], some 20% of patients suffering from Graves' disease have inactive or mild GO, 6% have active and moderate-to-severe GO, and only 0.3% develop dysthyroid optic neuropathy (DON). The remaining 73.7% of Graves' patients are free of ocular symptoms. The prevalence of GO tends to decline over the years, perhaps due to the decrease in smoking habits or to earlier diagnosis [4–7]. As only limited groups of GO patients are available for randomised clinical trials (RCTs), precise GO pathogenesis and targeted treatment remain uncertain [2, 8]. It is believed that higher TRAb titres are present in patients with more severe GO [9, 10]. Radioiodine treatment is associated with an increased risk of ophthalmopathy and more severe course than that after antithyroid medication [11, 12]. After radioiodine, persistent elevated TRAb levels were observed for over five years [13, 14].

The aim of our study was to evaluate the efficacy of immunosuppressive treatment of GO in patients who had earlier been treated with anti-thyroid drugs (the ATD group) or who had undergone radioiodine therapy (the 131-I group).

## Material and methods

Over the years 2000–2008 more than four hundred patients were admitted to our Endocrinology Department of the University Hospital, presenting with exacerbation of the eye syndrome in the course of Graves' disease. The patients who voluntarily participated in this prospective observational study signed their written consent and underwent follow-up for a period of 12 months after completion of their GO treatment. The drop-out rate was 46% (i.e. of 400 enlisted patients 214 completed this study). The study was approved by the Bioethics Committee of the Jagiellonian University. Before admission to our Department, patients had been treated elsewhere for hyperthyroidism, either with anti-thyroid drugs (ATD group) or with radioiodine (131 I-group). The ATD group consisted of 168 patients, 119 females and 49 males, of mean age 52.2 ± 11.2 years. The 131-I group consisted of 46 patients, 37 females and 9 males, of mean age 52.1 ± 13.3 years. All patients were evaluated by the same ophthalmologist. Severity and activity of GO were graded using the Clinical Activity Score (CAS) [15] ranging between 0 and 7 and the NOSPECS (No signs or symptoms; Only signs; Soft tissue involvement; Proptosis; Extraocular muscle involvement; Corneal involvement; Sight loss) classification [16], as expressed by the Orbitopathy Index (IO), ranging between 0 and 15. Orbital CT evaluations were performed for all patients. Patients were rendered euthyroid prior to application of methylprednisolone pulses. CAS and IO indices, TSH, FT4, and anti-TSH receptor antibody (TRAb) levels were evaluated prior to, and 1, 6, and 12 months after treatment. Patients were treated with intravenous methylprednisolone pulses (1 g for two consecutive days each week to a total dose of 8.0 g) followed by orbital irradiation a month later (20 Gy total dose in 10 daily fractions). No major side effects of glucocorticoid therapy were observed.

### *The ATD group*

The mean TSH and fT4 concentration before GO treatment was 2.34 ± 8.81 μU/mL and 18.34 ± 12.24 pmol/mL, respectively. Methimazole was used in 133 patients and L-thyroxin in 131 patients. Thyroidectomy was performed in 26 (15.8%) patients. Enlargement of the extraocular muscles was observed in the CT-scans of 85.1% of patients. The mean time of GO duration was 12.5 ± 20.3 months. The mean time of hyperthyroidism treatment was 31.5 ± 52.4 months. After IV pulses 41.1% of patients had to continue their therapy with oral glucocorticosteroids while awaiting their radiotherapy, during radiotherapy, and up to two months post therapy.

### *The 131-I group*

Radioiodine treatment was offered to patients with IO < 3 and CAS < 3. In this group, TSH blood concentration prior to 131-I treatment was 0.24 ± 0.58 μU/mL. The mean radioiodine activity administered was 496 ± 141 MBq. On admission to our Clinic due to GO, 35 of 46 patients (76%) were hypothyroid (mean TSH concentration 23.9 ± 24.5 μU/mL). To maintain euthyroidism, patients were treated with L-thyroxin. Prior to their radioiodine treatment thyroidectomy was performed in 17.4% of patients. Eight patients received I-131 treatment twice and one patient three times. In 87% of patients, enlargement of extraocular muscles was observed in orbital CT. The mean time of GO duration was 11.9 ± 17.8 months. The mean time of hyperthyroidism treatment was 72.3 ± 67.8 months. After IV methylprednisolone pulses, 28.3% of patients had to continue their therapy with oral glucocorticosteroids until radiotherapy, during radiotherapy, and up to two months post therapy.

### *Laboratory tests*

TSH concentrations were measured using radioimmunoassay (RIA) by Byk-Mallincrodt and immunoradiometric assay (IRMA) by Brahms GmbH, (normal range 0.4–4.0 μU/mL). fT4 concentrations were measured

using electrochemiluminescence immunoassay (ECLIA) by Roche Diagnostics (normal range 12–22.0 pmol/L). TRAb concentrations were measured using radioimmunoreceptor assay (RRA, TRAK human) by Brahms GmbH (normal value < 2.0 IU/L).

### *Statistical analysis*

We evaluated the efficacy of GO treatment. The ATD and 131-I groups were compared in terms of GO activity and severity, and with respect to TRAb levels during the follow-up period. Descriptive and inferential statistical data analysis was performed. Arithmetic means, standard deviations (± SD), and medians (min, max) were calculated. The Shapiro-Wilk test was used to test the normal distribution of data. For data with normal distribution, Student's *t*-test was applied. For data with non-normal distribution, U-Mann-Whitney and Wilcoxon signed-rank tests were applied. Since for non-Gaussian distributions the t-test may be valid if the number of samples exceeds 50 due to the property of robustness, the t-test was also applied in our analysis. The assumed level of significance was $\alpha < 0.05$. SoftStat Statistica, version 9.0 software was used.

## Results

### *The ATD group*

In this group, the median value of the CAS-0 index prior to treatment and CAS-1 one month after treatment was 4 points (min = 0, max = 7) and 1 point (min = 0, max = 7), respectively ($p < 0.05$) (Fig. 1, Table I).

The median IO-0 values in the ATD group prior to treatment and after 12 months (IO-12) of observation were 5 points (min = 1, max = 12) and 2 points (min = 0, max = 8), respectively ($p < 0.05$) (Table I). As based on t-test for paired samples and Wilcoxon signed-rank test, the IO values after 1, 6, and 12 months of observation were significantly lower than those before treatment ($p < 0.05$).

In the ATD group, no differences between median IO-0 values for female and male patients were observed: 5 points (min = 2, max = 12) and 5 points (min = 1, max = 12), respectively. However, after 12 months, these differences were significant: median IO-12 values were 2 points (min = 0, max = 7) and 3 points (min = 0, max = 8), respectively. Indeed, this difference remained significant during the follow-up period.

Before treatment, over 90% of patients experienced soft tissue swelling and extraocular muscle dysfunction. Within this subgroup, 30% developed severe (grade 3) symptoms. Proptosis was diagnosed in 53% of patients, of whom 6% experienced marked proptosis. Corneal pathology was observed in 21.4% of patients including severe damage in 1.2% of patients. Dysthyroid optic neuropathy (DON) was observed in 8.3% of patients, including severe DON, detected in 3.0% of patients. Aggravation of GO was observed in three patients one month after and in four patients six months after completion of treatment.

After 12-month follow-up upper lid retraction, soft tissue swelling, or extraocular muscle dysfunction (mainly grades 1 or 2) were present in 52.3%, 40.5%, and 60.7% of patients, respectively. In 46.7% of patients IO of 2 or 3 was stated, hence they still experienced symptoms of mild Graves' orbitopathy.

In the ATD group, TSH and fT4 concentrations did not differ significantly throughout the 12-month follow-up. The median value of TRAb-0 level was 5.6 IU/L (min = 0.1; max = 114.0) *vs.* 1.4 IU/L (min = 0.1; max = 75.3) for TRAb-12 ($p < 0.05$). TRAb concentrations at 1, 6, and 12 months after treatment were lower than the baseline level (Table I), (Wilcoxon signed-rank test, $p < 0.05$). Since the distribution of TRAb values was right-skewed, we took logarithms of these values and analysed their distribution using the Shapiro-Wilk test, followed by the t-test ($p < 0.05$) (Fig. 2).

### *The 131-I group*

In this group, the median values of CAS-0 index prior to treatment and CAS-1 one month after treatment were 4 (min = 2, max = 7) and 2 (min = 0, max = 6), respectively ($p < 0.05$) (Fig. 1, Table I).

The median values of IO-0 in this group prior to treatment and after 12 months (IO-12) of observation were 5 (min = 2, max = 12) and 2 (min = 0, max = 6), respectively ($p < 0.05$) (Table I). Based on the t-test for paired samples and the Wilcoxon signed-rank test, IO values after 1, 6, and 12 months of observation were significantly lower than those before treatment ($p < 0.05$).

About 90% of patients in this group presented with upper eyelid retraction, soft tissue swelling and extraocular muscle dysfunction before treatment. Among them, 21.7% and 24.0% had severe soft tissue swelling and extraocular muscle dysfunction (grade 3), respectively. Proptosis was present in 54.3% of patients, but none of them developed severe proptosis. Corneal damage was observed in 19.6% of patients. No symptoms of DON were found in this group. Aggravation of GO was observed in three patients one month after and in one patient six months after completion of the treatment.

After 12-month follow-up, upper lid retraction, soft tissue swelling and extraocular muscle dysfunction remained with various degrees of severity (mainly grade 1 and 2) in 56.5%, 43.5%, and 67.4% of patients, respectively. 20.5% of patients had an IO of 4, hence they still experienced symptoms of moderately severe orbitopathy.

**Table I.** *Comparison of TRAb levels and of IO and CAS scores in the ATD and 131-I groups*
**Tabela I.** *Porównanie stężenia TRAb, indeksów IO i CAS w grupie ATD i 131-I*

| Parameter | ATD median (min-max) | 131-I median (min-max) | Difference p-value |
|---|---|---|---|
| TRAb 0 [IU/L] | 5.6 (0.1–114.0) | 14.3 (0.6–90.0) | 0.0013 |
| TRAb 1 [IU/L] | 2.2 (0.01–51.9) | 7.7 (0.1–80.0) | 0.0001 |
| TRAb 6 [IU/L] | 1.6 (0.1–78.8) | 4.65 (0.1–96.0) | 0.0009 |
| TRAb 12 [IU/L] | 1.4 (0.1–75.3) | 3.65 (0.1–41.0) | 0.0129 |
| IO 0 | 5.0 (1.0–12.0) | 5.0 (2.0–9.0) | 0.7155 |
| IO 1 | 3.0 (0.0–10.0) | 4.0 (1.0–8.0) | 0.1610 |
| IO 6 | 3.0 (0.0–9.0) | 3.0 (0.0–7.0) | 0.8657 |
| IO 12 | 2.0 (0.0–8.0) | 2.0 (0.0–6.0) | 0.9461 |
| CAS 0 | 4.0 (0.0–7.0) | 4.0 (2.0–7.0) | 0.1588 |
| CAS 1 | 1.0 (0.0–7.0) | 2.0 (0.0–6.0) | 0.0151 |

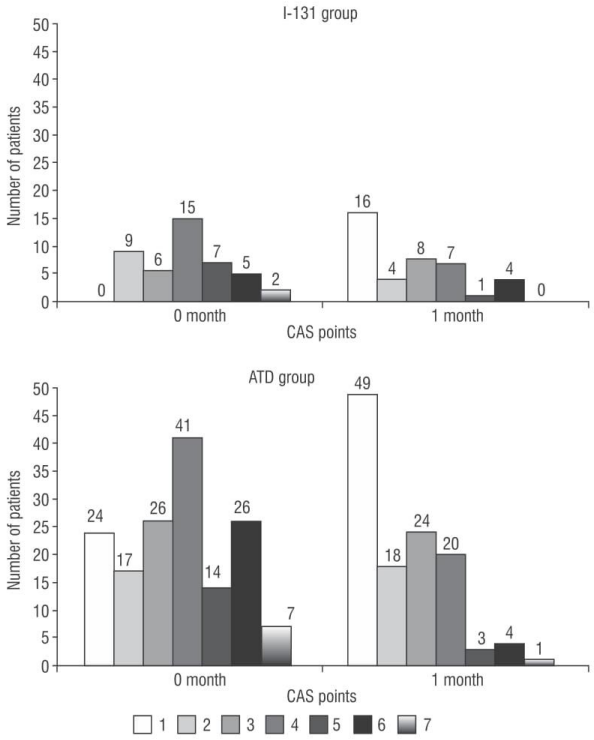


**Figure 1.** *Distribution of Clinical Activity Score (CAS) prior to and 1 month after GO treatment in the ATD and 131-I groups*
**Rycina 1**. *Rozkład indeksu CAS przed leczeniem i 1 miesiąc po leczeniu GO w grupie ATD i w grupie 131-I*

In the 131-I group, TSH and fT4 concentrations remained within normal range and did not differ significantly throughout the 12-month follow-up. The median TRAb-0 value prior to treatment was 14.3 IU/L (min = 0.6; max = 90.0) *vs.* TRAb-12 of 3.65 IU/L (min = 0.1; max = =41.0) ($p < 0.05$) (Table I). TRAb concentrations after 1, 6, and 12 months of treatment were significantly lower than the baseline level, as shown by t-test for paired

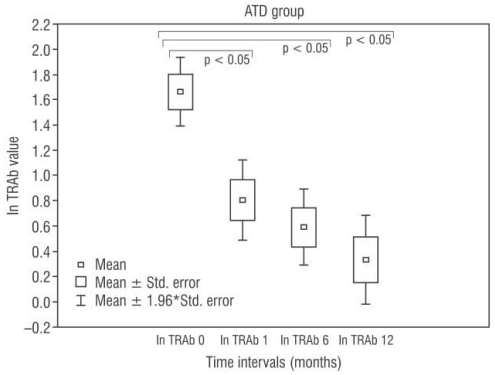


**Figure 2.** *TRAb concentration prior to, and 1, 6, and 12 months after treatment in the ADT group (t-test for paired samples, natural logarithms of variables)*

**Rycina 2.** *Stężenie TRAb przed leczeniem oraz po 1, 6, i 12 miesiącach w grupie ATD (test t dla prób zależnych dla zmiennych zlogarytmowanych)*

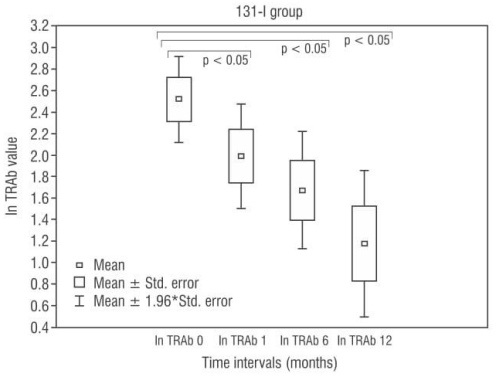


**Figure 3.** *TRAb concentration prior to, and 1, 6, and 12 months after treatment in the 131-I group (t-test for paired samples, natural logarithms of variables)*

**Rycina 3.** *Stężenie TRAb przed leczeniem oraz po 1, 6, i 12 miesiącach w grupie 131-I (test t dla prób zależnych dla zmiennych zlogarytmowanych)*

samples applied to natural logarithms of variables (Fig. 3) and Wilcoxon signed rank test ($p < 0.05$).

### *Comparison between ATD and 131-I groups (Table I)*

The activity and severity of GO in patients of the ATD and 131-I groups did not differ, despite differences in TRAb levels (Table I). We note that the percentage of patients after thyroidectomy in both groups did not differ significantly.

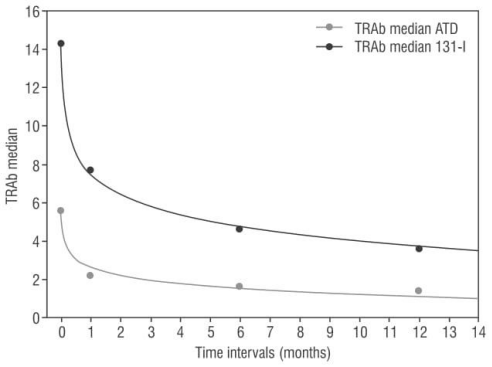


**Figure 4.** *Time-dependence of TRAb concentration over 12 months in the ATD group, $y = 2.6354–1.4099*log_{10}(x)$, and in the 131-I group, $y = 7.4554–3.4544*log_{10}(x)$*

**Rycina 4.** *Porównanie zmian stężenia TRAb w grupie ATD opisane równaniem $y = 2.6354–1.4099*log_{10}(x)$ i w grupie 131-I opisane równaniem $y = 7.4554–3.4544*log_{10}(x)$ w czasie 12-miesięcznej obserwacji*

After treatment, CAS-1 decreased significantly in both groups, with respect to CAS-0 ($p < 0.05$). The CAS-1 score was significantly higher in the 131-I group ($p < 0.05$).

Independently of the method of analysis chosen (mean or median), TRAb levels in the 131-I group were always above normal level and significantly higher than those in the ATD group throughout the follow-up period ($p < 0.05$) (Table I, Fig. 4).

In the ATD group the course of median TRAb level (y) *vs.* time, in months (x), could be approximated by the equation $y = 2.6354–1.4099*\log_{10}(x)$, while in the 131-I group the respective approximation was $y = 7.4554–3.4544*\log_{10}(x)$, as shown in Figure 4.

There was no significant difference in the course of IO between the ATD and 131-I groups (Table I). In the ATD group the course of the median IO (y) *vs.* time, in months (x), could be approximated by the expression $y = 4.0812*\exp(–0.0595*x)$. In the 131-I group the course of the median IO could be approximated by $y = 4.6481*\exp(–0.0713*x)$. The IO *vs.* time dependences over 12 months in both groups are shown in Figure 5.

Before treatment there was no significant difference between both groups with respect to age, sex, GO duration, IO, CAS-0 and fT4 levels. In the 131-I group, hyperthyroidism was significantly extended in time ($p < 0.05$), and pre-treatment levels of TSH were significantly higher than in the ATD group ($6.7 \pm 16.3$ *vs.* $2.34 \pm 8.81$ U/L, $p < 0.05$).

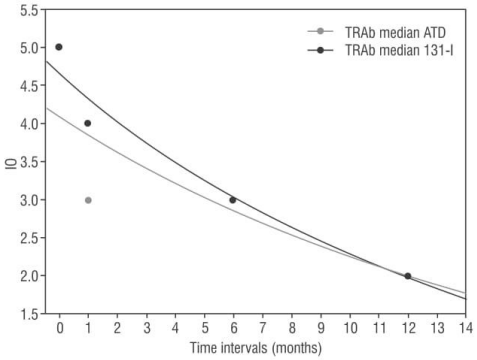


**Figure 5.** *Time-dependence of the IO over 12 months in the ATD group, y = 4.0812*exp (–0.0595*x), and in the 131-I group, y = 4.6481*exp (–0.0713*x)*

**Rycina 5.** *Porównanie zmian indeksów IO w grupie ATD opisane równaniem y = 4.0812*exp(–0.0595*x) i w grupie 131-I opisane równaniem y = 4.6481*exp(–0.0713*x) w czasie 12-miesięcznej obserwacji*

## Discussion

It is well known that radioiodine treatment may prolong thyrotropin receptor (TSH-R) autoimmunity in patients with Graves' disease, leading to *de novo* orbitopathy occurrence or to exacerbation of symptoms of concurrent GO. The likely pathogenesis of this phenomenon is the sudden release of thyroid antigens by 131-I-damaged thyroid cells, causing an increase in the production of autoantibodies, including TRAb [11, 13]. It is estimated that GO aggravation after 131-I treatment affects up to 20% of patients [11] and is more predominant in patients who had earlier manifested eye symptoms [2, 8] or in those with prolonged hypothyroidism following radioiodine treatment [17]. Male patients are more prone to GO exacerbation [18]. We also found significantly higher mean and median IO values in men in our ATD group over the follow-up period, suggesting a more severe and resistant course of GO in male patients. However, this trend was not observed in our 131-I group, presumably due to the small number of men (19.6%) in this group.

In a multi-centre study by Prummel et al. conducted in 2003 [19], proptosis was observed in 63% of newly referred patients with GO, while keratopathy was detected in 16% and optic nerve involvement in 21% of their patients. In our study, the percentage of the above-mentioned pathologies was comparable, except for DON, diagnosed in only 8.3% patients of our ATD group. The median values of IO prior to treatment and after 12 months of observation were 5 and 2, respectively, in both groups, thus the two groups did not differ with respect to severity, neither on admission to our Department nor at the end of the follow-up period, which is in agreement with a retrospective study conducted by Sisti et al. [20]. However, it is believed that patients after radioiodine treatment tend to develop more severe GO [11].

Thyroidectomy may have an impact on the course of GO. Weber et al. [21] observed either improvement or subsidence of eye symptoms after surgery. In contrast, our patients - despite their previous thyroidectomy — were still hyperthyroid and, for that reason, had been treated with methimazole or 131-I before admission to our Department to treat their GO exacerbation. Therefore, we assumed that prior thyroidectomy in these patients would not affect the efficacy of our GO treatment. We note that since the percentages of operated patients in the ATD and the 131-I groups did not differ, both groups are comparable with respect to the subject of our study.

Our treatment protocol, established in the year 2000, was based on our clinical experience and on published work available at the time, as summarised in the review of Zang et al. [22]. Like Macchia et al. in 2001 [23], we administered 1 g of methylprednisolone for two consecutive days each week, but we did not exceed the cumulative dose of 8.0 g.

In 2008 in the *Thyroid* journal, following reported cases of acute liver failure associated with high cumulative doses of methylprednisolone, EUGOGO recommended not to exceed a cumulative dose of methylprednisolone of 8.0 g [24]. By that time, we had completed our patient recruitment.

For orbital irradiation we applied a cumulative dose of 20 Gy, which was in line with the studies conducted by Marcocci et al. in 2001 [25] and by Ng et al. in 2005 [26].

We used a combination of methylprednisolone pulses followed by orbital irradiation, with good clinical outcome, low rate of recurrence, and no major clinical side effects. This treatment had already been proposed in 1983 by Bartalena et al. [27], who reported a better outcome of the combined therapy. Several studies confirmed the efficacy of orbital irradiation as based on the meta-analysis by Viani et al. [28], although Otsuka et al. [29] found no significant differences in terms of the therapeutic effect between groups treated with steroid pulses with or without orbital irradiation. There are no large RCTs comparing the advantage of this combined treatment over IV glucocorticosteroids alone [28, 30, 31]. As reported in a randomised study, lower orbital doses of 12 Gy were sufficient in reducing soft tissue changes, and higher doses were required in patients with ocular motility impairment [32]. It is generally accepted that orbital irradiation should be considered in patients with

diplopia and impaired motility [24, 26, 30, 32]. This has been confirmed by our clinical observation.

We found our treatment to be effective, since the rate of recurrence was low. Both groups responded well, and decreases in CAS and IO indices were significant. However, the CAS-1 score was significantly higher in the 131-I group, which might suggest that in patients with GO after radioiodine treatment glucocorticoid therapy is less effective. Following a recent multicentre randomised control trial [33], lower doses of IV glucocorticoids have been suggested. The efficacy and safety of different IV glucocorticosteroid therapy protocols are still under debate. Presently, there are no generally accepted recommendations or guidelines for treatment of GO [20, 34–36].

Elevation of TRAb blood concentration is an important GO risk factor [9]. TRAb levels increase after radioiodine treatment and remain elevated over considerably longer periods than in patients undergoing ATD therapy or surgery [10, 13, 14]. In our study, TRAb levels in the I-131 group were systematically above normal level and were always higher than those in the ATD group. In particular, we note that in our ATD group, TRAb levels returned to normal within one month after treatment.

We also note that despite the differences in TRAb levels, the efficacy of the applied immunosuppressive treatment was comparable in both studied groups. It is believed that higher TRAb titres are associated with more severe Graves' orbitopathy [9, 10]. However, this was not confirmed by our study. On the contrary, we found no statistically significant difference between the distribution of IO (which is relevant to the severity of GO) in the ATD and 131-I groups over respective time intervals.

## Conclusions

Treatment of Graves' Orbitopathy in its moderate to severe form remains difficult.

GO severity in the ATD and in the 131-I group of patients did not differ significantly over the course of their observation.

Although TRAb levels in the 131-I group were always above normal level and higher than those in the ATD group; the efficacy of GO treatment in both groups did not differ significantly.

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## 9.2 *Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy*

TERAPIE ZACHOWAWCZE | CONSERVATIVE TREATMENT

ARTYKUŁ ORYGINALNY | ORIGINAL RESEARCH STUDY

DOI: 10.24292/01.OT.190522

# Factors affecting the course of Graves' orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy

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**HIGHLIGHTS**

The course of Graves' Orbitopathy is affected by TRAb concentration. About 30% of patients receiving immunosuppressive treatment do not respond to the therapy. Patients with more severe Graves' Orbitopathy (NOSPECS >5) carry a higher risk of being a non-responder. Careful monitoring of GO patients and early referral to specialized centers is essential.

**ABSTRACT**

Graves' orbitopathy is a rare autoimmune disorder characterized by the inflammation of orbital tissues. The course of disease can be described in terms of its activity and severity.

**Aim:** The aim of our study was to determine the factors affecting the activity and severity of Graves' orbitopathy, as well as to identify the predictive factors of poor response to glucocorticoid treatment followed by orbital irradiation.

**Methods:** We performed a prospective observational study of 214 patients with Graves' orbitopathy who were divided into two groups depending on the treatment they had previously obtained for their Graves' disease. They received i.v. methylprednisolone pulses followed by orbital radiotherapy. They were examined and had their TSH, TRAb and $FT_4$ levels evaluated prior to treatment and after 1, 6 and 12 months.

**Results:** A pre-treatment TRAb concentration higher by one unit (U/L) implied a mean increase in the relative risk of active orbitopathy by 4.7% (p = 0.0362). A TRAb concentration higher by one U/L 1 month after treatment implied a mean increase in the relative risk of moderate-to-severe and severe GO by 8.7% (p = 0.0167) 6 months after treatment. As regards poor response to treatment, patients with moderate-to-severe and severe Graves' orbitopathy on admission carried a higher risk of being non-responders. Each point scored on the NOSPECS scale prior to treatment increased the relative risk of the patient being a non-responder by 30%.

**Conclusions:** Patients with higher TRAb levels have a higher risk of active Graves' orbitopathy and moderate-to-severe and severe Graves' orbitopathy. Monitoring TRAb serum concentration in those patients is of great importance. Patients with more severe Graves' orbitopathy carry a higher risk of being poor responders to immunosuppressive treatment. Therefore, careful monitoring of patients with Graves' orbitopathy and their early referral to specialized centers is essential.

**Key words:** Graves' orbitopathy, Graves' disease, TRAb, NOSPECS, glucocorticoid treatment, orbital radiotherapy

## INTRODUCTION

Graves' orbitopathy (GO) is an autoimmune disorder closely related to autoimmune thyroid disease. The autoimmune response is triggered by antibodies (TRAbs) against the thyroid-stimulating hormone receptor (TSH-R), which is expressed in orbital fibroblasts. This leads to an inflammatory infiltration of orbital tissues, increased production of glycosaminoglycans (GAGs) and adipogenesis. The insulin-like growth factor 1 receptor (IGF-1R) is also expressed by orbital fibroblasts and plays an essential role in GO pathogenesis [1–3]. The evolution of GO is self-limiting and may be described by the Rundle curve [4]. The first three phases of GO correspond to an active inflammation of orbital tissues. The first phase involves worsening signs and symptoms. During the second, plateau phase, no further exacerbation is observed. The third phase of gradual improvement is followed by the final, inactive phase with no further progression. As the active inflammation resolves, orbital tissue fibrosis ensues [5, 6].

Disease *activity* can be assessed by the Clinical Activity Score (CAS), which comprises the typical signs of inflammation: redness, pain, swelling and impaired function. The scored signs and symptoms include: eyelid swelling, eyelid erythema, conjunctival redness, chemosis, inflammation of the caruncle or plica, pain behind the globe and pain on gaze [7]. A CAS ≥ 3 indicates an active disease [8].

Within disease *severity* the functional and cosmetic changes related to the inflammation and fibrosis of the extraocular muscles and soft tissues are evaluated. The features considered in GO severity assessment are: palpebral aperture, soft-tissue involvement, exophthalmos, extraocular muscle dysfunction, corneal pathology and sight loss due to optic nerve compression. GO severity can be evaluated by means of the NOSPECS and EUGOGO classifications [9, 10].

Precise assessments of GO activity and severity are of great importance as they determine the choice of treatment. Surgical interventions, except for orbital decompression in dysthyroid optic neuropathy (DON), should not be undertaken while there is active inflammation of orbital tissues. On the other hand, all immunomodulatory therapies are of benefit during active GO [1, 11]. Patients with mild GO should be monitored, while patients with moderately severe and active GO should be treated with intravenous steroid pulses as a treatment of choice. Glucocorticoid treatment may be followed by orbital radiotherapy to enhance its efficacy [6, 12]. Both treatment modalities have an anti-inflammatory effect and have proved to be effective [13, 14].

Steroids induce anti-inflammatory proteins, inhibit key inflammatory mediators, decrease glycosaminoglycan production and suppress the function of immunocompetent cells [15]. They have a positive impact on visual acuity, orbital tissue oedema, inflammation of extraocular muscles and ocular motility [16]. Radiotherapy particularly improves ocular motility in GO patients by diminishing eye muscle enlargement and soft-tissue swelling [17]. It acts on lymphocytes, which are radiosensitive and infiltrate the orbit [18]. Radiotherapy also inhibits nitric oxide production, which reduces inflammatory pain and oedema [1].

Although effective in terms of reducing inflammation, both glucocorticoids and radiotherapy are not targeted treatments for GO. This may be the reason why ⅓ of all patients do not respond to these treatment modalities [6, 19, 20].

The aim of our study was to determine the factors affecting the activity and severity of GO and to search for predictive factors which determine poor response to glucocorticoid treatment followed by orbital irradiation. Since radioiodine ($^{131}$I) treatment for Graves' disease (GD) may result in *de novo* GO or may exacerbate the course of concurrent GO [21–23], we divided our patients into two groups, depending on the treatment they received for GD: the ATD group – treated with anti-thyroid drugs, and the $^{131}$I group – additionally treated with radioiodine.

## MATERIALS AND METHODS

We performed a prospective observational study of 214 patients treated between 2000 and 2008 for GO. The study was conducted in accordance with Helsinki Declaration. Patients gave their written informed consent to take part in the study and the study was approved by the Jagiellonian University Bioethics Committee. Patients were divided into two groups depending on the treatment they had previously received for their GD: a group, consisting of 168 patients, who had been treated with antithyroid drugs (the ATD group) and another, including 46 subjects, who had been treated with radioiodine (the $^{131}$I group). For a detailed description and comparison of the two groups see table 1 in our previous article [24].

Patients were examined by the same ophthalmologist and had their TSH, TRAb and $FT_4$ levels evaluated prior to treatment and after 1, 6 and 12 months. The activity and severity of GO were evaluated by means of CAS and NOSPECS classifications, respectively. GO severity was expressed by the Orbitopathy Index (IO). All patients were rendered euthyroid and received 1.0 g of methylprednisolone intravenously for 2 consecutive days each week, up to a total dose of 8.0 g. The treatment was followed by orbital irradiation with a total dose of 20 Gy in 10 daily fractions 1 month later.

We assumed that CAS ≥ 3 and IO ≥ 3 represented active GO and proceeded to search for factors affecting GO activity. Using logistic backward step-wise regression, we analysed whether radioiodine therapy, age, gender and pre-treatment TSH, $FT_4$ and TRAb (TSH-0, $FT_4$-0, TRAb-0) levels had any influence on GO activity.

We determined the factors affecting GO severity. We assumed that IO > 5 represents moderate to severe and severe

GO [25]. Using logistic regression, we evaluated the risk of moderate-to-severe and severe GO (IO > 5) with respect to TRAb concentration.

Finally, we analysed the relationship between CAS and IO using the chi-squared test for independence. Before treatment, all patients were divided into three groups with respect to IO (IO 1–2, IO 3–8, IO 9–15) [26] and assigned to subgroups of CAS ≤ 3 or CAS > 3.

In our study, patients who required additional treatment with oral glucocorticoids after intravenous methylprednisolone pulses and subsequent orbital radiotherapy were classified as non-responders. To identify the predictive factors of poor response to combined immunosuppressive treatment in both groups we performed a discriminant analysis with a backwards variable selection, which included age, gender, duration of hyperthyroidism, duration of GO, pre-treatment TSH, $FT_4$ and TRAb (TSH-0, $FT_4$-0, TRAb-0) levels, as well as GO activity and severity prior to treatment (CAS-0 and IO-0 respectively).

## RESULTS

We found that the only factors affecting GO activity in our patients were pre-treatment TRAb levels (TRAb-0) and age. As seen in table 1, TRAb-0 concentration higher by one unit (U/L) in both groups (ATD and $^{131}$I) analysed together, implied a mean increase in the relative risk of active orbitopathy of 4.7% (p = 0.0362). In turn, each year of age increased the mean relative risk of active GO by 2.8% (p = 0.0603).

TABLE 1

Influence of age and TRAb-0 on GO activity.

| Variable | n | Relative risk | 95% confidence interval | p |
|---|---|---|---|---|
| Age | 171 | 1.028 | 0.999–1.059 | 0.0603 |
| TRAb-0 | 171 | 1.047 | 1.003–1.092 | 0.0362 |

The correlation between TRAb concentration and GO severity expressed by the IO was investigated using Spearman's rank correlation coefficient. The values of TRAb concentration and their logarithms were analysed over all observation times. A positive correlation between TRAb and IO over time observation was found in both $^{131}$I and ATD groups. In both these groups, TRAb-0 concentrations prior to treatment correlated positively with TRAb concentrations after 1, 6 and 12 months (p < 0.05). A similar correlation was observed with respect to the ophthalmopathy index IO. Higher IO-0 and TRAB-0 values resulted in higher ophthalmopathy index values and TRAb concentrations over the time of observation.

As shown in table 2, in the $^{131}$I group, TRAb concentration 1 month after treatment (TRAb-1) correlated with the parallel IO-1 and was a good predictor of the IO value after 6 months (IO-6). TRAb-1 concentration higher by one U/L implied a mean increase in relative risk of moderate-to-severe and severe GO (IO-1 > 5) by 5.6 % (p = 0.0498), and of IO-6 > 5 by 8.7% (p = 0.0167).

TABLE 2

Risk of moderate to severe and severe GO (IO > 5) with respect to TRAb concentration prior to and 1 month after GO treatment – logistic regression. The coefficients with an asterix are statistically significant; p < 0.05.

| Independent variable | Dependent variable | n | Relative risk | 95% confidence interval | p |
|---|---|---|---|---|---|
| TRAb-0 | IO-0 > 5 | 40 | 1.009 | 0.980–1.039 | 0.5230 |
| | IO-1 > 5 | 38 | 1.019 | 0.988–1.051 | 0.2294 |
| | IO-6 > 5 | 36 | 1.049 | 1.005–1.094 | 0.0284* |
| | IO-12 > 5 | 35 | 0.971 | 0.847–1.114 | 0.6676 |
| TRAb-1 | IO-1 > 5 | 32 | 1.056 | 1.000–1.115 | 0.0498* |
| | IO-6 > 5 | 32 | 1.087 | 1.016–1.162 | 0.0167* |
| | IO-12 > 5 | 29 | 0.987 | 0.865–1.126 | 0.8405 |

*Coefficients with an asterix are statistically significant; p < 0.05.*

As shown in table 3, we also found that IO depends on CAS. All patients with very severe GO (IO 9-15) on admission had a CAS- 0 > 3 and all patients with mild GO (IO 1–2) on admission had a CAS-0 ≤ 3.

TABLE 3

Relationship between the CAS and IO index.

| IO-0 p = 0.0000 | CAS-0 ≤ 3 (n), (%) | CAS 0 > 3 (n), (%) |
|---|---|---|
| IO 1–2 | 10<br>10.99% | 0<br>0.00% |
| IO 3–8 | 81<br>89.01% | 106<br>90.60% |
| IO 9–15 | 0<br>0.00% | 11<br>9.40% |
| Total | 91<br>100% | 117<br>100% |

As regards poor response to treatment, 28% (13/46) of patients in the $^{131}I$ group were classified as non-responders. A discriminant analysis with backwards variable selection revealed that only TSH-0 (p = 0.0498) and IO-0 (p = 0.0574) remained as variables likely to predict patients' poor response to treatment in this group. Since the efficiency of correct classification was only 73%, TSH-0 and IO-0 were not the decisive factors. In the ATD group, 41% (69/167) of patients were classified as non-responders. The only variable likely to predict a patient's poor response to treatment in this group was IO-0 (p < 0.05). Since the efficiency of correct classification was only 60%, IO-0 was probably not a decisive factor. As shown in table 4, the final model of logistic regression revealed that each IO-0 point scored prior to treatment increased the relative risk of the patient being a poor responder by 30% (RR = 1.30; 95% CI 1.10–1.54; n = 163); p < 0.05.

FIGURE 1

Theoretical log-normal distributions of IO-0 in subgroups of patients who required (poor response, n = 82) or did not require (n = 131) oral glucocorticoid treatment after methylprednisolone pulses (total: n = 213 patients).

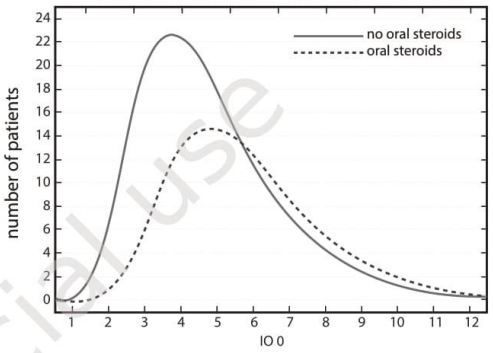


TABLE 4

Variables likely to predict a patient's poor response to treatment in the ATD group. Model of logistic regression. Coefficients with an asterix are statistically significant; p < 0.05.

| N = 109 | Gender | Age | Duration of GO | TSH-0 | FT4-0 | TRAb-0 | CAS-0 | IO-0 |
|---|---|---|---|---|---|---|---|---|
| RR | 1.08 | 0.98 | 1.00 | 1.01 | 1.01 | 1.01 | 0.70 | 1.87 |
| 95% CI | 0.41–2.88 | 0.94–1.02 | 0.98–1.02 | 0.93–1.10 | 0.97–1.05 | 0.99–1.03 | 0.46–1.07 | 1.25–2.79 |
| p | 0.8745 | 0.2470 | 0.8208 | 0.7300 | 0.5508 | 0.4423 | 0.0980 | 0.0020* |

**Coefficients with an asterix are statistically significant; p < 0.05.*

We analysed the empirical distribution and theoretical log-normal distributions of IO-0 of non-responders (n = 82) and responders (n = 131) in both groups obtaining a cut-off value of IO-0 > 5, which means that patients whose IO-0 exceeded 5 carried a higher risk of being a non-responder. As shown in figure 1, the proposed cut-off value takes into account that poor responders comprised approximately 40% of all patients (sensitivity 47%, specificity 71%, positive predictive value 51%, negative predictive value 68%).

## DISCUSSION

The objective of our study was to identify factors likely to influence GO activity, severity and poor response to combined immunosuppressive treatment (intravenous methylprednisolone pulses and subsequent orbital radiotherapy).

Previous studies have already found that TRAb concentration has an impact on the course of Graves' orbitopathy. Higher TRAb serum levels increase the risk of active GO and moderate-to-severe or severe GO [25]. We performed TRAb measurements using a second-generation thyrotropin-binding inhibitor immunoglobulin (TBII) assay (TRAK human by BRAHMS GmbH, Germany); however, TRAb can also be evaluated by means of different functional TSH-receptor stimulating immunoglobulin (TSI) bioassays [27].

In our study, a TRAb level higher by one unit (U/L) before treatment implied a mean increase in relative risk of active GO by 4.7%. Gerding et al. measured TSH binding inhibiting immunoglobins (TBII) and TSH-receptor stimulating immunoglobins (TSI) in 63 patients with GO. They compared 2 measurement modalities and found that TBII and TSI serum levels were strongly related to each other, as well as strongly correlated with the CAS value. Gerding et al. also found a correlation between proptosis and both TBII and TSI [28]. Similar results were obtained by Jang et al [27].

We found a positive correlation between TRAb and the severity of GO (IO) over the entire observation period. In the study by Eckstein et al., TBII concentrations were higher in subjects with severe GO than in subjects with mild GO during the entire observation time [25]. Just like in our study, the authors used a second-generation thyrotropin-binding inhibitor immunoglobulin (TBII) assay (TRAK human by BRAHMS GmbH, Germany) and established the TBII cut-off values for the prediction of severe GO. Prediction of se-

vere GO was possible after 4 months of observation, which is in line with our results. Our study showed that in the radioiodine-treated group, TRAb concentration 1 month after treatment was a good predictor of GO severity after 6 months. TRAb concentration higher by one U/L implied a mean increase in relative risk of moderate-to-severe and severe GO 6 months after treatment by 8.7% (tab. 2). These results again confirm the role TRAb play in the pathogenesis of Graves' orbitopathy. TRAb level before treatment is a better predictor for GO activity. High TRAb concentrations at the onset of Graves' orbitopathy may occur in patients with a mild and with a severe course of GO; however, in patients with a severe course of the disease, TRAb levels remain elevated for a considerably longer period of time. It should be noted, though, that in long-lasting GO, in an inactive fibrotic stage, TRAb levels no longer correlate with GO severity [28].

Given that a higher TRAb level is a risk factor of active and severe GO, careful monitoring of TRAb serum concentration in GO patients is essential.

The limitation of this study is the use of high-dose intravenous methylprednisolone pulses of 1 g for 2 consecutive days each week. This treatment protocol is no longer recommended. The reason for this choice of treatment in our study was that the data were collected between 2000 and 2008, when high-dose i.v. glucocorticoids were still commonly used [16, 29]. Since lower doses have the same or only slightly lower efficacy and a much lower rate of adverse events, the current regimen recommended by the European Group on Graves' Orbitopathy (EUGOGO) starts with 0.5 g of methylprednisolone once weekly for 6 weeks, followed by 0.25 g once weekly for 6 weeks, up to a cumulative dose of 4.5 g. The high-dose regimen is still used, but it is now reserved for more severe cases with diplopia. This regimen begins with 0.75 g once weekly for 6 weeks, followed by 0.5 g once weekly for 6 weeks, up to a cumulative dose of 7.5 g [6]. The cumulative dose is almost the same as in the old protocols (8 g), but it is more staggered and single doses are not administered on consecutive days, which increases treatment safety. The only exception is DON, which should be treated as follows: 0.5–1.0 g of methylprednisolone for 3 consecutive days for 2 weeks [6, 30].

Cumulative doses exceeding 8 g can cause acute liver damage and should be avoided [31, 32]. In our study, we did not exceed the cumulative dose of 8.0 g of methylprednisolone. Since the efficacy of higher doses of steroids is the same, even outperforming the new regimen in eye motility improvement, we believe that application of the old treatment modality does not influence the subject of our study [33].

Another disadvantage of this study is our failure to investigate our patients' smoking habits, as this could have affected our results. Smoking has been proven to be a risk factor for the occurrence of GO and for its progression following $^{131}$I treatment. It increases the risk of severe GO and reduces response to treatment. The effect of smoking depends on the daily number of cigarettes smoked [34].

As regards poor response to treatment, in our study 28% of patients after radioiodine therapy administered for GD and 41% of patients on antithyroid drugs were classified as non-responders. This is in line with other studies, which confirm that about one third of patients do not respond to steroid immunosuppressive treatment [6, 19, 20]. There might be several reasons for this relatively low response rate.

The first one might be a wrong interpretation of patients' response to therapy. For patients in the first phase of the Rundle curve, during which the signs and symptoms are naturally worsening, lack of evident improvement after immunosuppressive treatment may in fact reflect the inhibition or delay of GO progression. This implies that the treatment is in fact effective [1, 35]. Another explanation for the low response rate might be incorrect qualification of patients for immunosuppressive treatment. Patients with an inactive fibrotic disease with very advanced GO and severe symptoms are unlikely to respond to treatment [28]. Occasionally, these patients end up being treated with glucocorticoids even though they would better benefit from surgical approach [16]. This is why a very meticulous evaluation of disease activity using the Clinical Activity Score is so important. There are some patients, however, who score low in the CAS classification, yet their disease is visibly progressing. They present with a "white eye phenotype" with minimal signs or symptoms of inflammation, but with progressing proptosis, diplopia or restriction of eye movements. Some of these patients do not respond to steroids [36].

We aimed at identifying predictive factors of poor response to the applied treatment for GO. In the $^{131}$I group, the pre-treatment TSH level (TSH-0) and the severity of GO before treatment (IO-0) were the only variables likely to predict a patient's poor response to treatment. In the ATD group, the only factor influencing patients' response to treatment was IO-0. Each IO-0 point scored prior to treatment increased the relative risk of the patient being a poor responder by 30%. We obtained a cut-off value of IO-0 > 5 for being a non-responder, which means that patients whose IO-0 exceeded 5 carried a higher risk of being a non-responder.

Proptosis is one of the main symptoms of GO and plays an important role in assessing the severity of GO. Patients with more severe GO tend to have more pronounced proptosis. However, the beneficial effect of steroids and orbital radiotherapy on proptosis is limited [13, 17, 37, 38], which may be the reason why patients with more advanced GO were less likely to improve after treatment [39, 19]. In our study, 54% of patients in the $^{131}$I group and 53% of patients

in the ATD group had proptosis – a result which is similar to those reported in other studies [40, 41].

Other risk factors which increase the probability of a poor response to glucocorticoid treatment mentioned in the literature are smoking and a low-density lipoprotein cholesterol (LDLc) serum level exceeding 190 mg/dL [42, 43]. Early response to treatment is also a predictive factor of later response. In the study by Bartalena et al., patients who deteriorated at 6 weeks did not improve at 12 or 24 weeks. Of patients whose condition remained unchanged at 6 weeks, only 28% did later improve [35].

Both intravenous steroid pulses and orbital radiotherapy are based on non-specific immunosuppression, which may also explain the high rate of poor response to treatment. IV glucocorticoids affect dendritic and T-cells, inhibit the function and reduce the number of immune cells in orbital tissues. IV glucocorticoids also decrease the synthesis of prostaglandins and pro-inflammatory proteins [15]. Radiotherapy affects radiosensitive lymphocytes and fibroblasts in the orbit, thus reducing inflammation [13, 38].

Fortunately, a more targeted treatment already exists and will hopefully decrease the number of non-responders. The TSH-R and IGF-1-R play a crucial role in active inflammation, adipogenesis and increased production of glycosaminoglycans, leading to the expansion of ocular muscles and to the build-up of orbital fat [2, 3]. They form a signalling complex present in orbital fibroblasts that was not targeted by any previously available GO treatment until January 2020, when the US Food and Drug Administration (FDA) approved teprotumumab for the management of Graves' orbitopathy. Teprotumumab is a human IGF-1R inhibitory monoclonal antibody reacting with the IGF-1-R/TSH-R complex and attenuating the signalling initiated by IGF-1, TSH and thyroid-stimulating immunoglobulins [37]. Teprotumumab was tested in 2 randomised double-blind placebo-controlled multicentre trials [44], where 84 patients were treated with teprotumumab and 84 received placebo. They were followed up for 24 weeks. Patients in the teprotumumab group had a significant improvement in CAS and diplopia as compared to the placebo group. Moreover, 77% of patients in the active treatment group achieved a reduction in proptosis of at least 2 mm as compared to 13% in the placebo group [44].

Although these results are very promising, we still lack randomized studies comparing the efficacy of teprotumumab and i.v. glucocorticoids. The long-term efficacy and safety of teprotumumab remains unknown. Another clear obstacle is the cost of the new drug, which leaves steroids as a perhaps imperfect but current gold standard of treatment. Given that GO severity is a risk factor of poor response to immunosuppressive treatment, careful monitoring of GO patients and early referral to specialized centers is essential.

## CONCLUSIONS

Patients with higher TRAb levels have a higher risk of active GO.

Higher TRAb concentrations increase the risk of moderate-to-severe and severe GO.

Monitoring of TRAb serum concentration in patients with GO is essential.

Older patients have a higher risk of active GO.

Patients with a more active disease tend to have more severe GO.

Patients with more severe GO carry a higher risk of being poor responders to immunosuppressive treatment.

Careful monitoring of GO patients and early referral to specialized centers is essential.

A high percentage of GO patients do not respond to intravenous steroid pulses and orbital radiotherapy.

Fortunately, new more targeted treatments are already becoming available.

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**Authors' contributions:**
Agnieszka Jagiełło-Korzeniowska: conceptualization, analysis and interpretation of data, writing-original draft preparation. Andrzej Sokołowski: analysis and interpretation of data, writing-review and editing. Alicja Hubalewska-Dydejczyk: supervision, writing-review and editing. Bożena Romanowska-Dixon: supervision, writing-review and editing. Agata Bałdys-Waligórska: conceptualization, analysis and interpretation of data, supervision, writing-review and editing.
All authors have made the final approval of the version to be submitted.

**Conflict of interest:**
None.

**Financial support:**
The study was performed under statutory grant of the Jagiellonian University Medical College No WŁ/470/KL/L.

**Ethics:**
The content presented in the article complies with the principles of the Helsinki Declaration, EU directives and harmonized requirements for biomedical journals.

## 9.3 Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy

Journal of *Clinical Medicine*

MDPI

*Article*

# Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy

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**Abstract:** Background: The aim of the study was to perform a functional and structural evaluation of the anterior visual pathway in patients with Graves' Orbitopathy (GO) using electrophysiological tests and OCT, as well as to identify potential parameters that could be useful in detecting early optic nerve damage. Methods: 47 GO patients were enrolled in the study and divided into three groups, depending on their disease severity: Group 1 with mild GO, Group 2 with moderate-to-severe GO, and Group 3 with dysthyroid optic neuropathy (DON). Pattern visual evoked potential (PVEP), flash visual evoked potential (fVEP), pattern electroretinogram (pERG), and optical coherence tomography (OCT) findings were compared between the groups. Results: In the DON Group (Group 3), N75, P100, and P2 latencies were significantly extended, whereas P100, P50, and N95 amplitudes were significantly reduced as compared to the non-DON group (Groups 1 and 2). Group 3 also had significantly thinner peripapillary retinal nerve fiber layer (RNFL) and macular ganglion cell complex (GCC). In Group 2, as compared to Group 1, P100 amplitudes were significantly reduced for all check sizes, while P100 latency was elongated for the check size of 0.9°. Group 2 also had a significantly thinner average GCC and GCC in the superior quadrant. Conclusions: Electrophysiological examinations may be of use in diagnosis of DON. OCT findings and electrophysiological responses vary in patients with different GO severity. Including regular electrophysiological evaluation and OCT in the examination of patients with GO could be of benefit. However, more research is needed to establish the true significance of pVEP, fVEP, pERG, and OCT in monitoring patients with GO.

**Keywords:** Graves' Orbitopathy; dysthyroid optic neuropathy; visual evoked potential; pattern electroretinogram; optical coherence tomography; retinal nerve fiber layer; retinal ganglion cell complex



**Citation:** Jagiełło-Korzeniowska, A.; Bałdys-Waligórska, A.; Hubalewska-Dydejczyk, A.; Romanowska-Dixon, B. Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy. *J. Clin. Med.* **2022**, *11*, 4095. https://doi.org/10.3390/jcm11144095

Academic Editor: Ivo Guber

Received: 25 May 2022
Accepted: 13 July 2022
Published: 15 July 2022



## 1. Introduction

Graves' Orbitopathy (GO) is the most common extrathyroidal expression of Graves' disease (GD). It is an autoimmune, antibody-mediated disorder leading to the inflammation and remodeling of orbital tissues. Excessive production of glycosaminoglycans (GAGs), adipogenesis, oedema, and inflammatory infiltration induce muscle enlargement and orbital fat expansion within the constrained space of the orbit. This process may result in apical crowding and in the direct compression of the optic nerve or its blood supply [1].

Dysthyroid optic neuropathy (DON) is a rare condition with an insidious onset, affecting approximately 5–8% of patients with Graves' Orbitopathy. It requires urgent management as it can potentially lead to irreversible visual loss [2,3]. A thorough ophthalmological examination, including visual acuity and color vision assessment, pupillary test, perimetry, and fundus examination is needed to make a diagnosis of dysthyroid optic neuropathy. A relative pupillary defect (RAPD) and optic nerve edema are highly specific

of DON. However, RAPD may be absent in 50% of DON cases due to bilateral optic nerve involvement. Optic nerve oedema, in turn, is not present in 45–80% of DON cases [4,5]. Vision loss, which is the most common sign of DON, is highly unspecific. It may just as well result from ocular surface abnormalities associated with GO [6]. However, even a mild decrease in visual acuity or blurred vision should not be underestimated, as 50–70% of patients with confirmed DON have visual acuity of 20/40 or better [3]. The diagnosis of DON may be delayed at the subclinical stage, which is why electrophysiological assessment of the optic nerve and retinal ganglion cell function comprising flash and pattern visual evoked potentials (fVEP, pVEP) along with pattern electroretinogram (pERG) may be of use in monitoring patients with Graves' Orbitopathy. While pattern visual evoked potentials have been extensively studied, there are very few studies analyzing fVEP in patients with DON [7]. Several researchers have reported that patients with DON have prolonged N75 and P100 latencies as well as a decreased P100 amplitude [8,9]. Tsaloumas et al. observed a smaller amplitude of P2 in DON patients [9]. It has been postulated that electrophysiological examinations, in particular pVEP and pERG, can be used to detect the presence of subclinical DON. There are several studies comparing pVEP and pERG in GO patients without confirmed DON with healthy controls, but the results are inconsistent [10–13]. In addition to the above-mentioned electrophysiological techniques for evaluating the optic nerve and retinal ganglion cell function, morphological assessment by means of optical coherence tomography (OCT) may also be of use in monitoring patients with GO. OCT enables an analysis of the optic nerve head (ONH), retinal ganglion cell complex (GCC), and peripapillary retinal nerve fiber layer (RNFL). However, studies analyzing OCT findings in GO patients are scarce and their results are contradictory [14–16]. To the best of our knowledge, no attempts have been made so far to perform a simultaneous functional and structural assessment of the anterior visual pathway in patients with Graves' Orbitopathy.

The objective of our research was to perform a functional and morphological examination of the visual pathway in patients with Graves' Orbitopathy using pVEP, fVEP, pERG, and OCT, as well as to compare the results in patients with different degrees of severity of the disease.

## 2. Materials and Methods

47 patients with Graves' Orbitopathy were enrolled in the study, including 13 men (28%) and 34 women (72%). The patients' mean age was 52.1 years ± 14.8 SD. The research protocol was approved by the Jagiellonian University Bioethical Committee (Approval No. 1072.6120.163.2017) and the study adhered to the tenets of the Declaration of Helsinki. All patients signed a written informed consent. The exclusion criteria were other ocular diseases, high myopia greater than—6.0 D, intraocular pressure above 21 mmHg, neurological diseases (such as multiple sclerosis, Alzheimer's disease, Parkinson' s disease, and brain tumors), diabetes with polyneuropathy or diabetic retinopathy, and other diseases that could have an impact on electrophysiological or OCT examinations. The ophthalmological examination included visual acuity assessment, color vision assessment using Ishihara tables, tonometry, biomicroscopic anterior segment examination, cover test and Hess's screen, indirect fundus examination, Hertel exophthalmometry, and manual kinetic Goldmann perimetry. GO severity was evaluated according to the European Group on Graves' Orbitopathy (EUGOGO) classification (Table 1) [17]. Patients suspected of DON had orbital imagining with the measurements of particular extraocular muscles performed. Six patients had magnetic resonance imagining (MRI) and thirteen patients had Computed Tomography (CT) of the orbits. The electrophysiological examinations were performed using an EP-1000 device by TOMEY GmbH (Nuremberg, Germany). All examinations were conducted without pupil dilation. For the pVEP, the full-field, pattern-reversal protocol was used with four different check sizes, i.e., 0.4°, 0.9°, 1.5°, and 2.5°. Given that most patients with Graves' Orbitopathy have severe ocular surface abnormalities that influence visual acuity and cause blurred vision, we decided against using a small check size of 0.25°. We applied a mid check size of 0.4° instead and a large check size of 0.9° as stated in the In-

ternational Society for Clinical Electrophysiology of Vision (ISCEV) recommendations [18]. Additionally, very large checks of 1.5° and 2.5° were used in the study. The electrodes were placed as follows: the active electrode at Oz- 5 cm above the inion, the reference electrode at Fz- 11 cm above the nasion, and the ground electrode at the earlobe. Monocular stimulation was performed with full optical correction. Flash visual evoked potentials were elicited using the Ganzfeld bowl. The placement of electrodes was the same as in the pVEP examination. Monocular stimulation was performed with the fellow eye patched by a black obturator. We performed a transient pattern electroretinogram using the Ganzfeld bowl for stimulation. Binocular recording was conducted with an appropriate optical correction for the test distance. Fiber recording electrodes were positioned at the lower conjunctival fornix after topical anesthesia with proxymetacaine. The reference electrodes were placed on the skin at the outer canthus of each ipsilateral eye and the ground electrode was attached at the earlobe. The conditions of all the electrophysiological examinations were in line with ISCEV recommendations [18,19].

**Table 1.** Graves' Orbitopathy (GO) severity assessment according to European Group on Graves' Orbitopathy (EUGOGO).

| **EUGOGO Classification:** |
| --- |
| **Mild GO:** Patients whose features of GO have only a minor impact on daily life that have insufficient impact to justify immunomodulation or surgical treatment. They usually have one or more of the following: minor lid retraction (<2 mm), mild soft-tissue involvement, exophthalmos <3 mm above normal for race and gender, no or intermittent diplopia, and corneal exposure responsive to lubricants |
| **Moderate-to-severe GO:** Patients without sight-threatening GO whose eye disease has sufficient impact on daily life to justify the risks of immunosuppression (if active) or surgical intervention (if inactive). They usually have two or more of the following: lid retraction $\geq$ 2 mm, moderate or severe soft-tissue involvement, exophthalmos $\geq$ 3 mm above normal for race and gender, inconstant or constant diplopia |
| **Sight-threatening (very severe) GO:** Patients with dysthyroid optic neuropathy and/or corneal breakdown |

Optical coherence tomography was performed after pupil dilation. An RTVue OCT device (Model RT-100, version 6.3, OPTOVUE, Fremont, CA, USA) was used. An RNFL scan of 3.45 diameters, centered on the optic nerve head, was performed. The analysis of the peripapillary retinal nerve fiber layer thickness was presented in µm as Average RNFL, Superior RNFL, and Inferior RNFL.

The greatest number of ganglion cells is found in the central 8 degrees of the retina; therefore, a macular ganglion cell complex (GCC) analysis was performed. The GCC consists of 3 layers of the retina, i.e., the retinal nerve fiber layer, the retinal ganglion cell layer, and the inner plexiform layer. The GCC protocol comprised 15 vertical scans and 1 horizontal scan covering an area of 7 by 7 mm, localized 1mm temporally from the macula. The analysis of GCC thickness was presented in µm as Average GCC, Superior GCC, and Inferior GCC. Additionally, the Focal Loss Volume (FLV) and Global Loss Volume (GLV) were analyzed.

The patients were divided according to the EUGOGO severity classification: into mild (Group 1), moderate-to-severe (Group 2), and sight-threatening (Group 3). The groups comprised 16, 23, and 8 patients, respectively. We compared the results of electrophysiological examinations and OCT of patients with no clinical evidence of DON (Groups 1 and 2) with patients with confirmed DON (Group 3). Subsequently, we compared patients with mild GO (Group 1) with patients with moderate-to-severe GO (Group 2) in terms of their electrophysiological responses and OCT parameters.

*Statistical Analysis*

A Student's t-test for independent samples was used to test differences between groups of patients. To test if the variances of two populations are equal, an F-test for equality of two variances was used additionally. Age and sex differences of patients between the groups were estimated using Student's *t*-test and Mann–Whitney's u-test (for independent samples).

## 3. Results

### *3.1. Comparison between GO Patients with No Clinical Evidence of DON (Groups 1 and 2) and Patients with Confirmed DON (Group 3)*

The non-DON Group (Groups 1 and 2) comprised of 39 patients (9 men and 30 women). The mean age of patients in this group was 50.4 ± 14.7. Group 3 comprised 8 patients (4 men and 4 women). The mean age of patients in this group was 60.5 ± 12.8. The differences between the groups in terms of patients' sex and age were statistically insignificant ($p = 0.2402$ and $p = 0.0771$, respectively)

#### 3.1.1. Pattern Visual Evoked Potentials

We compared the latencies of N75 and P100, as well as the amplitudes of P100, in confirmed DON patients (Group 3) with GO patients with no clinical evidence of DON (Groups 1 and 2). The comparison revealed a significant increase in N75 and P100 latencies for all check sizes and a significant reduction of P100 amplitudes for all check sizes except for check size of 1.5° in Group 3 (Table 2). The differences in latencies were much more pronounced than the differences in P100 amplitudes.

**Table 2.** A comparison of visual evoked potential (VEP) components between GO patients with no clinical evidence of dysthyroid optic neuropathy (DON) (Groups 1 and 2) and patients with confirmed DON (Group 3).

| Parameter | Check Size | GROUPS 1 and 2 NON-DON (Mean ± SD) | GROUP 3 DON (Mean ± SD) | *p-Value* |
|---|---|---|---|---|
| N75 latency (ms) | *0.4°* | 69.1 ± 6.6 | 79 ± 11.9 | *p = 0.0001* * |
| | *0.9°* | 64.3 ± 6.7 | 75.6 ± 8.2 | *p < 0.0001* * |
| | *1.5°* | 62.8 ± 9.0 | 79.6 ± 15.2 | *p < 0.0001* * |
| | *2.5°* | 64.1 ± 12.3 | 83.5 ± 16.6 | *p < 0.0001* * |
| P100 latency (ms) | *0.4°* | 95.8 ± 8.6 | 111.2 ± 13.3 | *p < 0.0001* * |
| | *0.9°* | 94.5 ± 8.5 | 110.3 ± 15.0 | *p < 0.0001* * |
| | *1.5°* | 94.0 ± 10.7 | 110.6 ± 13.8 | *p < 0.0001* * |
| | *2.5°* | 94.0 ± 12.5 | 110.8 ± 14.4 | *p = 0.0001* * |
| P100 amplitude (μV) | *0.4°* | 11.5 ± 6.0 | 7.5 ± 3.8 | *p = 0.0317* * |
| | *0.9°* | 10.7 ± 5.9 | 7.4 ± 3.1 | *p = 0.0414* * |
| | *1.5°* | 9.9 ± 5.0 | 7.2 ± 2.9 | *p = 0.0607* |
| | *2.5°* | 9.0 ± 4.0 | 5.7 ± 3.4 | *p = 0.0089* * |

* Statistically significant results are marked with an asterisk.

#### 3.1.2. Flash Visual Evoked Potentials

As for fVEP, the mean latency of P2 was 133.4 ± 16 ms vs. 118.0 ± 13.7 ms ($p = 0.0001$) in the DON group (Group 3) and in the non-DON Group (Groups 1 and 2), respectively. There was no statistically significant difference in the P2 amplitude between the two groups (10.1 ± 7.0 vs. 12.0 ± 6.5 $p = 0.2828$).

#### 3.1.3. Pattern Electroretinogram

There was a significant reduction in N95 and P50 amplitudes in Group 3 (Table 3). P50 latency was significantly delayed whereas the latency of N95 was shorter in Group 3 (Table 3).

**Table 3.** A comparison of pattern electroretinogram (PERG) components between GO patients with no clinical evidence of DON (Groups 1 and 2) and patients with confirmed DON (Group 3).

| Parameter | GROUPS 1 and 2 NON-DON (Mean ± SD) | GROUP 3 DON (Mean ± SD) | *p-Value* |
|---|---|---|---|
| N95 amplitude (μV) | 8.3 ± 4.1 | 4.0 ± 2.3 | *p = 0.0001* * |
| P50 amplitude (μV) | 6.3 ± 2.8 | 3.5 ± 2.0 | *p = 0.0003* * |
| P50 latency (ms) | 49.4 ± 4.8 | 54.3 ± 8.9 | *p = 0.0022* * |
| N95 latency (ms) | 101.5 ± 11.4 | 93.8 ± 15.8 | *p = 0.0244* * |

* Statistically significant results are marked with an asterisk.

#### 3.1.4. Optical Coherence Tomography

A macular ganglion cell complex analysis revealed significant differences between the groups. The average GCC as well as inferior and superior quadrant GCC were significantly thinner in Group 3 (Table 4). In contrast, FLV and GLV indices representing the focal and global ganglion cell loss volume, respectively, were significantly greater in Group 3. The average peripapillary RNFL thickness as well as the RNFL thickness in the superior and inferior quadrants were also significantly smaller in Group 3; however, these differences were not so pronounced (Table 4).

**Table 4.** A comparison of optical coherence tomography (OCT) parameters between GO patients with no clinical evidence of DON (Groups 1 and 2) and patients with confirmed DON (Group 3).

| Parameter | GROUPS 1 and 2 NON-DON (Mean ± SD) | GROUP 3 DON (Mean ± SD) | *p-Value* |
|---|---|---|---|
| Average RNFL (μm) | 108.2 ± 9.6 | 99.3 ± 17.2 | *p = 0.0069* * |
| Superior RNFL (μm) | 107.0 ± 10.2 | 98.2 ± 21.4 | *p = 0.0178* * |
| Inferior RNFL (μm) | 109.4 ± 11.8 | 100.4 ± 14.7 | *p = 0.0137* * |
| Average GCC (μm) | 95.7 ± 5.9 | 83.8 ± 7.9 | *p < 0.0001* * |
| Superior GCC (μm) | 95.1 ± 6.5 | 84.1 ± 9.4 | *p < 0.0001* * |
| Inferior GCC (μm) | 96.5 ± 6.0 | 83.6 ± 8.7 | *p < 0.0001* * |
| FLV (%) | 0.7 ± 0.9 | 3.6 ± 3.8 | *p < 0.0001* * |
| GLV (%) | 3.7 ± 3.2 | 14.6 ± 7.4 | *p < 0.0001* * |

* Statistically significant results are marked with an asterisk.

### *3.2. Comparison between MILD GO Patients (Group 1) and MODERATE-TO-SEVERE GO Patients (Group 2)*

Group 1 comprised of 16 patients (1 man and 15 women). The mean age of patients in this group was 47.1 ± 16.2.

Group 2 comprised of 23 patients (8 men and 15 women). The mean age of patients in this group was 52.7 ± 13.5. There were more men in Group 2 ($p = 0.0420$), which was to be expected since men tend to have more severe GO. The percentage of men in the groups in this study increased along with GO severity. The age differences between the groups were statistically insignificant ($p = 0.2490$).

#### 3.2.1. Pattern Visual Evoked Potentials

We found that P100 amplitudes were significantly reduced in patients with moderate-to-severe GO for all check sizes. There was a statistically elongated P100 latency for check size of 0.9° in those patients. However, the differences in P100 amplitudes were much more pronounced. (Table 5). There were no differences in N75 latencies between the groups.

**Table 5.** A comparison of VEP components between GO patients with moderate-to-severe GO (Group 2) and patients with mild GO (Group 1).

| Parameter | Check Size | Group 2 (Mean ± SD) | Group 1 (Mean ± SD) | *p-Value* |
|---|---|---|---|---|
| P100 latency (ms) | *0.4°* | 96.6 ± 9.0 | 94.7 ± 8.2 | *p = 0.3427* |
| | *0.9°* | 96.2 ± 9.4 | 92.1 ± 6.6 | *p = 0.0443* * |
| | *1.5°* | 95.5 ± 12.0 | 92.0 ± 8.8 | *p = 0.1770* |
| | *2.5°* | 94.9 ± 14.6 | 93.0 ± 9.5 | *p = 0.5562* |
| P100 amplitude (μV) | *0.4°* | 9.4 ± 3.9 | 14.5 ± 7.0 | *p = 0.0001* * |
| | *0.9°* | 8.6 ± 3.6 | 13.6 ± 7.0 | *p = 0.0002* * |
| | *1.5°* | 7.9 ± 3.5 | 12.2 ± 5.5 | *p = 0.0002* * |
| | *2.5°* | 7.6 ± 3.6 | 10.8 ± 3.9 | *p = 0.0014* * |

* Statistically significant results are marked with an asterisk.

#### 3.2.2. Flash Visual Evoked Potentials

The groups did not differ in terms of P2 amplitude and latency.

#### 3.2.3. Pattern Electroretinogram

The groups did not differ in terms of P50 or N95 amplitudes and latencies.

#### 3.2.4. Optical Coherence Tomography

The groups differed in terms of the average GCC and GCC in the superior quadrant.

There were no statistically significant differences in terms of inferior quadrant GCC, peripapillary RNFL, FLV, or GLV (Table 6).

**Table 6.** A comparison of OCT parameters between GO patients with moderate-to-severe GO (Group 2) and patients with mild GO (Group 1).

| Parameter | Group 2 (Mean ± SD) | Group 1 (Mean ± SD) | *p-Value* |
|---|---|---|---|
| Average GCC (μm) | 94.5 ± 5.6 | 97.6 ± 5.9 | $p = 0.0282$ |
| Superior GCC (μm) | 93.6 ± 6.2 | 97.4 ± 6.3 | $p = 0.0138$ |

## 4. Discussion

In the present study, we performed a thorough investigation of the functional and structural changes of the visual pathway in patients with Graves' Orbitopathy. We found significant differences in electrophysiological responses and OCT parameters in patients with different GO severity.

A visual evoked potential is produced by activated neurons of the occipital cortex. It is generated in response to visual stimulation: a flashing light (fVEP) or an alternating checkboard pattern (pVEP). The fVEP consists of several positive and negative components, among which P2 is the most commonly used in clinical assessment. We found a significantly prolonged P2 latency in patients with dysthyroid optic neuropathy, as compared to GO patients without evident signs of DON (133.4 ± 16 ms vs. 118.0 ± 13.7 ms), but we did not find any differences in P2 amplitudes between the groups (10.1 ± 7.0 vs. 12.0 ± 6.5 $p = 0.2828$). Our results are in contradiction to those of Tsaloumas et al., who reported no

difference in P2 latency between the DON and thyroid-associated orbitopathy (TAO) groups (112.0 ± 4.46 ms vs. 110.1 ± 2.65 ms) but found a significantly smaller P2 amplitude in DON patients (6.83 ± 0.92 ms vs. 12.4 ± 1.05 μV). Tsaloumas et al. admitted, however, that in their study, reductions in amplitude occurred more frequently than delays as compared to other studies [9]. Our results are in line with Setälä et al., who identified a significant increase in the latency of the main positive fVEP component in patients requiring orbital decompression [20]. Studies concerning the fVEP in patients with GO are scarce, however, and they are also difficult to compare due to the heterogenous conditions under which the examinations were conducted. The fVEP is less sensitive and more variable between individuals than the pVEP, which might be the reason why it is less commonly used in research. However, it has some advantages over the pVEP, being less dependent on the macular input and patients' cooperation. It may be particularly useful in some patients with GO, who have severe ocular surface changes, poor visual acuity, or symptoms like tearing and diplopia, which impede proper concentration and make it difficult to obtain a reliable response to checkboard stimuli [21,22].

We did not find any differences in fVEP responses between patients with mild and moderate-to-severe GO.

The pVEP is a triphasic waveform with a negative N75, positive P100 and negative N135 components. P100 latency and amplitude are the most commonly used parameters in clinical practice. In the present study, pVEP examination in patients with DON revealed significantly extended N75 and P100 latencies, as well as significantly reduced P100 amplitudes, as compared to GO patients without DON symptoms. This was in accordance with previous studies [7,9,23]. While comparing the mild GO group and the moderate-to-severe GO group, we found notably reduced P100 amplitudes and prolonged P100 latency for 0.9 check size in the latter group. We did not find any changes in N75 latencies. Previous reports in this matter are inconsistent. Shawkat et al. and Tsaloumas et al. found no differences in pVEP responses between GO patients and normal controls, whereas Salvi et al. and Acaroğlu et al. found significant delays of the P100 component [7,9,10,24]. Pawłowski et al. described both N75 and P100 latency increase in GO patients without any symptoms of optic nerve dysfunction in comparison to healthy controls [12]. In contrast, Ambrosio et al. emphasized the importance of the P100 amplitude as a sensitive indicator of compressive nerve damage in patients with GO and concomitant glaucoma [8]. The discrepancies found in the earlier reports may result from the fact that the authors failed to take account of GO severity. There were some attempts to correlate proptosis with electrophysiological findings; however, proptosis only represents one of the factors assessed in GO severity classifications [12,25]. We believe that patients with a less severe disease may demonstrate no changes in electrophysiological responses, their optic nerve may not be endangered, and they may not differ from normal controls. The situation, however, may change insidiously along with GO progression.

It was shown in several studies that spatial frequency (check size) has an impact on pVEP responses [26]. Some authors emphasize that higher spatial frequencies target more foveal retinal ganglion cells, whereas larger checks stimulate better peripheral vision [21,27,28]. We used additional low spatial frequencies in our protocol to see if they could be of use in monitoring patients with GO. We were unable to confirm that using larger check sizes when performing a pVEP examination in patients with Graves' Orbitopathy is more beneficial in everyday practice than the standard procedure. Also, pVEP latencies for all check sizes were prolonged in patients with DON in comparison to patients without clinical features of DON, while pVEP amplitudes were reduced in the moderate-to-severe GO group as compared to mild GO group regardless of the check size used for the examination. The P100 latency in patients with moderate-to-severe GO was prolonged only for a check size of 0.9° as compared to patients with mild GO. Therefore, higher spatial frequencies seem to be more suitable for detecting subclinical, functional changes in the optic nerve in patients with GO, which is in line with other studies [8,29]. However, we

do not find that longer pVEP protocols with different check sizes are of benefit in patients with GO, who often experience severe ocular surface abnormalities.

The pattern visual evoked potential is not specific to optic nerve damage as it depends on good macular function. Pattern electroretinogram is a complementary technique to visual evoked potentials. It enables simultaneous assessment of retinal ganglion cell and macular function. The P50 component of the pERG is generated by inner- and outer-retinal neurons, whereas the N95 component represents retinal ganglion cell function [29,30]. To the best of our knowledge there are very few studies analyzing pERG in patients with GO and no studies whatsoever describing pERG in patients with DON.

We found significantly reduced P50 and N95 amplitudes in patients with DON as compared to GO patients without confirmed DON. The P50 latency was significantly prolonged, whereas the N95 latency was reduced in those patients. The differences in latencies, however, were much less pronounced than those in amplitudes (Table 2) and were absent when the right and the left eye were compared separately. Pawłowski et al. postulated that the P50 component could be used as a marker of early optic nerve dysfunction in GO patients, as in their study, its amplitude was reduced in GO patients without evident DON [13]. We did not find any differences in PERG parameters while comparing patients with moderate-to-severe GO (Group 2) and patients with mild GO (Group 1). Our results are in line with those obtained by Spadea et al. They reported that patients with very severe GO had a significant reduction in pERG and pVEP amplitudes and a significant increase in pVEP latencies, whereas patients with less severe GO only demonstrated a reduction in the P100 amplitude [11]. They suggested that a pVEP amplitude reduction represented a decrease in normally functioning optic nerve fibers, thus being more sensitive in early optic nerve dysfunction. Meanwhile, they attributed the pVEP delay and pERG amplitude reduction to retrograde axonal degeneration. Some authors emphasize that dysthyroid optic neuropathy is not only a compressive optic neuropathy characterized by direct nerve compression impairing axoplasmic flow, but also a sort of ischemic optic neuropathy with compromised vascular perfusion due to enlarged ocular muscles and increased intraorbital pressure [13]. The pVEP amplitude reduction in patients with moderate-to-severe GO may be due to early ischemic changes in the optic nerve [29–34]. The P50 component of the pERG may also occasionally be affected in eyes with ischemic optic neuropathy, suggesting a dysfunction at the level of macular photoreceptors or bipolar cells [29].

In patients with DON, a significant reduction in P50 amplitude may be secondary to retrograde damage of the retinal ganglion cells or may be due to ischemic changes in the outer retinal layers. The elongated P50 latency, which is characteristic of macular involvement, is more suggestive of the latter [35,36]. We cannot explain the N95 latency reduction in patients with DON.

Our analysis of OCT parameters in GO patients revealed that patients with DON had a significantly thinner average peripapillary RNFL, as well as RNFL in the upper and lower quadrants. Our results are in agreement with the study by Park et al., who reported a significantly smaller mean temporal peripapillary RNFL thickness in patients with long-lasting DON ($\geq$6 months) in comparison to healthy controls and patients with acute DON [14]. In contrast, Meirovitch et al. found increased peripapillary RNFL thickness in superior, inferior, and nasal quadrants in patients with GO [16]. Peripapillary RNFL is not a reliable indicator of axon loss in patients with Graves' Orbitopathy. The compression of the optic nerve by overgrown orbital tissues may impede axoplasmic flow, leading to the swelling of the axons. RNFL thickening may be misleading in accurate neuronal loss assessment [37]. The discrepancies between previous studies may result from the fact that patients at different stages of the disease were enrolled in the studies. Increased RNFL thickness in patients with GO may indicate disc edema, which may not be detectable in fundus examination, whereas RNFL thinning in patients with DON may indicate the onset of the optic nerve atrophy.

When comparing the macular ganglion cell complex in DON patients and in GO patients without any clinical signs of DON, we found significant differences in all the

parameters. The average GCC, as well as inferior and superior quadrant GCC, were significantly thinner in DON patients. Both the foveal loss volume (FLV) and the global loss volume (GLV) were significantly increased in DON patients. Our results are in line with the study by Romano et al., who compared patients with optic nerve compression due to GO with normal controls and found a significantly thinner average GCC, inferior GCC, as well as significantly thinner RNFL in the superior and inferior quadrant in the first group [38]. Since the retinal ganglion cell complex is not affected by axon swelling, its analysis is better suited for assessing axon loss in the course of GO than RNFL [37]. In our study, the differences in GCC between patients with DON and GO patients were much more pronounced than those in RNFL thickness. The differences in RNFL were not statistically significant when analyzed for the left and for the right eye separately. Additionally, while comparing OCT parameters in patients with mild GO and moderate-to-severe GO, the only statistically significant differences that we found between the groups were in average GCC and GCC in the superior quadrant.

Orbital MRI was not performed in each patient, which is a limitation of this study as such scans would have revealed the factors which contributed to the changes found in the electrophysiological tests. Moreover, an additional comparison with a healthy control group would have given us a more complete picture of the morphological and functional changes in patients with GO.

## 5. Conclusions

Dysthyroid optic neuropathy may have an insidious onset. Hence, assessing optic nerve function in electrophysiological examinations may be of use in the diagnosis of DON. In our study, we observed significantly reduced P100, P50, and N95 amplitudes, as well as significant increased N75, P100, and P2 latencies in patients with DON. Combining pVEP with pERG may be helpful in distinguishing between changes resulting from macular and ganglion cell dysfunction. fVEP may be useful in patients with severe eye surface disorders and corneal or lens opacities. Previous studies have shown that changes in electrophysiological and OCT results are likely to be present in GO patients without clinical signs of DON. In this study, patients with moderate-to-severe GO had significantly reduced P100 amplitudes, a significantly longer P100 latency for the check size of 0.9°, and reduced average and superior GCC thickness as compared to patients with mild GO. Therefore, we conclude that electrophysiological responses and OCT parameters vary in patients with different GO severity. Including regular electrophysiological evaluation and OCT in the examination of patients with GO could be of benefit. However, more research is needed to establish the true significance of pVEP, fVEP, pERG, and OCT in monitoring patients with GO.

**Author Contributions:** Conceptualization, A.J.-K. and A.B.-W.; methodology, A.J.-K. and B.R.-D.; validation, A.J.-K. and B.R.-D.; formal analysis, A.J.-K.; investigation, A.J.-K.; resources, B.R.-D. and A.H.-D.; data curation, A.J.-K.; writing—original draft preparation, A.J.-K.; writing—review and editing, A.B.-W., B.R.-D., A.H.-D. and A.J.-K.; supervision, A.B.-W. and B.R.-D. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki, and approved by the Jagiellonian University Bioethical Committee, Kraków, Poland (Approval No. 1072.6120.163.2017, 29 September 2017).

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

**Data Availability Statement:** Not applicable.

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

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# 10 Oświadczenia współautorów

Kraków, dnia 5 grudnia 2022 r.

Prof. dr hab. Andrzej Sokołowski

**OŚWIADCZENIE**

Jako współautor pracy pt. **„The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to przeprowadzenie analizy statystycznej otrzymanych wyników i nadzór merytoryczny nad odpowiednią ich interpretacją. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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Kraków, dnia 06.12.2022 r.

Dr n. med. Anna Krzentowska-Korek

**OŚWIADCZENIE**

Jako współautor pracy pt. **„The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to pomoc przy opracowywaniu bazy danych. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

Anna Krzentowska-Korek

/podpis współautora/

Kraków, dnia 02.11.22

Lek. med. Grażyna Miklaszewska

**OŚWIADCZENIE**

Jako współautor pracy pt. **„The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to przeprowadzenie badań okulistycznych. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

lek. med. Grażyna Miklaszewska

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/podpis współautora/

Kraków, dnia 21.01.2023

Dr hab. n. med
Agata Bałdys- Waligórska

**OŚWIADCZENIE**

Jako współautor pracy pt. **„The efficacy of immunosuppressive treatment of Graves' orbitopathy is not affected by previous anti-thyroid drugs or by radioiodine therapy of Graves' disease."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to prowadzenie pacjentów z orbitopatią tarczycową będących przedmiotem badania, pomoc przy opracowaniu koncepcji badania oraz udział w opracowaniu ostatecznej wersji artykułu. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.
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/podpis współautora/

Kraków, dnia 5 grudnia 2022 r.

Prof. dr hab. Andrzej Sokołowski

**OŚWIADCZENIE**

Jako współautor pracy pt. **„ Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to przeprowadzenie analizy statystycznej otrzymanych wyników i nadzór merytoryczny nad odpowiednią ich interpretacją. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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/podpis współautora/

Kraków, dnia 16.12.2022

Prof. dr hab. n. med. Alicja
Hubalewska-Dydejczyk

**OŚWIADCZENIE**

Jako współautor pracy pt. **„ Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to nadzór nad badaniami przeprowadzanymi u pacjentów w Oddziale Endokrynologii oraz przegląd przygotowanego artykułu pod kątem istotnej zawartości intelektualnej. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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/podpis współautora/

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Prof. dr hab. n. med. Bożena
Romanowska-Dixon

**OŚWIADCZENIE**

Jako współautor pracy pt. **„ Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to nadzór nad przeprowadzanymi badaniami okulistycznymi oraz przegląd przygotowanego artykułu pod kątem istotnej zawartości intelektualnej. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

B. Romanowska-Dixon

/podpis współautora/

Kraków, dnia 04.01.2023

Dr hab. n. med
Agata Bałdys- Waligórska

**OŚWIADCZENIE**

Jako współautor pracy pt. **„ Factors affecting the course of Graves' Orbitopathy and poor response to glucocorticoid treatment followed by orbital radiotherapy."** oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to prowadzenie pacjentów z orbitopatią tarczycową będących przedmiotem badania, pomoc przy opracowaniu koncepcji badania oraz udział w opracowaniu ostatecznej wersji artykułu. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

A. Wal...

/podpis współautora/

Kraków, dnia 04.01.2023

Dr hab. n. med
Agata Bałdys- Waligórska

**OŚWIADCZENIE**

Jako współautor pracy pt. **„Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy ."**oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to pomoc przy opracowaniu koncepcji badania oraz udział w opracowaniu ostatecznej wersji artykułu. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.
Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek. Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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/podpis współautora/

Kraków, dnia 16.12.2022

Prof. dr hab. n. med. Alicja
Hubalewska-Dydejczyk

**OŚWIADCZENIE**

Jako współautor pracy pt. **„Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy ."**oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to nadzór nad badaniami przeprowadzanymi u pacjentów w Oddziale Endokrynologii oraz przegląd przygotowanego artykułu pod kątem istotnej zawartości intelektualnej. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych. Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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/podpis współautora/

Kraków, dnia 5.12.22

Prof. dr hab. n. med. Bożena
Romanowska-Dixon

**OŚWIADCZENIE**

Jako współautor pracy pt. **„Functional and Morphological Changes in the Visual Pathway in Patients with Graves' Orbitopathy ."**oświadczam, iż mój własny wkład merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz przedstawienie pracy w formie publikacji to nadzór nad przeprowadzanymi badaniami okulistycznymi oraz udział w opracowaniu ostatecznej wersji artykułu. Jednocześnie wyrażam zgodę na przedłożenie w/w pracy przez lek. Agnieszkę Jagiełło-Korzeniowską jako część rozprawy doktorskiej w formie spójnego tematycznie zbioru artykułów opublikowanych w czasopismach naukowych.

Oświadczam, iż samodzielna i możliwa do wyodrębnienia część ww. pracy wykazuje indywidualny wkład lek Agnieszki Jagiełło-Korzeniowskiej przy opracowywaniu koncepcji, wykonywaniu części eksperymentalnej, opracowaniu i interpretacji wyników tej pracy.

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/podpis współautora/