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Joanna Anna Sarbinowska

Rola manometrii przełykowej wysokiej rozdzielczości i specyficznych biomarkerów zapalenia w diagnostyce pacjentów z dysfagią i podejrzeniem eozynofilowego zapalenia przełyku.

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SPIS TREŚCI

STRESZCZENIE

WSTĘP: Rosnąca częstość zachorowań na eozynofilowe zapalenie przełyku (EoE), kosztowne, źle tolerowane i inwazyjne badania kontrolne pod postacią powtarzanych okresowo ezofagogastroduodenoskopii (EGD) z pobraniem wycinków z przełyku do oceny histopatologicznej, wraz z utrzymującymi się i nawracającymi objawami dysfunkcji przełyku pod postacią dysfagii, nie tylko obniżają poziom jakości życia pacjentów, ale skłaniają liczne grupy badaczy do poszukiwania alternatywnych małoinwazyjnych metod monitorowania EoE.

CELE: Podstawowym celem projektu była ocena korelacji wyników manometrii przełykowej wysokiej rozdzielczości (HRM) i stężeń specyficznych biomarkerów zapalenia (eotaksyny 3, głównego białka zasadowego - MBP, interleukiny 5 - IL-5 i interleukiny 13 - IL-13 oraz transformującego czynnika wzrostu β1 - TGF-β1 we krwi obwodowej) z oceną kliniczną, endoskopową i histologiczną, a także oceną jakości życia pacjentów z EoE. Dodatkowym celem pracy była weryfikacja hipotezy sugerującej powstawanie pierścienia Schatzkiego (SR) jako efektu zaawansowanego włóknienia i trachealizacji przełyku w przebiegu EoE.

MATERIAŁ I METODY: Prospektywne badanie kohortowe przeprowadzono w dniach od 1.11.2017 r. do 30.04.2020 r. w Klinice Gastroenterologii i Hepatologii oraz Otolaryngologii, Chirurgii Głowy i Szyi Uniwersytetu Medycznego we Wrocławiu. Do projektu zrekrutowano 58 pacjentów z dysfagią, wykluczając chorych obciążonych schorzeniami przebiegającymi z eozynofilią oraz z rozpoznaną chorobą nowotworową przełyku. Każdy uczestnik wypełnił ankietę dotyczącą nasilenia objawów i jakości życia, miał wykonaną HRM, EGD z pobraniem wycinków z przełyku poddanych weryfikacji histopatologicznej i oceną EoE Endoscopic Reference Score (EREFS) całkowitego, zapalnego i fibrostenotycznego oraz oznaczone w surowicy krwi stężenia: IL-5 i IL-13, TGF-β1, MBP i eotaksyny 3 za pomocą testu immunoenzymatycznego. Pacjenci spełniający kryterium histologiczne EoE zostali przez 8 tygodni leczeni inhibitorami pompy protonowej, a następnie ponownie poddani diagnostyce jak przy włączeniu do projektu. Udział w badaniu był dobrowolny, a projekt otrzymał pozytywną opinię Komisji Bioetycznej. Uzyskane wyniki poddano ocenie statystycznej, a przyjęty poziom istotności wynosił $p \leq 0.05$. Numer badania w rejestrze ClinicalTrials.gov to: NCT04803162.

WYNIKI: Stężenie MBP i TGF-β1 istotnie korelowało z rozpoznaniem EoE, natomiast MBP i eotaksyny 3 ze szczytową ilością eozynofilii. Wartości EREFS były dodatnio skorelowane z wyjściowym stężeniem MBP i stężeniem eotaksyny 3 po leczeniu. IL-13 ujemnie koreluje z EREFS fibrostenotycznym, natomiast TGF-β1 po leczeniu wykazuje ujemną korelację z EREFS zapalnym i dodatnią z fibrostenotycznym. EoE, erozyjna postać choroby refluksowej przełyku (ERD), jak i SR, korelują dodatnio z nieefektywną motoryką (IEM), choć istotność statystyczną uzyskano jedynie dla ERD. W EoE rozpoznanie IEM lub utrudnienia przepływu żołądkowo-przełykowego (EGJ outfow obstraction) poprzedza rozwój choroby, natomiast konsekwencją jest brak kurczliwości (absent contractility), w przeciwieństwie do ERD, gdzie IEM rozwija się wtórnie do zmian erozyjnych. SR nie wiąże się zaawansowaną trachealizacją przełyku, jednak jest przyczyną najcięższej dysfagii. Najniższą ocenę jakości życia wśród pacjentów z dysfagią uzyskali chorzy z prawidłowym obrazem endoskopowym i histopatologicznym przełyku.

WNIOSKI: Nie można wyłonić jednego biomarkera surowicy ani zidentyfikować swoistego wzorca motorycznego, pozwalającego z czułością i swoistością porównywalną do stosowanych obecnie badań endoskopowych z pobraniem wycinków do oceny histopatologicznej, jednocześnie diagnozować i monitorować przebieg EoE. Potencjalna rola MBP w przewidywaniu rozpoznania, eotaksyny 3 w przewidywaniu zaawansowania i korelacji IL-13 i TGF-β1 w różnicowaniu przebiegu zapalnego i fibrostenotycznego choroby, przy jednoczesnym uwzględnieniu ich wzajemnych zależności, a być może także wzorców i parametrów manometrycznych skorygowanych długością opóźnienia diagnostycznego, może posłużyć do skonstruowania algorytmu rokującego poprawę precyzji i indywidualizacji terapii EoE.

ABSTRACT

INTRODUCTION: The increasing incidence of eosinophilic esophagitis (EoE), costly, poorly tolerated and invasive follow-up examinations in the form of periodically repeated esophagogastroduodenoscopy (EGD) with the collection of esophageal specimens for histopathological evaluation, and persistent and recurrent symptoms of esophageal dysfunction in the form of dysphagia lower the quality of life of patients and induce numerous groups of researchers to look for alternative minimally invasive methods of monitoring EoE.

OBJECTIVES: The main goal of the project was to assess the correlation of high-resolution esophageal manometry (HRM) results, and the concentrations of specific biomarkers of inflammation (eotaxin 3, the main basic protein – MBP, interleukin 5 – IL-5 and interleukin 13 – IL-13, and transforming growth factor β1 – TGF-β1 in peripheral blood) with clinical, endoscopic and histological assessments, as well as the assessment of the quality of life of patients with EoE. An additional aim of the study was to verify the hypothesis suggesting the formation of the Schatzki ring (SR) as an effect of advanced esophageal fibrosis and trachealisation in the course of EoE.

MATERIAL AND METHODS: A prospective cohort study was conducted from 1.11.2017 to 30.04.2020 at the Department of Gastroenterology and Hepatology and the Department of Otolaryngology, Head and Neck Surgery at Wroclaw Medical University. 58 patients with dysphagia were recruited for the project, excluding patients with eosinophilia and diagnosed esophagus cancer. Each participant completed a questionnaire regarding the severity of symptoms and quality of life. HRM, EGD with esophageal biopsy for histopathological examination, assessment of total, inflammatory and fibrostenotic Eosinophilic Esophagitis Reference Score (EREFS) and serum levels of IL-5 and IL-13, TGF-β1, MBP and eotaxin 3 by enzyme immunoassay were performed. Patients who met the histological criterion of EoE were treated with proton pump inhibitors for 8 weeks and then re-diagnosed as at the beginning of the project. Participation in the study was voluntary, and the project received a positive opinion from the Bioethics Committee. The obtained results were statistically assessed, and the adopted level of significance was $p<0.05$. The study number in the ClinicalTrials.gov registry is: NCT04803162.

RESULTS: The concentration of MBP and TGF-β1 significantly correlated with the diagnosis of EoE, while MBP and eotaxin 3 with the peak eosinophil count. EREFS values were positively correlated with the baseline MBP concentration and the concentration of eotaxin 3 after treatment. IL-13 negatively correlates with fibrostenotic EREFS, while TGF-β1 after treatment shows a negative correlation with inflammatory EREFS and positive with fibrostenotic EREFS. EoE, erosive gastroesophageal reflux disease (ERD), and SR correlate positively with ineffective esophageal motility (IEM), although statistical significance was only obtained for ERD. In EoE, diagnosing IEM or esophagogastric junction outflow obstruction (EGJOO) precedes disease development, while the consequence is absent contractility, unlike ERD where IEM develops secondary to erosive changes. SR is not associated with advanced esophageal trachealisation but is the cause of the most severe dysphagia. The lowest quality of life scores among patients with dysphagia was obtained by patients with normal endoscopic and histopathological images of the esophagus.

CONCLUSIONS: It is impossible to select a single serum biomarker or identify a specific motility pattern that allows sensitivity and specificity comparable to current endoscopic examinations with histopathological specimens while diagnosing and monitoring the course of EoE. The potential role of MBP in predicting the diagnosis, eotaxin 3 in predicting the advancement and correlation of IL-13 and TGF-β1 in the differentiation of the inflammatory and fibrostenotic course of the disease, considering their interrelationships, and perhaps also manometric patterns and parameters adjusted by the length of the diagnostic delay, can be used to construct an algorithm that looks promising to improve the precision and individualization of EoE therapy.

WSTĘP

Schorzeniem gastroenterologicznym będącym niewątpliwym wyzwaniem naukowym i klinicznym ostatnich dziesięcioleci jest eozynofilowe zapalenie przełyku (EoE). Ponad 30-krotny wzrost częstości zachorowań w ostatnich dwóch dekadach [1], a także przewlekły, postępujący [2-3] i nawrotowy przebieg choroby o nieswoistych, zależnych od wieku pacjenta objawach [4], szereguje EoE jako istotny problem kliniczny i główną przyczynę dysfagii nienowotworowej, zarówno w grupie pacjentów dorosłych, jak i w populacji pediatrycznej [5].

Dynamiczny rozwój badań nad EoE, znajduje potwierdzenie w wytycznych diagnostyczno-terapeutycznych, które od pierwszych doniesień o chorobie, aktualizowano aż 6-krotnie [4, 6-10]. Obecna definicja schorzenia opracowana przez UEG, EAACI ESPHAGAN i EUREOS w 2017 r. [4], a następnie podtrzyma w aktualizacji międzynarodowego konsensusu z 2018 r. [9] i w najnowszych rekomendacjach terapeutycznych z 2020 r. [10], precyzuje EoE jako pierwotne schorzenie przełyku o podłożu immunologicznym, manifestujące się objawami dysfunkcji przełyku pod postacią dysfagii i utykania kęsów pokarmowych, a histologicznie – przewlekłym naciekiem zapalnym z przewagą śródnabłonkowych eozynofili.

Poznana dotychczas patofizjologia choroby uwzględnia rozwój wyzwalanego w odpowiedzi na działanie refluksu kwaśnego oraz alergenów pokarmowych i wziewnych procesu immunologicznego, który prowadzi do indukcji odpowiedzi Th2-zależnej, wzrostu stężenia cytokin stymulujących migrację wewnątrztkankową i degranulację eozynofili – interleukiny 5 (IL-5), 13 (IL-13) i eotaksyny 3, a także produktów degranulacji eozynofili - głównego białka zasadowego (MBP) i transformującego czynnika wzrostu β (TGF-β) - biomarkerów zaangażowanych w zwiększenie reaktywności mięśni gładkich, rozwój włóknienia i remodelingu, a w konsekwencji zaburzeń motoryki przełyku [11-12]. Wpływ działania kwasu solnego, początkowo uważany wyłącznie za mechanizm spustowy rozwoju choroby refluksowej (GERD), poprzez zmniejszenie integralności bariery nabłonkowej przełyku ułatwia ekspozycję na antygen, co z kolei stymuluje rozwój reakcji zapalnej z udziałem eozynofilii [9]. Nie bez wpływu pozostaje również samo EoE na rozwój GERD, prowadząc poprzez włóknienie przełyku i zaburzenia motoryki do wtórnego refluksu [9]. Związek patofizjologiczny tych dwóch schorzeń uważanych do niedawna za wykluczające się, zmusza do analizy patogenezy powstawania dolnego pierścienia przełyku, zwanego pierścieniem Schatzkiego (SR), który dotychczas wiązany był z reakcją autoochronną organizmu przed narażeniem na aspirację treści kwaśnej [13-15], a potencjalnie mógłby być

również efektem zaawansowanego procesu zapalnego i fibrostenotycznego prowadzącego do powstawania pierścieni przełyku w przebiegu EoE (tzw. proces trachealizacji).

Choć aktualne rozumienie patogenezy EoE sugeruje przypuszczalne znaczenie diagnostyczne biomarkerów reakcji zapalnej z udziałem eozynofilii oraz „złotego standardu" w ocenie zaburzeń motoryki przełyku – manometrii przełykowej wysokiej rozdzielczości (HRM), dotychczas nie udowodniono jednoznacznie roli tych badań w rozpoznawaniu i monitorowaniu schorzenia [16]. W świetle aktualnych wytycznych, na rozpoznanie choroby pozwala tylko wykrycie ≥15 eozynofili w polu widzenia przy dużym powiększeniu (na powierzchni ~0,3 mm^2) w wycinkach z błony śluzowej przełyku pobranych podczas ezofagogastroduodenoskopii (EGD)), przy jednoczesnym współistnieniu objawów klinicznych EoE i wykluczeniu innych stanów związanych z miejscową lub ogónoustrojową eozynofilią [4, 9]. Wykonanie panendoskopii z pobraniem wycinków do oceny histopatologicznej jest zatem badaniem obligatoryjnym zarówno w diagnostyce, jak i monitorowaniu skuteczności terapii, jednocześnie jest jednak badaniem kosztownym i inwazyjnym, co w wraz z nieustępującymi lub nawracającymi objawami EoE, znacznie obniża poziom jakości życia chorych [17].

CEL I ZAŁOŻENIA PRACY

Celem projektu badawczego jest ocena korelacji wyników HRM i stężeń specyficznych biomarkerów zapalenia (eotaksyna 3, MBP, IL-5, IL-13, TGF-β1 we krwi obwodowej) z objawami dysfagii, cechami endoskopowymi, histologicznymi i oceną jakości życia u pacjentów z podejrzeniem EoE.

Szczegółowe cele i założenia projektu, realizowane przez poszczególne artykuły wchodzące w skład niniejszej rozprawy:

1. Sarbinowska JA, Waśko-Czopnik D. **High-resolution manometry in diagnostics and evaluation of therapy effectiveness in patients with eosinophilic esophagitis - underestimated breakthrough or dead end?** Prz Gastroenterol. 2020;15(1):22-26.

   Celem artykułu przeglądowego było przedstawienie obecnego stanu wiedzy na temat miejsca HRM w diagnostyce i monitorowaniu EoE, korelacji wzorców manometrycznych z występowaniem objawów klinicznych i cech endoskopowych. Krytyczna ocena literatury oraz nakreślenie perspektywy dalszych badań, miały stanowić podstawę dalszych rozważań i analiz ujętych w cyklu publikacji.

2. Sarbinowska J, Wiatrak B, Waśko-Czopnik D. **Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis.** Eur J Gastroenterol Hepatol. 2021;33(9):1167-1173.

   Przegląd systematyczny z metaanalizą przeprowadzono celem oceny ryzyka wystąpienia SR wśród pacjentów z EoE, w porównaniu do populacji bez rozpoznanego EoE. Identyfikacja wszystkich prac badawczych oceniających współwystępowanie EoE i SR miała stanowić wstęp do dalszych analiz nad patofizjologią obu schorzeń i weryfikacją hipotezy o rozwoju SR jako zaawansowanej trachealizacji przełyku w przebiegu EoE.

3. Sarbinowska J, Wiatrak B, Waśko-Czopnik D. **Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring.** Adv Med Sci. 2021;66(2):279-283.

Celem pracy była ocena stężeń biomarkerów reakcji zapalnej z udziałem eozynofilii: IL-5 i IL-13, eotaksyny 3, MBP i izoformy 1 TGF-β u pacjentów diagnozowanych z powodu dysfagii, u których rozpoznano SR. W artykule tym scharakteryzowano także pacjentów z rozpoznanym SR w porównaniu z populacją pacjentów bez SR pod względem cech różnicujących GERD i EoE, w tym cech endoskopowych i obciążeń atopowych.

4. Sarbinowska J, Wiatrak B, Waśko-Czopnik D. **Searching for noninvasive predictors of the diagnosis and monitoring of eosinophilic esophagitis – the importance of biomarkers of the inflammatory reaction involving eosinophils.** Biomolecules. 2021;11(6):890.

   Koncepcją pracy była próba oceny użyteczności biomarkerów surowicy krwi (IL-5, IL-13, eotaksyny 3, MBP i TGF-β1) w diagnostyce i monitorowaniu EoE poprzez ocenę ich korelacji z występowaniem choroby oraz zaawansowaniem endoskopowym i histopatologicznym u pacjentów diagnozowanych z powodu dysfagii.

5. Sarbinowska J, Wiatrak B, Waśko-Czopnik D. **Esophageal motility disorders in the natural history of acid-dependent causes of dysphagia and their influence on patients' quality of life – a prospective cohort study.** Int. J. Environ. Res. Public Health 2021; 18(21):11138.

   Założeniem pracy była ocena porównawcza wzorców i cech manometrycznych, nasilenia dysfagii zobiektywizowanej kwestionariuszem The Eating Assessment Tool (EAT-10) [18] oraz jakości życia ocenianej przy pomocy kwestionariusza the Gastrointestinal Quality of Life Index (GIQLI) [19] w populacji pacjentów z rozpoznaniem schorzeń przełyku zależnych od narażenia na działanie kwasu solnego soku żołądkowego: postaci nadżerkowej choroby refluksowej przełyku (ERD), EoE i SR. Celem pracy było także umiejscowienie zaburzeń motoryki w historii naturalnej tych chorób oraz ocena wpływu ich współwystępowania na kształtowanie jakości życia pacjentów z ERD, EoE i SR.

MATERIAŁ I METODY

Badanie naukowe będące podstawą niniejszej rozprawy doktorskiej przeprowadzono w Klinice Gastroenterologii i Hepatologii oraz w Klinice Otolaryngologii, Chirurgii Głowy i Szyi Uniwersytetu Medycznego im Piastów Śląskich we Wrocławiu w dniach od 1.11.2017 r. do 30.04.2020 r. W trakcie trwania badania do udziału zrekrutowano 58 pacjentów skierowanych celem diagnostyki dysfagii do ww. ośrodków. Kryterium wykluczenia z udziału w projekcie były już rozpoznane choroby przewlekłe z możliwym naciekiem eozynofilowym przewodu pokarmowego (eozynofilowe zapalenie przełyku, eozynofilowe zapalenie żołądka i jelit, choroba Leśniowskiego-Crohna, celiakia), choroby reumatologiczne, dermatologiczne i genetyczne z możliwą eozynofilią obwodową, a także dysfagia spowodowana rozpoznanym naciekiem nowotworowym przełyku. Każdy uczestnik włączony do projektu wyraził pisemną zgodę na udział w badaniu, a projekt uzyskał pozytywną opinię Komisji Bioetycznej Uniwersytetu Medycznego we Wrocławiu dnia 17.08.2017 r. (KB no. 544/2017), z późniejszym poszerzeniem o kolejny ośrodek dnia 6.12.2018 r. (KB no. 730/2018).

Pacjent włączany do projektu wypełniał ankietę dotyczącą stanu zdrowia i obciążeń chorobowych ze szczególnym uwzględnieniem atopii, oceny nasilenia dysfagii zobiektywizowanej kwestionariuszem EAT-10 [18] oraz jakości życia ocenianej przy pomocy kwestionariusza GIQLI [19]. Wszyscy mieli wykonaną HRM, a dane manometryczne rejestrowano i analizowano przy użyciu oprogramowania ManoViewTM ESO 3.0, natomiast interpretacji wyników dokonano zgodnie z klasyfikacją Chicago v3.0 [20]. Celem uniknięcia terapeutycznego działania endoskopu i ewentualnego wpływu na parametry manometryczne wpustu oraz ocenę motoryki przełyku, w przypadku wszystkich chorych, wykonanie HRM poprzedzało EGD. W trakcie panendoskopii monitorowano obecności endoskopowych cech zapalenia przełyku, przepukliny rozworu przełykowego i SR, a retrospektywnie na podstawie uzyskanego materiału fotograficznego i opisów badań oceniono obecność i zaawansowanie cech zawartych w systemie klasyfikacji EoE Endoscopic Reference Score (EREFS), tj. obrzęku, pierścieni, wysięków, bruzd i zwężeń oraz tzw. przełyku bibułowatego, czyli kruchości i krwawliwości kontaktowej błony śluzowej przełyku po przejściu endoskopu diagnostycznego [21]. Punktacja uzyskana w ocenie obecności wysięku, obrzęku i bruzd stanowiła podtyp EREFS zapalny, natomiast zsumowana za rozpoznanie pierścieni i zwężeń – fibrostenotyczny [22]. Podczas EGD, bez względu na obecność zmian makroskopowych, każdy uczestnik projektu miał pobrane sumarycznie minimum 6 wycinków błony śluzowej z co

najmniej dwóch obszarów przełyku, a uzyskany materiał poddano podwójnej weryfikacji histopatologicznej. W ciągu maksymalnie 7 dni od wykonanego badania endoskopowego od każdego uczestnika pobrano także próbkę krwi żylnej, celem oznaczania w surowicy krwi stężenia IL-5 i 13, TGF-β1, eotaksyny 3 oraz MBP. Ilościowe oznaczenie stężeń wykonano przy pomocy testów immunoenzymatycznych, a protokoły testów i wartości referencyjne dla populacji ogólnej przyjęto zgodnie z zaleceniami producentów.

Po wykonanej diagnostyce uczestników podzielono na podstawie spełnienia kryterium histopatologicznego rozpoznania EoE. Chorzy posiadający ≥15 eozynofili w polu widzenia przy dużym powiększeniu stanowili grupę pacjentów z EoE, natomiast pozostali grupę kontrolną. Pacjenci z rozpoznaniem EoE byli przez następne 8 tygodni leczeni inhibitorami pompy protonowej w dawce 20 mg omeprazolu stosowanej dwa razy na dobę (zgodnie z aktualnymi wytycznymi terapeutycznymi) [10], a po 8 tygodniach przeszli ponowną ocenę ankietową, manometryczną, endoskopową, histopatologiczną i biochemiczną według protokołów badań tożsamych z realizowanymi przy włączaniu pacjentów do projektu.

Uzyskany materiał poddano opracowaniu statystycznemu, a zastosowaną w analizie wyników metodologię szczegółowo opisano w rozdziałach „Materiał i metody" każdej publikacji stanowiącej podstawę rozprawy doktorskiej. Przyjęty poziom istotności wynosił $p \leq 0.05$.

Niniejsze badanie zostało także retrospektywnie zarejestrowane w rejestrze badań klinicznych ClinicalTrials.gov (nr badania: NCT04803162), data rejestracji: 17.03.2021 r.

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IF = 4,879; Pkt. MNiSW/KBN = 100,0

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Review paper

High-resolution manometry in diagnostics and evaluation of therapy effectiveness in patients with eosinophilic esophagitis – underestimated breakthrough or dead end?

Joanna A. Sarbinowska, Dorota Waśko-Czopnik

Department of Gastroenterology and Hepatology, Wroclaw Medical University, Wroclaw, Poland

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Key words: dysphagia, eosinophilic esophagitis, esophageal motility disorders, high-resolution manometry.

Address for correspondence: Joanna A. Sarbinowska MD, Department of Gastroenterology and Hepatology, Wroclaw Medical University, 213 Borowska St, 50-556 Wroclaw, Poland, phone: +48 71 733 21 20, e-mail: sarbinowska.joanna@gmail.com

Abstract

Eosinophilic esophagitis (EoE) is a chronic disease with non-specific symptoms, among which dysphagia is a prevailing one. The observed increase of EoE rate, its chronic and recurrent character, as well as invasive follow-up examination (periodical panendoscopy with specimen collection for histopathology), compel optimization of both the diagnostics algorithm and disease monitoring through searching for new, unique methods and tools so far not applied, including high-resolution manometry (HRM). Mentioned investigations result from advances in comprehension of disease pathogenesis, in which it is suggested that development of a chronic inflammatory reaction of the esophageal wall may lead to consecutive fibrosis and motility disorders. In research published to date one manometric pattern characteristic for EoE was not obtained, whereas the obtained inconsistent and at times contradictory results do not correlate either with symptoms exacerbation or endoscopic scan. Numerous constraints of discussed studies as well as current knowledge in disease etiopathology and esophagus biomechanics prompt further investigation of HRM significance in diagnostics and therapy monitoring of patients with EoE.

Introduction

In recent years a significant increase in the number of reported eosinophilic esophagitis (EoE) has been observed. It has been estimated that over the last two decades disease prevalence has increased 30-fold, and the frequency of occurrence varies between 13 and 49 cases out of 100 000 inhabitants. Thus, EoE is classified in the group of diseases constituting an common clinical problem [1–3]. As research shows that occurrence dynamics exceeds the increase of diagnostic test frequency 20-fold, consequently increased recognition does not solely result from improved disease identification, its more effective detection or establishment of unambiguous diagnostic criteria, but from existing environmental changes (hypotheses concern changes in food and airborne allergens, reduction of *Helicobacter pylori* infections, increase in administration of proton pump inhibitors, as well as exposure to dangers in early lifetime that might influence microbiome modifications) [2, 4, 5].

The EoE is a chronic disease with non-specific symptoms that may vary depending on patients' age. Due to early occurrence of signs and their distinctness in adults and children, EoE was originally regarded as an exclusively pediatric issue, whereas dysphagia in adult patients, episodes of food impaction, pyrosis or retrosternal chest pain were treated as gastroesophageal reflux disease (GERD) [1, 3, 6]. Today it is assumed that undiagnosed EoE may constitute 10% of cases of so-called reflux disease refractory to treatment [7].

Due to the chronic process and recurrent disease character patients require constant gastroenterological monitoring including follow-up examinations that will allow one to evaluate the effectiveness of conducted treatment, which in accordance with present standards means periodical panendoscopy with specimen collection for histopathology [1]. Invasive clinical tests in re-

lation to disease symptoms, which frequently do not respond to standard treatment, significantly downgrade patients' quality of life [8], yet simultaneously stimulate numerous groups of researchers to investigate alternative diagnostic methods and EoE therapies.

Eosinophilic esophagitis diagnostics

The latest UEG, EAACI ESPHAGAN and EUREOS (2017) guidelines define EoE as a chronic esophageal disease with an immunological background, clinically distinguished as esophageal dysfunctions with swallowing disorders, and histologically distinguished as inflammatory infiltration of the esophagus wall with predominant eosinophils [1]. Definition modification arises from changes referring to suspected disease etiology, and consequently they modify the previous diagnostic approach. Until now, besides clinical and histological EoE features diagnostic criteria involved an 8-week trial of treatment with proton-pump inhibitors (PPIs) with a complete therapeutic dosage applied twice a day. Patients with an improved clinical and histopathological response following 8-week therapy were not diagnosed with EoE; however, they were classified as patients with proton-pump inhibitor-responsive esophageal eosinophilia (PPI-REE), or as patients with diagnosed GERD with esophageal eosinophilia connected with hydrochloric acid [3]. Since it still remains unknown whether the immunological response in the esophagus wall in predisposed patients is triggered as reaction to food and airborne allergens, hydrochloric acid or a combination of the two, the term 'antigene' was removed from the valid disease definition, and application of PPIs was classified as a therapeutic method, not a diagnostic EoE [1, 9]. Differential diagnostics is still perceived as a necessary procedure in EoE identification. It is maintained and emphasized as a procedure to exclude systemic and topical causes of esophageal eosinophilia other than EoE (including eosinophilic gastritis and enteritis, Leśniowski-Crohn disease, parasitic infection, achalasia, hypereosinophilic syndrome, hypersensitivity to medicines, connective tissue diseases, vasculitis, graft-versus-host disease, pemphigus) [1].

Changes in present guidelines also refer to histological identification of EoE. The required description in esophagus biopsy specimen ≥ 15 eosinophilia per high power field magnification (in the area ~0.3 mm^2) remained unaltered; however, the number of biopsies was increased from 2–4 (suggested in numerous guidelines including ACG from 2013) to a minimum of 6 from at least two different parts of the esophagus (distal and proximal half of the esophagus) [1, 3, 10, 11]. Recommended sites for esophagus endoscopic biopsy ought to be the areas with macroscopic changes such as circular folds, mucous membrane rings (esophagus trachealization), longitudinal furrows, white exudates, lack of vascular pattern, hyperemia, mucosa edema, or stricture [1]. Correct scan of esophagus mucous membrane does not rule out detection (it is estimated that in approximately 10% of adult patients EoE might proceed without visible macroscopic changes), which is why in the case of clinical signs it is advisable to collect the specimen from esophagus mucosa not subject to macroscopic changes [1, 12]. In the opinion of some authors recognition cannot be excluded from specimens with the number of eosinophils between 1 and 14 in a high-power field. They also state that repeated histopathological evaluation of such specimens in 22% of cases will allow errors to be avoided and enable assessment of eosinophils in order to meet histological criteria indispensable for EoE detection [13].

Although endoscopy with specimen collection for histopathology is an invasive procedure with numerous restrictions relating to financial expenditures, time consumption, significant impact of the human factor, uneven position of lesions (false negative results), or diversity and quality of endoscopic and microscopic equipment (for example high power field (HPF) magnification), is not only a diagnostic method for EoE, but also a monitoring one [1, 13]. The latest guidelines referring to monitoring of therapy effectiveness in patients with EoE demand endoscopic and histopathological follow-up examination already implemented in 6–12 weeks following therapy commencement, but also in case of modification of the therapeutic approach or medicine dosage due to disease exacerbation or symptoms recurrence [1, 14, 15]. Evaluation of clinical signs exacerbation is generally insufficient because a constant correlation of symptoms scale and inflammatory reaction in the histopathological scan was not reported [1, 16]. Endoscopic examination in dysphagia diagnostics is highly advised out of consideration for its potential organic background, yet due to the invasive character of endoscopic tests combined with biopsy specimen collection, as well as possible complications and patients' concerns, it seems to be justified to reduce the frequency of endoscopic follow-up tests. On the other hand, reduction of these procedures might delay therapeutic decisions such as modification of patient diet or medicine dosage, and consequently it might have an impact on obtaining and sustaining remission [15]. Since UEG, EAACI ESPHAGAN and EUREOS (2017) guidelines due to insufficient evidence neither recommend other monitoring methods in remission of esophageal inflammatory changes (scanning studies, laboratory tests) nor advise

esophagus functional tests including high-resolution manometry, it is necessary to conduct research aiming at investigation for an effective, less invasive and well-tolerated method monitoring response to treatment [1, 17, 18].

Pathogenesis of esophageal motility disorders in eoe and their monitoring

Significant changes in the present diagnostic algorithm and further investigation for other promising methods and tools facilitating EoE detection and monitoring are the result of the latest progress in understanding disease pathogenesis [5].

Many interfering mechanisms, environmental factors, genetic and ontogenetic immunological features participate in EoE development. In predisposed patients a chronic inflammatory reaction of the esophageal wall with predominant eosinophilia develops. It has not been unequivocally determined whether the immunological process is solely triggered in response to airborne and food allergens, or in consequence of hydrochloric acid effects as well. However, the latest research suggests that exposure to acid reflux might interfere with esophageal mucous membrane integrity, thus facilitating transfer of interepithelial allergens [1, 19].

Exposure to allergens induces a Th2-mediated response and leads to increase in interleukins 13 and 4, thereby increasing the concentration of eotaxin 3, which entails migration of eosinophils to esophagus, induction of tissue remodeling combined with collagen deposition, angiogenesis, as well as damage of the epithelium barrier via desmoglein 1 degradation [5, 20]. As it is induced by IL-13, interleukin 5 affects eosinophil migration and their degranulation with release of many proteins and mediators, especially major basic proteins (MBP), eosinophilic cation protein, eosinophilic peroxidase, eosinophilic neurotoxin, TGF-β, interleukin 13, and the factor activating platelets [20]. Although all the factors play an important role in tissue damage and remodeling, a crucial role belongs to major basic protein that stimulates fibroblast activity and proliferation, direct epithelium damage, and mast cell degranulation with release of proteolytic enzymes and TGF-β, which has an influence on disruption of esophageal mucosal barrier, fibrosis, remodeling of esophageal mucosa and deterioration of smooth muscle functioning [20]. At present, esophagus remodeling is evaluated with histological parameters of epithelium, including hypertrophy and elongation of stratum basale papillae, broadening of the intercellular space as well as fibrosis of stratum basale of the mucosal membrane [1, 5]. Development of an inflammatory reaction leads to fibrosis and esophageal motility disorders [21, 22].

High-resolution manometry

A modern tool aiming at differentiation of neuromotor dysfunction from functional dysphagia is high-resolution manometry (HRM) involving precise measurement of real segmental pressure in reference to bolus dynamic movement, with the possibility of correlation of all components beginning with the superior esophageal sphincter, through trunk parameters with contribution of functions of both gastroesophageal connection and diaphragm branches.

The HRM was introduced into clinical practice in the year 2000. Since that time a few both prospective as well as retrospective studies have been published with the objective to establish manometric pattern characteristic for EoE patients.

It is estimated that irregularities in the manometric record occur in 20–76% of patients with EoE [23–29]. Among motility disorders mentioned in Chicago classification criteria the most frequently quoted were the patterns of weak peristalsis (17–27%), frequent failed peristalsis (7–12%) [23–27], as well as functional esophagogastric junction (EGJ) obstruction, rapid contraction with normal latency, absent peristalsis, hypertensive peristalsis [23], or even Jackhammer esophagus [25]. Although esophageal motility disorders occurred more frequently in patients with EoE than in control groups, both frequency and type of described manometric irregularities were similar to those observed in patients with GERD [23, 24], and in the case of patients with PPI-REE proved to be nearly identical, which might indicate that both diseases share similar pathogenetic mechanisms [29]. Yet, as opposed to patients with GERD, patients with EoE are symptomatically more prone to exposure to abnormal bolus pressurization in the esophagus (20–48%), such as early pan-esophageal pressurizations (15–17%) or compartmentalized esophageal pressurizations (5–19%) [23, 26, 28]. It was specified that pan-esophageal pressurizations correlate with episodes of bolus impaction in this group of patients, but do not correlate with dysphagia occurrence [28]. Another study did not find a connection between deterioration of signs and manometric features, yet it defined disease duration as a risk factor responsible for esophageal motility disorders. In this research frequency of irregularities described in HRM rose from 36% in the first 5 years of disease duration to 83% in the case of patients suffering for at least 16 years [24]. Along with disease duration and progression of changes evolving from chronic inflammation and resulting in esophageal wall fibrosis [21, 22], in accordance with the endoscopic reference system (EREFS), there were distinguished inflammatory and fibrostenotic subtypes of EoE [30]. Some authors claim that manometric features

also differentiate between these two subtypes. It was confirmed that patients with EoE have increased intrabolus pressure (IBP) [23], but patients with fibrostenotic subtype in HRM have not only significantly higher IBP (determined cut-off value in phenotype differentiation up to 16 mm Hg) but also a larger reduction of medium IBP following administration of site steroid therapy than in the case of patients with inflammatory subtype [25, 26]. At the same time, there are reports of no meaningful manometric differences between subtypes in EoE [27]. Apart from higher IBP values, patients with EoE reported essentially higher resting pressure of the EGJ and the UES, and more breaks of the peristaltic wavefront in the 20 mm Hg and in the 30 mm Hg isobaric contour, which proves ineffectual motoric function in this group of patients. However, no relation was confirmed between these irregularities and noticeable discomfort during swallowing [27].

Although no single optimal HRM parameter was defined to monitor the response to treatment, still regardless of the EoE subtype, in the group of patients with incorrect esophageal motility diagnosed prior to treatment, histological and clinical remission correlates with recovery from esophageal motility disorders [26].

Conclusions

With regard to the increased number of EoE reported cases there is a continued effort to optimize the invasive nature of present diagnostic workup and to monitor the disease through exploration of unique methods and diagnostic tools involving HRM. Insufficiently acknowledged and regularly updated alleged disease pathophysiology indicates that development of a chronic inflammatory reaction of the esophageal wall may entail subsequent fibrosis and motility disorders.

Although to date seven research studies devoted to investigation of HRM significance in diagnostics of EoE patients' therapy monitoring have been published, due to obtaining incoherent and sometimes contradictory results, a manometric pattern for EoE has not been established yet. Studies have numerous constraints resulting from, among other factors, changes in disease definition, modifying precise criteria for including and excluding patients from projects. Unquestionable drawbacks of all projects taking into consideration manometric evaluation in patients with EoE conducted so far are the small study group of 20 to 52 patients with EoE (including patients with PPI-REE) [23–29], as well as differences in gender distribution and participants' age. The impact of mentioned drawbacks must be taken into account when considering esophageal motility disorders [23, 24]. What is more, constraints also emerge as far as research conduct is concerned. Not all the projects included a control group [26], and patient position was not described during HRM examination [25, 28]. Only a few projects considered the potential therapeutic influence of gastroscopy in scheduling succession of conducted procedures in time [25], or introduced a break between tests [28] in order not to decrease the diagnostic effect or compromise the HRM result. Most of the researchers collected specimens from the stomach and duodenum in order to eliminate other EoE causes [26–29], and only a few took into account possible interference of EoE with GERD [23], verifying with histopathological tests specimens of esophageal mucosa in patients with GERD [29] or excluding pathological reflux with 24-hour pH-metry with impedance evaluation in patients with EoE [24, 26]. In the light of increasing significance of hydrochloric acid in EoE etiopathogenesis it might undoubtedly contribute to obtained test results. The lack of objectification of results correlated with HRM, the data obtained from the patient, related to occurrence and exacerbation of signs, as well as disease duration and diagnostic delay due to application of unvalidated questionnaires, seem to be considerable constraints [26]. Bearing in mind the fact that dysphagia evaluation in EoE depends on type and texture of foods, and discomfort is generally related to solid food swallowing, water swallowing during manometric examination may serve as an explanation of lack of an essential correlation between dysphagia exacerbation and manometric results [27].

To conclude, HRM results in patients with EoE are non-specific and incoherent, and moreover do not correlate with dysphagia exacerbation and endoscopic signs. Numerous constraints of discussed studies as well as present knowledge on etiopathology and esophageal biomechanics encourage further investigation. However, significance of HRM in diagnostics and monitoring of EoE patients may not be unequivocally ignored.

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Conflict of interest

The authors declare no conflict of interest.

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2. Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis.

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Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis

Joanna Sarbinowska[a], Benita Wiatrak[b] and Dorota Waśko-Czopnik[a]

Background/objective The involvement of hydrochloric acid in the etiology of eosinophilic esophagitis and numerous reports on its coexistence and interaction with reflux disease, as well as the rings of the esophageal mucosa formed with the advancement of the disease, suggest a potential association of eosinophilic esophagitis with another disorder of esophageal morphology potentially caused by exposure to acid reflux–Schatzki ring. Therefore, it seems reasonable to check the relationship of eosinophilic esophagitis with the coexistence of the Schatzki ring as a potential effect of advanced esophageal trachealization, which is the subject of this systematic review with a meta-analysis.
Methods The protocol of this meta-analysis was performed according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis. A systematic search of the indexed literature in the *MEDLINE* and *Scopus* databases from early to December 2019 was performed to identify all original research articles on the association between the occurrence of the Schatzki ring and eosinophilic esophagitis in adults.
Results Out of 68 searched studies, after the analysis and evaluation of the works, only 4 met the criteria set according to the protocol and were included in the meta-analysis. Based on the performed meta-analysis, no relationship was found between the occurrence of Schatzki ring and eosinophilic esophagitis.
Conclusion The present study did not show a significant relationship between the occurrence of the Schatzki ring and eosinophilic esophagitis in the adult population, which suggests that these are two independent causes of dysphagia in this patient population. Eur J Gastroenterol Hepatol 33: 1167–1173

Introduction

The subjective sensation of difficulty swallowing or the difficult passage of food from the mouth into the stomach is referred to as dysphagia, it may affect 3–22% of the adult population [1], and the actual incidence is difficult to assess and depends on the age of patients or the local prevalence of diseases that maybe its cause in a given population [2].

According to the World Gastroenterology Organization Global Guidelines – Dysphagia, both in North America and Western Europe, there is a decrease in the importance of gastroesophageal reflux disease (GERD) and its complications as the main cause of dysphagia, with a significant increase in the incidence of eosinophilic esophagitis (EoE) [2]. It is currently estimated that EoE may be the leading cause of dysphagia in both pediatric and adult patients [3].

Guidelines United European Gastroenterology, European Academy of Allergy and Clinical Immunology, European Society of Pediatric Gastroenterology, Hepatology and Nutrition and European Society of Eosinophilic Oesophagitis (2017) define EoE as a chronic disease of the esophagus with an immunological background, clinically characterized by symptoms of esophageal dysfunction and histologically by inflammatory infiltration of the esophageal wall dominated by eosinophils [4]. So far, it has not been established whether the immune response of the esophagus wall in predisposed individuals is triggered by food and inhalation allergens, hydrochloric acid or a combination of both factors, which undoubtedly suggests a relationship between the disease entities that have recently been considered mutually exclusive: EoE with GERD [5]. It is now known that these diseases can coexist; EoE can lead to secondary reflux due to decreased esophageal compliance or motility disorders. In contrast, GERD can lead to a reduction in the integrity of the epithelial barrier, allowing exposure to the antigen and then the development of an inflammatory reaction leading to subsequent fibrosis and dysfunction esophageal motility [1,5]. A consequence of the course of the inflammatory and fibrostenotic process are the changes observed in the endoscopic image of the esophagus in the form of whitish deposits, swelling of the mucosa, linear furrows, to rings of the mucosa (so-called trachealization of the esophagus) and strictures arising with the advancement of the disease and the development of fibrosis [4–7].

Another esophageal disease that is potentially related to exposure to gastric reflux and is a significant cause of dysphagia and choking in the adult population is the esophageal 'B' ring, usually called the Schatzki ring [8]. This lower esophageal ring is located at the junction of the

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Keywords: dysphagia, eosinophilic esophagitis, esophageal stenosis, lower esophageal ring, meta-analysis, Schatzki ring

[a]Department of Gastroenterology and Hepatology and [b]Department of Pharmacology, Wroclaw Medical University, Wroclaw, Poland

Corresspondence to Joanna Sarbinowska MD, Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska Street 213, 50-556 Wrocław, Poland
Tel: +71 733 21 20; e-mail: sarbinowska.joanna@gmail.com

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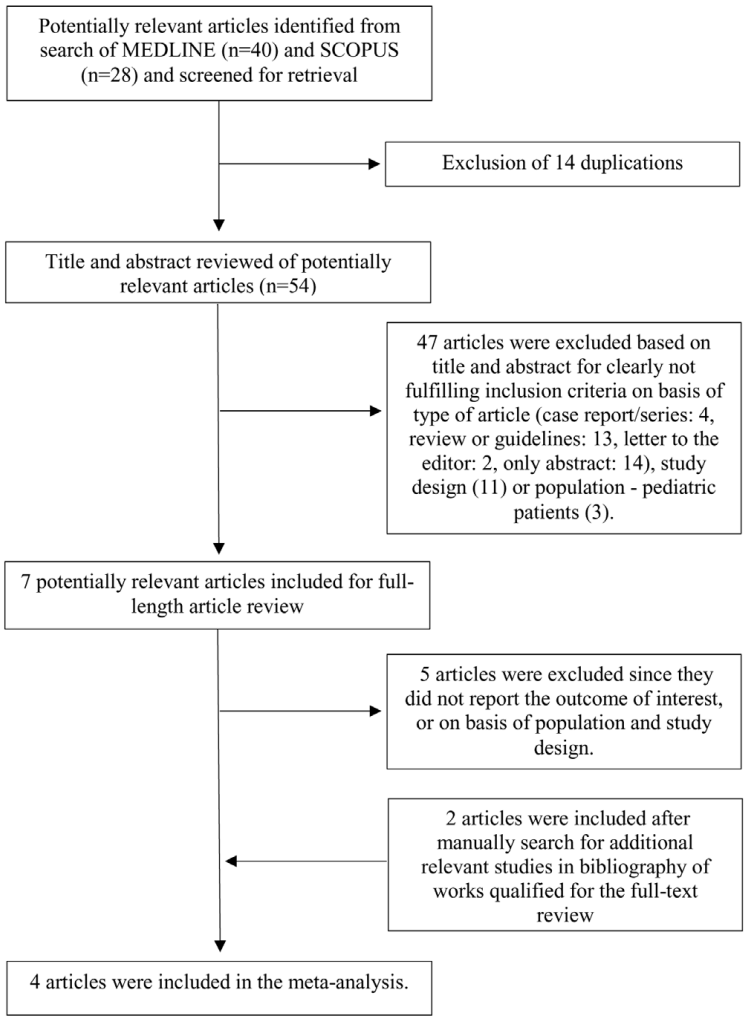


Fig. 1. Literature review process.

squamous and cylindrical epithelium, forming a circular nonmuscular structure composed of the mucosa and the submucosa [8]. The etiology and pathogenesis of Schatzki ring remain unclear, and little is known about their relationship to other structural and functional abnormalities of the esophagus [9]. In 57% of symptomatic patients, Schatzki ring is associated with other esophageal disorders, in particular hiatal hernia [9]. Research also suggests that Schatzki ring formation is the body's response to frequent acid exposure and will naturally prevent complications from acid reflux disease, including Barrett's esophagus [10,11]. However, the relationship of Schatzki ring with reflux disease has been proven only in less than two-thirds of patients [12], which necessitates the further search for other pathogenic factors. On the basis of endoscopic observations, patients with EoE tend to have narrower Schatzki ring than patients without EoE [10], it seems reasonable to check the relationship between EoE and the coexistence of Schatzki ring as a potential effect of advanced esophageal trachealization.

This systematic review with meta-analysis was performed to identify all studies assessing the risk of Schatzki ring in EoE patients, compared to a population without known EoE, and to summarize the results obtained.

Methods

Research identification and search strategy

The protocol of this meta-analysis was carried out according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis-PRISMA (Supplemental digital content 1, Supplemental digital content 1, *http://links.lww.com/EJGH/A658*) [13]. Two

Table 1. Characteristics of the studies included in this meta-analysis

Study	Study design	Country, year	Participants	Diagnosis of EoE	Diagnosis of Schatzki ring	Newcastle–Ottawa scale
Desai *et al.* [19]	Cross-sectorial study	USA, 2005	Subjects (31) were patients with acute esophageal food impaction who underwent upper endoscopy with biopsies in the esophagus in Baumont Hospital between January 2000 and December 2003.	During endoscopic examinations, mucosal pinch biopsy specimens of the upper and the lower esophagus, the stomach, and the duodenum were taken. Lower esophageal samples were obtained within 3 cm above the squamocolumnar junction; the proximal esophageal specimen was obtained at least 9 cm above the squamocolumnar junction. Esophageal biopsy in EoE demonstrated >20 eosinophils/HPF, eosinophilic microabscesses, or superficial eosinophil layering.	Schatzki ring was diagnosed based on the endoscopic view as a circumferential thin membranous ring of mucosa in the distal esophagus just above the gastroesophageal junction.	Selection: 3 Comparability: 1 Outcome: 3
Mackenzie *et al.* [20]	Cross-sectorial study	USA, 2008	Subjects (261) were patients with dysphagia who underwent upper endoscopy with biopsies in the esophagus at The University of Utah and Salt Lake Veterans Affairs Medical Center between December 2005 and January 2007.	4 quadrant biopsies were performed in the distal and mid-esophagus. EoE was diagnosed when specimens demonstrated >20 eosinophils/HPF in the proximal esophagus and/or biopsies demonstrating >20 eosinophils/HPF while on high dose PPIs. Exclusion criteria included inability to consent, previously diagnosed EoE, inability to safely undergo endoscopy, incarcerated patients, esophageal varices, or a contraindication for esophageal biopsies.	Schatzki ring was diagnosed based on the endoscopic view.	Selection: 2 Comparability: 2 Outcome: 3
Moawad *et al.* [21]	Cross-sectorial study	USA, 2015	Subjects (68) were patients with symptoms of esophageal dysfunction and dense esophageal eosinophilia on tissue biopsies identified from a database for 1 year. The study population included male and female military health care beneficiaries age 18 years and older who had an established diagnosis of EoE, PPI-REE, or GERD.	EoE was defined by symptoms of esophageal dysfunction, ≥15 eosinophils/HPF, and lack of response to at least an 8-week course of high dose PPIs.	Schatzki ring was diagnosed based on the endoscopic view.	Selection: 2 Comparability: 2 Outcome: 3
Truskaite *et al.* [22]	Cross-sectorial study	Sweden, 2016	Subjects (185) were patients who underwent upper endoscopy with biopsies in the esophagus because of a present or recent episode of food bolus impaction. This study included adult patients referred to the Endoscopy Unit at Karolinska University Hospital, Stockholm, Sweden, in the period from August 2011 to April 2016.	The presence of ≥15 eosinophils/HPF in the esophageal mucosa was considered as EoE. The presence of ≥15 eosinophils/HPF that normalized after 6–12 weeks of PPIs treatment combined with a normal pH monitoring was considered as PPI-REE.	Schatzki ring was diagnosed based on the endoscopic view.	Selection: 2 Comparability: 1 Outcome: 3

EoE, eosinophilic esophagitis; GERD, gastroesophageal reflux disease; HPF, high-power field; PPIs, proton-pump inhibitors; PPI-REE, proton-pump inhibitor-responsive esophageal eosinophilia.

researchers (J.S. and D.W-C.) independently systematically searched the indexed literature in *MEDLINE* and *Scopus* databases from early to December 2019 to identify all original research article on the association between Schatzki ring and EoE in adults. The search strategy included the terms "eosinophilic esophagitis", "eosinophilic oesophagitis", "Schatzki ring", "Schatzki", "lower esophageal ring" and is described in Supplemental digital content 2, Supplemental digital content 2, *http://links.luww.com/EJGH/A658*. No language restrictions have been applied. Articles were selected for a full-text review on the basis of their title and abstract. The bibliography of works qualified for the full-text review was manually searched for the possible identification of additional eligible publications.

Inclusion and exclusion criteria

Cross-sectional, case–control and cohort studies assessing the coexistence of EoE and Schatzki ring were qualified for the meta-analysis. The diagnosis of Schatzki ring was based on endoscopic identification, and works using only radiological assessment methods were excluded. The diagnosis of EoE, due to the variability and numerous updates of the diagnostic and therapeutic guidelines at the turn of recent years, was based on the guidelines valid for the year of publication of the study, with all studies using the histopathological criterion confirming the presence of >15 eosinophils in a large field of view in sections esophageal (number, location of samples taken, additional symptomatological criteria and criteria for excluding other causes of esophageal eosinophilia were consistent with 2007, 2011, 2013, 2017 and 2018 guidelines update) [4,5,14–16]. The research could be carried out both in a hospital setting and on an outpatient basis. Only studies on adult patients were included in the review. All duplicate publications have been identified and removed. All researchers analyzed the identified articles, and qualification discrepancies were established by consensus. The Newcastle–Ottawa Quality Assessment Scale was used to evaluate the quality of the studies in terms of topic selection, group comparability and treatment outcomes for cohort studies or exposure to case–control studies [17], and for the qualitative assessment of cross-sectional studies and its dedicated modifications [18].

Data extraction

All researchers independently extracted data from articles using a predesigned data form, and any disputes were resolved by referencing the content of the article. The form used for data extraction contained: the title of the study, the name of the first author, the year or years in which the research was conducted, type of study, country, number of participants, and the method used to diagnose EoE and Schatzki ring.

Statistical analysis

All analyzes were performed using STATISTICA v. 13. A bivalent meta-analysis model was performed using their 2×2 tables and the total sensitivity, specificity and the odds ratio (OR) were calculated together with their 95% confidence intervals (95% CI).

Results

Search results

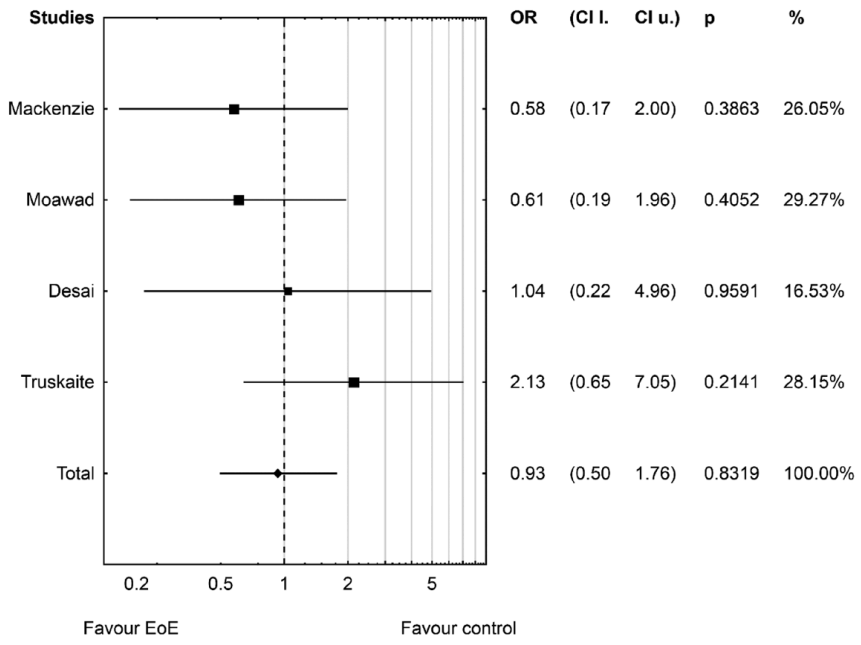
The search yielded titles and abstracts, respectively, a total of 40 from *MEDLINE* and 28 from *SCOPUS*, 54 of which were unique after removing duplicates (Fig. 1). Then, on the basis of titles and abstracts, 47 articles were excluded from the analysis due to a clear failure to meet the inclusion criteria – 33 articles were excluded due to the type of article (case report/series: 4; review papers or guidelines: 13; letters to the editor: 2; abstracts without full-text studies: 14) and another 14 studies were excluded due to the project implementation or the population (3 studies concerned a pediatric or mixed population – both pediatric and adults). In the last step, the full texts of the remaining seven reports were downloaded, evaluated further and their bibliography was manually searched for possible identification of additional eligible publications (two such articles were identified). Another five reports were excluded from the analysis because they did not report the association of interest, or the population included or the study design did not meet the inclusion and exclusion criteria. Finally, four articles [19–22] were included in the meta-analysis.

Characteristics of the included studies

Summary of information on EoE and Schatzki ring four of the included works are listed in Table 1. Selected works were published in 2005–2016. All studies were cross-sectional studies and included 545 participants in total. Although the diagnosis of Schatzki ring in all analyzed studies was based on endoscopic evaluation, the diagnosis of EoE depended on the diagnostic guidelines valid for the year of each study, which resulted in discrepancies in the number of samples taken from the esophageal mucosa, their location, taking samples from the stomach or duodenum in order to exclude eosinophilic infiltration, the amount of eosinophilia per high-power field (HPF), determining the diagnosis (in all studies it was at least 15 eosinophils/HPF), the importance of clinical symptoms in the diagnosis, excluding other causes of esophageal eosinophilia, as well as qualifying or not qualifying for the diagnosis of EoE patients achieving clinical and histopathological improvement after proton-pump inhibitors (PPI) therapy [PPI-responsive esophageal eosinophilia(PPI-REE)]. For the qualitative assessment of the studies, a modification of the Newcastle–Ottawa scale (Table 1) dedicated to cross-sectional studies was used. In this meta-analysis, the risk of occurrence Schatzki ring in the group of patients diagnosed with EoE was not found to be significantly higher than the risk of coexistence of a ring in the control group. The pooled ORs was 0.93 (95% CI, 50–1.76) (Fig. 2).

Evaluation for publication bias and sensitivity

No obvious bias was found in the review. Extensive searches were carried out in two research bases: *MEDLINE* and *Scopus* without language restrictions. Two gastroenterologists independently assessed selected articles and extracted data for study. All the authors of the work evaluated the importance of each article. The evaluation of the publication using the Begg and Mazumdar rank correlation test



EoE (eosinophilic esophagitis); CI l. (lower confidence interval); CI u. (upper confidence interval); OR (odds ratio); SR (Schatzki ring)

Fig. 2. Forest plot diagram of the systematic review and meta-analysis.

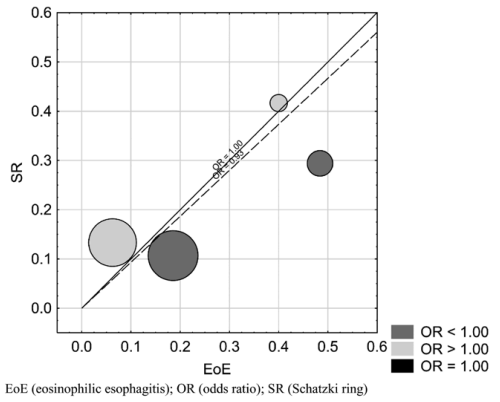


Fig. 3. The L'Abbe chart – relationship between the risk of SR in the group of patients with EoE and in the control group.

showed a *P* value of 1.0, that is, NS and Egger also were NS ($P = 0.95$).

Heterogeneity analysis

To graphically assess the heterogeneity of the studies, the L'Abbe chart was used, showing the relationship between the risk of Schatzki ring in the group of patients diagnosed with EoE and the risk of Schatzki ring in the control group (Fig. 3). The solid line represents the equilibrium level (OR = 1.00), and the dashed line represents the total effect determined in the meta-analysis (OR = 0.93). The size of the markers is proportional to the participation of a given study in the meta-analysis. There was no evidence of great variability in the results.

Discussion

The presented systematic review with meta-analysis is the first study to assess the coexistence of Schatzki ring in patients with EoE, compared to patients without diagnosed EoE. The analysis of the studies included in the study did not show a relationship between the occurrence of these two important causes of dysphagia in the adult population. This conclusion also confirms the discrepancy in the histopathological images of Schatzki ring and EoE [23]. So far, only one study has attempted to compare the histopathological picture of the esophageal mucosa in patients diagnosed with EoE with Schatzki ring sections in the group of patients without EoE, showing a statistically significant difference between the mean number of eosinophils in esophageal biopsies of patients with EoE (82/HPF, range 23–557 eosinophils/HPF) compared with negligible eosinophilic infiltrate by Schatzki ring sections (0.3 eosinophils/HPF; range 0–5 eosinophils/HPF) and no epithelial

hypertrophy by Schatzki ring sections compared to EoE [23]. The discrepancy of the histopathological features is, therefore, in contradiction with the suggested common pathophysiology of both diseases.

So far, no systematic review and meta-analysis of this topic in pediatric populations have been conducted, even though we have a much greater wealth of scientific reports on EoE in this particular group of patients because the disease was initially considered typical only for childhood. EoE is characterized by nonspecific symptoms, which vary with the age of patients, even in the pediatric group itself – from nonspecific feeding difficulties or inhibition of weight gain in young children to dysphagia, food impaction, long chewing time, sipping meals with plenty of fluids or retrosternal pain in the group of elderly children and adult patients [4]. This diversity makes it difficult to implement and identify studies that include both the pediatric population and the adult patient population, and also meet the inclusion and exclusion criteria for this systematic review and meta-analysis. For this reason, the presented review has been limited only to studies based on adult populations.

However, this study is not without limitations. Only four studies that met the previously assumed inclusion and exclusion criteria were included in the meta-analysis, and the analyzed patient populations were sparse [19–22]. Few studies are assessing the coexistence of eosinophilic esophagitis and other structural causes of dysphagia. In numerous studies in the population of patients in whom the esophageal ring was visualized during panendoscopy, identifying it as the cause of the ailments, the removal of specimens from the esophageal mucosa for histopathological evaluation was omitted, making it impossible to extend the diagnosis [10]. Numerous works in the assessment of the presence of Schatzki ring were based on the contrast radiological examination with the use of barite, which, allowing the diagnosis of fibrostenotic changes, does not allow the diagnosis of inflammatory changes requiring histopathological assessment [24]. The meta-analysis also ruled out studies in which esophageal mucosa samples were taken for histopathological evaluation only from patients who had already been diagnosed based on the endoscopic picture of Schatzki ring – which, if included in the meta-analysis, could falsely confirm the relevance of the relationship between Schatzki ring and EoE [9,10]. Conducting a thorough systematic review was hampered by numerous changes to the EoE guidelines, which in the years included in the publication review, that is, 2005–2016, were updated three times. The latest diagnostic guidelines and their update, including in the diagnosis of EoE the previously excluded group of patients with clinical and histopathological improvement after therapy PPI (PPI-REE), was published in 2017 and 2018 [4,5]. The publication of subsequent guidelines, this time raising the topic of EoE therapy, developed and published by the American Gastroenterological Association and the Joint Task Force on Allergy-Immunology Practice Parameters in 2020 [25] testifies to the dynamic development of knowledge of this increasingly common disease. Another possible limitation of this study is the inclusion of as many as three analyses carried out in the USA [19–21], which increases the risk of including the same patients in the analysis.

In conclusion, this study did not show a significant relationship between the incidence of Schatzki ring and EoE in the adult population, which suggests possibly different pathophysiology of both diseases, and the occurrence of Schatzki ring in patients with EoE may indicate the superimposition of EoE and GERD rather than advanced trachealization in the course of EoE.

Acknowledgements

All authors had access to the data and a role in writing the manuscript.

Conflicts of interest

There are no conflicts of interest.

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PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4-5
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5-6
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	6
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	6-7
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	6-7
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6-7
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6-7
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6-7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Table 1.
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Table 1.
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Table 1.
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Figure 2.
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	9
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	9
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	9-10
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	10-11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	11
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	1

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Search Strategy

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3. or/1-2
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5. Schatzki.mp.
6. lower esophageal ring.mp.
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3. Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring.

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Original research article

Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring

Joanna Sarbinowska [a,*], Benita Wiatrak [b], Dorota Waśko-Czopnik [a]

[a] Department of Gastroenterology and Hepatology, Wroclaw Medical University, Wroclaw, Poland
[b] Department of Pharmacology, Wroclaw Medical University, Wroclaw, Poland

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ABSTRACT

Purpose: The study aimed to assess the level of inflammatory biomarkers related to eosinophilia: interleukins 5 (IL-5) and 13 (IL-13), eotaxin 3, major basic protein (MBP) and transforming growth factor β1 (TGF-β1) in patients with dysphagia and Schatzki ring (SR), as well as the characteristics of this group of patients in terms of the features differentiating gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EoE).
Patients and methods: We analyzed 42 patients with dysphagia, each of whom underwent panendoscopy with an assessment of the occurrence of SR, retrospectively assessed EoE Endoscopic Reference Score (EREFS) total, inflammatory and fibrostenotic and serum concentrations of IL-5 and 13, TGF-β1, eotaxin 3 and MBP. All of them completed a symptom and comorbid questionnaire. Patients diagnosed with SR constituted the SR group ($n = 8$), the rest – the non-SR group. The quantification of the biomarkers was performed by enzyme immunoassay (ELISA). In the data analysis, $p \leq 0.05$ was considered statistically significant.
Results: We demonstrated a significant increase in terms of exceeding the reference values of TGF-β1 (37.5% vs 8.8%) and MBP (75% vs 35.3%) in patients with SR compared to the non-SR group (qualitative analysis). There was also a statistically significant increase in the concentration of each of the determined biomarkers (quantitative analysis) in the SR group.
Conclusions: The increase in TGF-β1 and MBP concentrations indicates the inflammatory and probably fibrostenotic pathogenesis of SR. Obtained results do not allow for an unequivocal classification of SR as a complication typical only for GERD or EoE.

1. Introduction

One of the significant causes of both dysphagia (13.3%) and food impaction (approximately 7%) in the general population is the esophageal "B" ring, usually called the lower esophageal ring or the Schatzki ring (SR) [1–3]. Morphologically, it is a circular non-muscle structure composed of the mucosa and submucosa located in the esophagus at the junction of the squamous and cylindrical epithelium [4], and its diameter determines the severity of the symptoms associated with it [4,5].

Although almost 70 years have passed since the first description of SR [5], the etiology and pathogenesis of this structural disorder of the esophagus have still not been established. Apart from hereditary and developmental theories, numerous reports have linked the development of SR with other esophageal pathologies (it is estimated that SR is associated with additional esophageal disorders in 57% of symptomatic patients) [6]. According to the plication theory, the development of SR is associated with the presence of a sliding hiatal hernia, which, by generating a longitudinal shortening of the esophagus, would lead to folding of the excess mucosa [6,7]. The coexistence of esophageal motility disorders in spastic disorders is also suggested as an independent theory of the development of SR or a plication theory [7]. However, in recent reports, SR is most commonly associated with gastroesophageal reflux disease (GERD). In GERD, the coexistence of SR is considered an auto-protective reaction of the organism [8]. Numerous reports suggest that through this form of esophageal stricture, the body naturally defends itself against exposure to acid aspiration, thus preventing the development of GERD complications, including Barrett's esophagus [8–10]. This theory's validity is confirmed by the coexistence of pathological gastroesophageal reflux in 2/3 of SR patients, but it does not explain SR's etiology in all patients [11]. Eosinophilic esophagitis (EoE) is another condition associated with exposure to gastric reflux that may define SR as a potential effect of advanced inflammatory and fibrostenotic processes.

* Corresponding author. Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska 213, 50-556, Wroclaw, Poland.
E-mail address: sarbinowska.joanna@gmail.com (J. Sarbinowska).

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The immune reaction in the esophageal wall of patients with EoE is triggered in response to food and inhalation allergens, and damage to the esophagus by hydrochloric acid and subsequent epithelial integration disorders allow easy exposure to the antigen and development of an inflammatory reaction [12]. Exposure to the allergen leads to the induction of a Th2-dependent response, an increase in interleukin 13 (IL-13), 5 (IL-5) and 15 (IL-15). Increased secretion of eotaxin 3 by the esophageal epithelium, in turn, promotes tissue migration of eosinophils. A similar effect is demonstrated by IL-5, which also affects the degranulation of eosinophils and the release of, e.g., major basic protein (MBP), increasing the reactivity of the esophageal muscle, as well as transforming growth factor β (TGF-β), which affects the fibrosis and remodeling of the esophageal mucosa and deterioration of functions smooth muscles [13, 14]. As a consequence of the inflammatory and fibrostenotic processes, changes in mucosal rings forming (so-called esophageal trachealization) are observed in the endoscopic image of the esophagus [12,15,16]. The Schatzki ring existence theory resulting from advanced trachealization in the course of EoE is currently the subject of numerous studies and observations of these two common causes of dysphagia [6,10,17,18].

This study focuses on the previously unknown relationship between the concentration of eosinophilic inflammatory biomarkers (IL-13, IL-5, eotaxin 3, MBP and TGF-β1) and the incidence of SR in patients diagnosed with dysphagia. The observation aimed to characterize patients diagnosed with SR compared to the population of patients without SR in terms of features differentiating GERD and EoE (endoscopic picture, atopic history).

2. Material and methods

2.1. Patient population

Fifty eight adult patients (over 18 years of age) were included in the study. All patients had esophagogastroduodenoscopy performed due to dysphagia at the Department of Gastroenterology and Hepatology and the Department of Otolaryngology, Head and Neck Surgery (Wrocław, Poland), between November 2017 and April 2020. Serum levels of cytokines IL-5 and IL-13, TGF-β1 and eotaxin 3, and the product of eosinophilia degranulation – MBP, were measured in each study participant. Additionally, all patients completed a questionnaire on symptoms and comorbidities, including atopic diseases. We excluded from the study 16 patients with already diagnosed chronic diseases with possible eosinophilic infiltration of the gastrointestinal tract (EoE, eosinophilic gastroenteritis, Crohn's disease, celiac disease), rheumatological and dermatological disorders and symptoms, as well as genetic diseases with possible peripheral eosinophilia, and also patients with known or diagnosed neoplastic infiltration of the esophagus. Patients diagnosed with SR in panendoscopy constituted the SR group (n = 8), while the remaining patients – the non-SR group (n = 34).

2.2. Endoscopy

All study participants underwent diagnostic esophagogastroduodenoscopy performed by one endoscopist – a specialist in gastroenterology (co-author D. W–C.). Endoscopic examinations were performed with the use of Olympus GIF-Q180 endoscope (Olympus, Tokyo, Japan). The examination was performed through the mouth in patients placed on their left side after prior anesthesia of the throat with a 10% lidocaine spray solution. The patients subjected to the study were fasting (they had not taken food for 6 h and fluids for 4 h before the procedure). During the diagnostic examination, the presence of SR, defined as a peripheral thin membranous ring of the mucosa in the distal part of the esophagus, just above the gastroesophageal junction (Fig. 1) [4], as well as the presence of endoscopic esophagitis (the severity of the lesions was assessed according to the Los Angeles classification) was carefully evaluated and monitored [19].

Based on the obtained photographic material and research descriptions, a retrospective analysis was performed. Endoscopic images of all patients were assessed according to the six key features included in the EoE Endoscopic Reference Score (EREFS). This score system enables the consistent recognition and reporting of endoscopic features of EoE activity [20]. The major features included in the current modified EREFS are: edema, rings, exudate, furrows, and strictures. Edema was recorded as absent (grade 0) or present (loss of vascular pattern; grade 1). Similarly, longitudinal furrows and strictures were reported as absent (grade 0) or present (grade 1). Rings were recorded as absent (grade 0), mild (slight peripheral fringes: grade 1), moderate (prominent rings that did not prevent standard endoscope from passing: grade 2), or severe (distinct rings that prevented standard endoscope from passing: grade 3), while exudates were reported as absent (grade 0), mild (involving <10% esophageal mucosa area: grade 1), or severe (involving > 10% esophageal mucosa area: grade 2). The so-called "crêpe-paper" esophagus, i.e., fragile and bleeding esophageal mucosa due to passage of a diagnostic endoscope (a minor feature of EREFS), obtained grade 1 if present, or grade 0 if absent [20]. The sum of the scores obtained for all 6 endoscopic features constituted the total EREFS, which could be from 0 to 9. This study also assessed the inflammatory and fibrostenotic subscores of EREFS, similar to the study by Dellon et al. [21], but based on the current modified EREFS classification system [20]. Thus, inflammatory EREFS was the sum of the scores for exudate, edema, and fissures (0–4), while fibrostenotic was the sum of the scores for the diagnosis of rings and strictures (0–4).

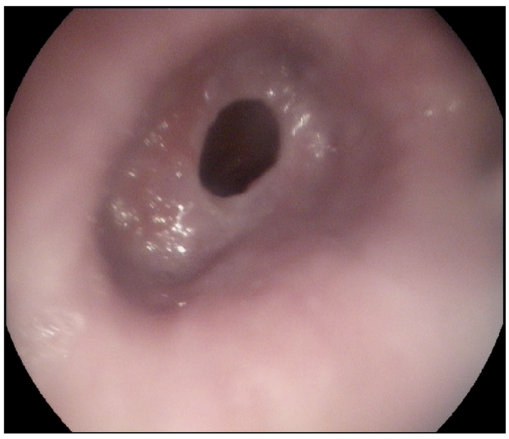

Fig. 1. Endoscopic image of the Schatzki ring.

2.3. Measurement of biomarker levels

Venous blood samples were collected from fasting patients within a maximum of 7 days from the endoscopic examination. Blood was collected by qualified medical personnel into centrifuged tubes without pyrogens/endotoxins. The serum was separated immediately after centrifugation. The blood was centrifuged at approximately 1000×*g* for 10 min, and the obtained serum was collected and stored at −70 °C. Quantitative determination of the concentration of IL-5, IL-13 and TGF-β1, both in the SR group and in the non-SR group, was performed using Diaclone enzyme immunoassays: Human IL-5 ELISA Kit, Human IL-13 ELISA Kit, and Human TGF-β1 ELISA Kit (Besançon, France), and eotaxin 3 and MBP using kits from Cloud-Clone (ELISA Kit for Macrophage Inflammatory Protein 4 Alpha; ELISA Kit for Major Basic Protein; Katy, TX, USA). The determinations were performed according to the protocols recommended by the manufacturers. The reference values for

Table 1
Characteristics of patients.

Parameters		SR	without SR	*p*
Patients [n (%)]		8 (19.05%)	34 (80.95%)	*NS*
Age median [n (range)]		49 (24 68)	34 (24 63)	0.02
Demography: male gender [n (%)]		6 (75%)	14 (41.2%)	*NS*
Atopy [n (%)]		4 (50%)	16 (47.1%)	0.03
Atopy:	inhalation allergies [n (%)]	3 (37.5%)	5 (14.7%)	*NS*
	food allergies [n (%)]	3 (37.5%)	3 (8.8%)	0.037
	bronchial asthma [n (%)]	2 (25%)	5 (14.7%)	*NS*
	allergic sinusitis [n (%)]	1 (12.5%)	2 (5.9%)	*NS*
	atopic dermatitis [n (%)]	2 (25%)	2 (5.9%)	*NS*
Clinical symptoms:	choking [n (%)]	5 (62.5%)	9 (26.5%)	0.05
	food impaction [n (%)]	6 (75%)	12 (35.3%)	0.04
	odynophagia [n (%)]	7 (87.5%)	11 (32.4%)	0.004
Biomarker:	IL-5 [patients with non-reference values (%)]	0	4 (11.8%)	*NS*
	IL-5 median [range]	5.085 (3.55 8.2)	4.3 (3.5 6.4)	
	IL-13 [patients with non-reference values (%)]	1 (12.5%)	8 (23.5%)	*NS*
	IL-13 median [range]	3.35 (1.615 4.7)	3 (1.45 5.0)	
	TGF-β1 [patients with non-reference values (%)]	3 (37.5%)	3 (8.8%)	0.03
	TGF-β1 median [range]	10027.5 (6960 15763.5)	7879.5 (6300 9855)	
	Eotaxin 3 [patients with non-reference values (%)]	3 (37.5%)	18 (52.9%)	*NS*
	Eotaxin 3 median [range]	29 (15.4 93.2)	53.7 (41 141.3)	
	MBP [patients with non-reference values (%)]	6 (75%)	12 (35.3%)	0.04
	MBP median [range]	679.5 (367 813)	492.5 (473 683)	
Endoscopic features:	inflammation [n (%)]	2 (25%)	2 (5.9%)	*NS*
	endoscopic features of a hiatal hernia [n (%)]	1 (12.5%)	2 (5.9%)	*NS*
	edema [n (%)]	3 (37.5%)	4 (11.8%)	*NS*
	rings [n (%)]	2 (25%)	6 (17.6%)	0.04
	exudates [n (%)]	2 (25%)	4 (11.8%)	*NS*
	furrows [n (%)]	0	2 (5.9%)	*NS*
	stricture [n (%)]	1 (12.5%)	0	0.03
	crepe paper esophagus [n (%)]	0	0	*NS*
EREFS:	inflammatory [n (%)]	3 (37.5%)	6 (17.6%)	0.04
	fibrostenotic [n (%)]	4 (50%)	6 (17.6%)	*NS*
	total [n (%)]	5 (62.5%)	8 (23.5%)	0.005

Abbreviations: EREFS - Eosinophilic Esophagitis Endoscopic Reference Score; IL-5 – interleukin 5; IL-13 – interleukin 13; MBP – major basic protein; NS – statistically non-significant; SR – Schatzki ring; TGF-β1 – transforming growth factor β1.

the general population were 0–18.49 pg/ml for IL-5, 0–7.28 pg/ml for IL-13, from 5222 to 13731 pg/ml for TGF-β1, from 18.6 to 51.2 pg/ml for eotaxin 3 and from 372.1 to 685.4 ng/ml for MBP. These reference values were given by the manufacturers of the ELISA kits used to evaluate the biomarkers tested.

2.4. Statistical methods

The obtained results were statistically analyzed with the Statistica v13.1 software (Dell Software Inc., Round Rock, TX, USA). The normality of the results was checked with the Shapiro-Wilk test. The statistical analysis was carried out using non-parametric tests – the Mann-Whitney *U* test and Spearman's rank correlation coefficients due to the lack of a normal distribution for all parameters. $P \leq 0.05$ was considered statistically significant.

2.5. Ethical issues

The Bioethics Committee of the Medical University of Wroclaw (Poland) approved the study on August 17, 2017 (KB no. 544/2017), with a subsequent extension on December 6, 2018 (KB no. 730/2018). All study participants gave their informed written consent to participate in the study. This study which involves human participants is in compliance with the 1964 Helsinki declaration and its later amendments.

3. Results

Among the 58 patients recruited for the study with dysphagia symptoms, 16 patients with diagnosed chronic diseases with possible eosinophilic infiltration of the gastrointestinal tract were excluded. Finally, 42 participants were included in the study. Based on the presence of SR visualized in the endoscopic examination, patients were divided into the group with SR - the SR group of 8 patients (19.05%) and the non-SR group of 34 patients (80.95%) (Table 1).

The overall median age of the population was 35.5 years (range from 24 to 68 years). The median age of patients diagnosed with SR (49 years, range from 24 to 68 years) was statistically significantly higher than that in the group of patients without SR (34 years, range from 24 to 63 years) ($p = 0.02$; $r = 0.35$ medium relationship in the assessment of the correlation) (Table 1). According to our results, men are much more likely to develop SR than women (21.43% *vs* 6.67%, $p = 0.08$). Patients with diagnosed SR were statistically significantly more likely to be burdened with atopy than the patients in the non-SR group ($p = 0.03$). Only food allergies were statistically significant in the SR group ($p = 0.037$) (Table 1). SR also predisposed to the occurrence of other symptoms accompanying dysphagia: choking with food (5.33% *vs.* 2.67%, $p = 0.05$; $r = 0.3$ $p = 0.022$), food impaction (4.57% *vs.* 3.42%, $p = 0.04$; $r = 0.27$ $p = 0.038$), and odynophagia (4.57% *vs.* 3.43%, $p = 0.004$; $r = 0.44$ $p = 0.00$) (Table 1).

Among the measured biomarkers of the inflammatory reaction involving eosinophilia, only the concentration of TGF-β1 in the blood serum statistically significantly exceeded the healthy population's reference values in the group of patients with SR ($p = 0.03$) (Table 1). In the case of the assessed biomarkers, a statistically significantly higher level of MBP concentration was also demonstrated in the SR group compared to the non-SR group ($p = 0.04$) (Table 1). Analyzing the determined concentrations of serum biomarkers, while ignoring the imposed reference values for the population of healthy people (reference values provided by the manufacturers of the ELISA kits used), based on the Mann-Whitney *U* test, it was found that in the group of patients with SR, the concentrations of the biomarkers were statistically significantly higher ($p = 0.0001$) compared to the non-SR group.

The endoscopic analysis showed no statistical significance for endoscopic esophagitis ($p = 0.28$) or endoscopic hiatal hernia ($p = 0.29$) when comparing the SR and non-SR groups. Among the 6 key features of endoscopic activity assessment included in the EREFS, endoscopic classification system assessed retrospectively, the presence of esophageal rings ($p = 0.04$) and strictures ($p = 0.03$) were statistically significantly more frequent in the group with SR (Table 1) than in the non-SR group. Patients in the SR group had statistically significantly higher inflammatory ($p = 0.04$) and total ($p = 0.005$) EREFS scores than the patients in the non-SR group (Table 1).

4. Discussion

This study is the first attempt to search for the etiology and pathogenesis of SR based on the assessment of the concentration of biochemical markers in the blood serum. So far, only a few studies assessing the concentration of biomarkers and immunological plasma profiles of patients with esophageal dysfunction focus on the causes of dysphagia of definite inflammatory etiology, such as eosinophilic esophagitis and reflux esophagitis, or the probable inflammatory etiology, such as achalasia [22]. Biomarkers were assessed to look for cytokines' role in the pathophysiology of the disease and their possible role in the diagnosis, monitoring, or differentiation of these disease entities.

In our study, among the assessed biomarkers, only the concentration of TGF-β1 significantly exceeded the reference values in the group of patients with diagnosed SR. At the same time, statistical significance was found for MBP ($p = 0.04$). A higher MBP concentration was observed in the SR patient group than in the non-SR group. Taking into account the function of TGF-β1 - as the main factor causing fibrogenesis in all organs by inducing epithelial-mesenchymal transformation, as well as MBP - responsible for changing the function and increasing the permeability of the epithelium and the reactivity of smooth muscles [23–25], it can be assumed that the pathogenesis of SR is related to the remodeling effect of the esophageal mucosa due to chronic inflammation leading to fibrosis. Such a model would, in turn, suggest a strong pathogenetic similarity between SR and EoE. Despite the great importance of TGF-β1 and MBP (and their increased concentrations in the assessment of esophageal tissues) in the development of EoE [24,26], no significant increase in the concentration of these biomarkers in the blood serum of patients has so far been proven [27]. However, it seems inappropriate to use the arbitrarily accepted norms for the concentration of these serum biomarkers established for a healthy population in the assessment of inflammation located in the esophagus. In this study, despite the lack of a statistically significant difference in terms of exceeding the norms of IL-5, IL-13, eotaxin 3 and MBP levels (qualitative analysis), we found a statistically significant increase in the concentration of each of the determined biomarkers (quantitative analysis) in the group of patients with diagnosed SR. This may also suggest the need to develop dedicated reference values of inflammatory biomarkers of blood serum for the esophagus' inflammatory diseases.

Gonsalves et al. [28] demonstrate lack of histological consistency (epithelial hypertrophy and/or eosinophilic infiltration) between the specimens taken from SR patients and esophageal mucosa specimens from EoE patients. However, in our present study, although without histopathological assessment, which is an unquestionable limitation of the study, we found numerous clinical and endoscopic features typical for EoE in the group of SR patients. In our study, SR was significantly more common in male patients (which is a risk factor for developing EoE) [29]. The incidence of SR was also significantly higher in patients with food allergies (occurring in up to 46–79% of EoE cases) [24]. The age of SR patients was significantly higher than the age of patients with dysphagia without SR, which may seem unusual for EoE - typical for young people, but in the context of the potential pathogenesis of SR as a progression of chronic inflammation (up to esophageal fibrosis), it is also typical for EoE [15,16]. Clinical symptoms related to the severity of the disease, characteristic of EoE [29], and also occurring in chronic or complicated GERD [30], such as choking on swallowing, food impaction or odynophagia, in our study occurred significantly more often in the SR patients than in the non-SR patients. Among the endoscopic features that often pathogenetically associate SR with GERD, in the plication theory [6,7] or the self-protective theory [8], there are endoscopic features of a hiatal hernia, as well as macroscopically visible features of esophagitis. In our study, the presence of any of these diagnoses was not significantly associated with the presence of SR, which questions the relationship between SR and GERD.

Rings and strictures were significantly more common in the group of patients with SR, which may justify the theory of SR formation as the effect of advanced esophageal trachealization. An additional argument indicating a common mechanism of ring formation in EoE and SR are endoscopic observations showing that patients with EoE tend to have narrower SR than patients without EoE [10]. Contrary to previous assumptions, the diagnosis of EoE was correlated with inflammatory and total EREFS and not correlated with fibrostenotic EREFS, which does not allow to clearly determine the pathogenesis of SR, but together with the increase in the levels of biochemical markers, it emphasizes its inflammatory nature.

4.1. Limitations of the study

The undoubted limitation of this study is the lack of an impedance pH-measurement, which would ultimately exclude the occurrence of reflux, as well as high-resolution esophageal manometry, which would allow to refine the diagnosis of hiatal hernia. Among the endoscopic features typical for EoE in the performed panendoscopies, the presence and advancement of major and minor features were assessed retrospectively, and total EREFS and its subtypes (inflammatory and fibrostenotic) were calculated [20,21].

5. Conclusions

Increased concentrations of serum biochemical markers, including mainly MBP (a pro-inflammatory product of eosinophilic degranulation) and TGF-β1 (mediating numerous fibrotic processes), indicate inflammatory and probably indirectly also fibrostenotic pathogenesis of SR. The described biochemical, clinical and endoscopic relationships between SR and EoE counterbalance the current theories and research confirming the relationship between SR and GERD. Numerous pathophysiological links, based on the action of hydrochloric acid in the development of these two, until recently considered to be mutually exclusive diseases – EoE and GERD, in the light of current knowledge, do not allow to clearly classify SR as an exclusive complication of only one of them.

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The Author Contribution

Study Design: Dorota Waśko-Czopnik, Benita Wiatrak, Joanna Sarbinowska.

Data Collection: Dorota Waśko-Czopnik, Joanna Sarbinowska.

Statistical Analysis: Benita Wiatrak.

Data Interpretation: Joanna Sarbinowska, Benita Wiatrak, Dorota

Waśko-Czopnik.

Manuscript Preparation: Joanna Sarbinowska, Benita Wiatrak, Dorota Waśko-Czopnik.

Literature Search: Joanna Sarbinowska.

Funds Collection: Joanna Sarbinowska, Dorota Waśko-Czopnik.

Declaration of competing interests

The authors declare no conflict of interests.

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4. Searching for noninvasive predictors of the diagnosis and monitoring of eosinophilic esophagitis – the importance of biomarkers of the inflammatory reaction involving eosinophils.

Joanna Sarbinowska, Benita Wiatrak, Dorota Waśko-Czopnik

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Searching for Noninvasive Predictors of the Diagnosis and Monitoring of Eosinophilic Esophagitis—The Importance of Biomarkers of the Inflammatory Reaction Involving Eosinophils

Joanna Sarbinowska [1], Benita Wiatrak [2,*] and Dorota Waśko-Czopnik [1]

[1] Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland; sarbinowska.joanna@gmail.com (J.S.); dorota.wasko-czopnik@umed.wroc.pl (D.W.-C.)
[2] Department of Pharmacology, Wroclaw Medical University, Mikulicza-Radeckiego 2, 50-345 Wroclaw, Poland
* Correspondence: benita.wiatrak@umed.wroc.pl; Tel.: +48-717-841-438

Abstract: Background: Invasive and costly endoscopic diagnosis is obligatory for the diagnosis and monitoring of eosinophilic esophagitis (EoE). This study aims to evaluate the usefulness of serum biomarkers involved in eosinophil-mediated inflammation in the management of EoE. Methods: A prospective cohort study was conducted in 58 patients with dysphagia. Each participant completed a health questionnaire, underwent esophagogastroduodenoscopy with esophageal biopsy for histopathological examination and assessment of total, inflammatory and fibrostenotic Eosinophilic Esophagitis Reference Score (EREFS). Serum levels of interleukin 5 (IL-5), interleukin 13 (IL-13), transforming growth factor β1 (TGF-β1), major basic protein (MBP), and eotaxin 3 were determined by enzyme immunoassays. Total of 16 patients meeting the histological criteria for EoE were treated with proton pump inhibitors for 8 weeks, and then the same diagnostics was performed again. Results: Statistically significantly higher concentrations of MBP and TGF-β1 were demonstrated in the group of patients with EoE, while MBP and eotaxin 3 correlated with the peak eosinophil count (PEC). Baseline MBP levels and eotaxin 3 after treatment significantly positively correlated with EREFS. There was a negative correlation between IL-13 and fibrostenotic EREFS. Additionally, after treatment, a negative correlation TGF-β1 was noted with the inflammatory EREFS and a positive correlation with the fibrostenotic EREFS. Conclusions: The potential role of MBP in predicting the diagnosis of EoE, eotaxin 3 in predicting the advancement and correlation of IL-13 and TGF-β1 in differentiating the inflammatory and fibrotic course of the disease may facilitate the management and individualization of EoE therapy.

Keywords: eosinophilic esophagitis; eotaxin 3; interleukin 5; interleukin 13; major basic protein; transforming growth factor beta 1

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1. Introduction

In recent decades, in response to global trends aimed at minimizing the invasiveness and the cost of treatments, we observe a significant development of innovative diagnostic methods and technologies to detect new biomarkers based on the study of pathomechanisms of diseases, especially those with a chronic nature and increasing morbidity.

Eosinophilic esophagitis (EoE) is a disease that has undoubtedly been a clinical challenge in the last two decades, not only due to the almost 30-fold increase in the incidence, more than 20-fold increase in the frequency of performing diagnostic tests [1,2], but also due to chronic course and recurrent nature of the disease, often leading to complications [2]. The dynamic development of research on EoE, which was initially considered a purely pediatric disease [3], is reflected in multiple changes to diagnostic and therapeutic guidelines that have been updated six times since the disease's first reports [4–9]. According to the

definition published in 2017 and maintained in 2020 in the latest recommendations of the American Gastroenterological Association and the Joint Task Force on Allergy-Immunology Practice Parameters, EoE is the primary immune-mediated esophageal disease manifested by esophageal dysfunction in the form of dysphagia and food impaction [9]. Histologically are observed chronic inflammatory infiltrates with a predominance of intraepithelial eosinophils [9]. Detection of ≥15 eosinophils/HPF (per high power field) in a biopsy of the esophageal mucosa, the coexistence of clinical symptoms and the exclusion of other conditions with systemic or local eosinophilic infiltration are therefore mandatory for the diagnosis of the disease, but also for monitoring the advancement and effectiveness of therapy [7–9]. Periodic panendoscopy with the collection of numerous specimens from the esophageal mucosa for histopathological evaluation, although considered the only "gold standard", is not a perfect solution. Apart from being cost-intensive and invasive, which, together with persistent or recurrent symptoms, significantly deteriorate patients' quality of life [10], they also carry a high risk of underdiagnosis and therapeutic delay. A too small number of samples taken (less than 6), as well as their incorrect location, limited to only one-half of the esophagus or only lesions (while a normal endoscopic image does not rule out the disease) [7,11,12] significantly reduce the chances of a correct diagnosis. Although precisely specified, the histological criterion is based on the pathologist's subjective assessment, which, as the research shows, in as many as 22% of cases, may lead to underestimation and erroneous exclusion of the diagnosis [13].

In response to the current needs, numerous studies are being developed to maximize the individualization of EoE management by assessing already recognized methods and completely newly developed and implemented technologies [14]. Among them, a minimally invasive targeting marker would be of strategic importance. Even in the case of non-specific clinical symptoms, it should be able to specifically suggest endoscopic and histopathological diagnostics (which are the only methods that ultimately differentiate from other causes of dysphagia, including neoplasm) and be sensitive enough to replace it in control and monitoring the effectiveness of the therapy [15]. All these criteria would be met by a biochemical marker detectable in the blood of patients. However, no substance with sufficient sensitivity and specificity to be included in the guidelines has yet been identified [7,9].

In this study, an attempt was made to assess the concentrations of serum biomarkers involved in the Th2-dependent immune response, and thus influencing the formation and advancement of EoE. These were the cytokines associated with stimulating intra-tissue migration and degranulation of eosinophils—interleukin 5 (IL-5), interleukin 13 (IL-13), and eotaxin 3, as well as biomarkers involved in increasing muscle reactivity, development of fibrosis, and remodeling—eosinophil major basic protein (MBP) and transforming growth factor β1 (TGF-β1) [16–18].

The aim of the study was, therefore, to evaluate the use of serum biomarkers (IL-5, IL-13, eotaxin 3, MBP, and TGF-β1) in the diagnosis and monitoring of EoE by assessing their correlation with the occurrence, as well as endoscopic and histopathological advancement of EoE in patients diagnosed with dysphagia.

This study is not the first attempt to assess the diagnostic and prognostic significance of serum markers with a recognized pathophysiological role in EoE. However, the design of this study was adjusted to consider the main allegations raised in the evaluation of previous studies analyzing the concentration of biomarkers in EoE—the prospective nature of the study was adopted, and the time between taking serum samples and performing endoscopic examinations with biopsies was shortened as much as possible [14].

2. Materials and Methods

2.1. Study Design and Population

This prospective cohort study was conducted at the Department of Gastroenterology and Hepatology and the Department of Otolaryngology, Head and Neck Surgery at Wroclaw Medical University in Poland. From 1 November 2017 to 30 April 2020, the

58 adult patients were recruited to the project for endoscopic diagnosis of dysphagia. The criterion of exclusion from participation in the study were already diagnosed chronic diseases with possible eosinophilic infiltration of the gastrointestinal tract (eosinophilic esophagitis, eosinophilic gastroenteritis, Crohn's disease, celiac disease), rheumatological, dermatological, infectious and genetic disorders with possible peripheral eosinophilia, as well as dysphagia caused by a diagnosed neoplastic infiltration of the esophagus. None of the project participants was a transplant recipient, and no one reported heartburn as an accompanying symptom of dysphagia. Before enrollment in the project, high-resolution esophageal manometry (HRM) was performed to rule out patients with achalasia as a potential cause of esophageal eosinophilia. HRM always precedes panendoscopy to avoid the therapeutic effect of the endoscope and possible effects on the manometric parameters and esophageal motility assessment.

Each project participant completed a questionnaire on health and existing diseases, with particular emphasis on atopy. Esophagogastroduodenoscopy was performed, and serum levels of cytokines IL-5 and IL-13, TGF-β1, and eotaxin 3, as well as the product of eosinophil degranulation—MBP, were determined. Diagnostic panendoscopies were performed by one endoscopist—gastroenterology specialist using an Olympus GIF-Q180 device (Olympus, Tokyo, Japan). During the medical examination, the presence of endoscopic features of esophagitis, hiatal hernia, and Schatzki ring were analyzed in detail. Retrospectively, based on the obtained photographic documentation and results description, the presence and advancement of features included in the EoE Endoscopic Reference Score (EREFS) were assessed, including edema, rings, exudates, furrows and strictures, and also crepe paper esophagus, i.e., mucosal fragility or laceration upon passage of diagnostic endoscope [19]. EREFS was assessed similarly to the study by Dellon et al. [20], taking into account the current modified EREFS classification system [19]. Total EREFS (rated on a scale from 0 to 9) was the sum of the points obtained in assessing all the EREFS classification features. The inflammatory subscore was the sum of the points given for the presence of exudate, edema and furrows (from 0 to 4), and fibrostenotic subscore for the diagnosis of rings and strictures (from 0 to 4). Regardless of the presence of the described macroscopic changes, from each participant during the study, six esophageal mucosa biopsy specimens were collected (two each for distal, middle, and proximal esophagus). The obtained material was sent for histopathological examination to assess peak eosinophil count (PEC) at each biopsy, interpreted as the maximum number of eosinophils per HPF (standard size ~0.3 mm^2). Each biopsy sample was re-verified by a second independent specialist—a pathologist. A venous blood sample was also collected from each participant within a maximum of 7 days after endoscopy, centrifuged, and the collected serum was stored at −70 °C. Quantification of IL-5, IL-13, and TGF-β1 levels was performed using Diaclone enzyme immunoassays (Diaclone SAS, Besancon, France), and eotaxin 3 and MBP using Cloud-Clone enzyme immunoassays (Cloud-Clone Corp., Houston, TX, USA). Both test protocols and reference values of biomarkers for the general population were adopted in accordance with the recommendations of the assay manufacturers (for IL-5 from 0 to 18.49 pg/mL, for IL-13 from 0 to 7.28 pg/mL, for TGF-β1 from 5222 to 13,731 pg/mL, for eotaxin 3 from 18.6 to 51.2 pg/mL, and for MBP from 372.1 to 685.4 ng/mL).

After completing medical examinations, the project participants were divided according to the histopathological criterion's fulfillment for the diagnosis of EoE. Patients with ≥15 eosinophils/HPF in the biopsy samples constituted the group of patients with EoE, while the remaining patients—the non-EoE group. EoE patients were then treated for 8 weeks with proton pump inhibitor (PPI)—omeprazole in the dose of 20 mg twice daily (following current therapeutic UEG, EAACI ESPGHAN, and EUREOS guidelines from 2017 with later amendments) [7–9]. After 8 weeks, each patient in the EoE group completed the health and symptom questionnaire again, had a second panendoscopy with distal, middle, and proximal esophagus biopsies for histopathological examination. Venous blood was collected again to determine eosinophil-mediated inflammatory biomarkers (the protocols were identical to those used for qualifying patients to the project).

2.2. Statistical Analysis

The sample size was calculated using the general linear model ($\alpha = 0.05$; power = 0.90; effect size = 0.25). The required number of patients was calculated as 39. We assumed a dropout rate of 30%, and a sample size of 58 patients was selected.

The data distribution was analyzed with the Shapiro–Wilk test, and it turned out that there was no normal distribution. Data are presented as median and interquartile ranges (IQR). The comparison of demographic data between the group of patients with EoE and the control group was performed using the chi-squared test. Quantitative values obtained in pre-treatment and post-treatment groups of patients were compared using the Wilcoxon test.

One-dimensional logistic models were used to assess the relationships and prediction potential of the studied biomarkers. The dependent variable was the variable representing the diagnosis of EoE, and the independent variable was the biomarker under study. Significant statistical models are marked in red. Sensitivity and specificity were calculated and presented for biomarkers for the EoE pre-diagnosis score. Spearman's rank correlation coefficients were used to investigate correlations between biomarker levels, PEC in esophageal biopsies and diagnosis of EoE.

Statistical analyses were performed using Statistica 13.0 software (Dell Software Inc., Round Rock, TX, USA). In the data analysis, $p < 0.05$ was used as the level of significance.

2.3. Ethical Considerations

The Bioethics Committee at the Wroclaw Medical University approved the project on 17 August 2017 (KB no. 544/2017), with a subsequent extension on 6 December 2018 (KB no. 730/2018). All project participants gave informed written consent to participate in the study.

3. Results

3.1. Study Population

During the 30 months of the study, taking into account the assumed exclusion criteria, 58 patients were recruited for endoscopic diagnosis due to dysphagia. Based on the first histopathological evaluation of the specimens from the esophageal mucosa collected during esophagogastroduodenoscopy, initially, 15 patients met the histopathological criteria for the diagnosis of EoE. However, in 16 patients (27.6%), EoE features were confirmed by microscopic examination after re-evaluating the specimens. The remaining 42 persons (72.4%) belonged to the non-EoE group, in which 6 patients were diagnosed with hiatal hernia, and 4 persons with erosive esophagitis and Schatzki ring as a possible cause of dysphagia reported upon admission. In 28 participants, the cause of the symptoms was not identified in the endoscopic and histopathological examination.

Despite the disproportion in both groups' size, no statistically significant differences were observed in terms of age, the burden of atopic diseases or clinical symptoms related to esophageal dysfunction (Table 1). The demographic feature differentiating the studied populations was gender—in the EoE group, a statistically significant majority of patients were men (68.75% vs. 40.48%, $p = 0.05$).

The described groups significantly differed in terms of histopathological, endoscopic, and biochemical features (Table 1, Figure 1). As predicted, EoE patients had significantly higher median PEC values than the non-EoE group ($p = 0.0001$). However, endoscopic features of esophagitis ($p = 0.33$), hiatal hernia ($p = 0.86$), and Schatzki's ring ($p = 0.12$) are not characteristic of patients with dysphagia in the course of EoE in the studied population. Among the six key features included in the EREFS, the presence of edema ($p = 0.026$), as well as all endoscopic features of fibrostenosis, i.e., esophageal rings ($p = 0.046$) and strictures ($p = 0.02$), was significantly more often observed in the group of patients with EoE. The results for both EREFS subscores—inflammatory ($p = 0.003$) and fibrostenotic ($p = 0.02$), and also total EREFS ($p = 0.0015$) turned out to be significantly higher in the EoE group compared to the control group.

Table 1. Characteristics of the study participants divided into the group with eosinophilic esophagitis (EoE) and without EoE (EREFS—Eosinophilic Esophagitis Endoscopic Reference Score, PEC—peak eosinophil count).

Parameters		EoE	Without EoE	*p*
Patients [*n* (%)]		16 (27.6)	42 (72.4)	-
Age median (range)		28.5 (20–50)	36.5 (24–68)	0.47
Male [*n* (%)]		11 (68.75)	17 (40.48)	0.05
Atopy [*n* (%)]		8 (50.00)	20 (47.62)	0.87
Atopy	inhalation allergies [*n* (%)]	4 (25.00)	10 (23.81)	0.92
	food allergies [*n* (%)]	4 (25.00)	5 (11.90)	0.21
	bronchial asthma [*n* (%)]	0 (0.00)	5 (11.90)	0.14
	atopic dermatitis [*n* (%)]	2 (12.50)	3 (7.14)	0.52
	allergic sinusitis [*n* (%)]	1 (6.25)	3 (7.14)	0.90
Clinical symptoms	choking [*n* (%)]	9 (56.25)	18 (42.86)	0.36
	food impaction [*n* (%)]	9 (56.25)	21 (50.00)	0.67
	odynophagia [*n* (%)]	7 (43.75)	18 (42.86)	0.95
Endoscopic features	inflammation [*n* (%)]	3 (18.75)	4 (9.52)	0.33
	endoscopic features of a hiatal hernia [*n* (%)]	2 (12.50)	6 (14.29)	0.86
	Schatzki ring [*n* (%)]	4 (25.00)	4 (9.52)	0.12
	edema [*n* (%)]	6 (37.50)	5 (11.90)	0.026
	rings [*n* (%)]	8 (50.00)	10 (23.38)	0.046
	exudates [*n* (%)]	3 (18.75)	5 (11.90)	0.49
	furrows [*n* (%)]	3 (18.75)	3 (7.14)	0.19
	strictures [*n* (%)]	1 (12.50)	0	0.02
	crepe paper esophagus [*n* (%)]	0	0	-
EREFS	inflammatory [*n* (%)]	9 (56.25)	8 (19.04)	0.003
	fibrostenotic [*n* (%)]	9 (56.25)	10 (23.81)	0.02
	total [*n* (%)]	12 (75.00)	12 (28.57)	0.0015
PEC	median (range)	45 (15–100)	0 (0–5)	0.0001

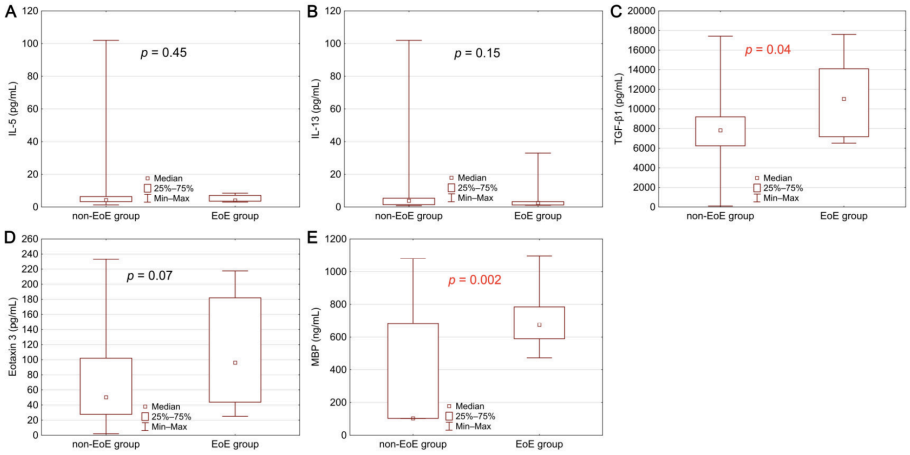

Figure 1. Comparison of serum biomarker concentrations between the group of patients with EoE and the non-EoE group: (**A**) interleukin 5—IL-5, (**B**) interleukin 13—IL-13, (**C**) transforming growth factor β1—TGF-β1, (**D**) eotaxin 3, (**E**) major basic protein—MBP. Statistical significance was evaluated with a Mann–Whitney U test.

3.2. *Biomarkers in the Prediction of Diagnosis and Histopathological Advancement*

The assessment of the concentration of biomarkers of the eosinophil-mediated inflammatory reaction in the blood serum revealed markers that could predict the disease's diagnosis. The median (IQR) concentrations determined during biomarker diagnostics are as follows: IL-5—4.25 (range 1.30–23.40) pg/mL, IL-13—3.00 (range 0.79–33.00) pg/mL, eotaxin 3—50.85 (range 1.98–233.10) pg/mL, MBP—682.5 (range 299.0–1096.0) ng/mL and TGF-β1—7995 (range 3150–17,604) pg/mL.

The concentration of the studied biomarkers was compared between the group of patients with EoE and the control group. Statistically significantly higher concentrations of MBP ($p = 0.002$) and TGF-β1 ($p = 0.04$) were demonstrated in the EoE patients (Figure 1). A higher level was also observed in the case of eotaxin 3, where the difference was close to statistical significance ($p = 0.07$).

Similar results were obtained from the analysis in terms of exceeding the reference values of individual biomarkers. Obtained serum levels of TGF-β1 ($p = 0.04$) and MBP ($p = 0.0001$) exceed the upper limit of the general population's reference values. The described dependence was also observed for eotaxin 3, but without statistical significance ($p = 0.31$).

Relationships between biomarkers, diagnosis of EoE and PEC were evaluated using Spearman's rank correlation coefficients (Table 2). Levels of IL-5 and IL-13 showed a positive, statistically significant correlation between them. Simultaneously, there were weak negative correlations between these cytokines and PEC, diagnosis of EoE and concentrations of other biomarkers (for eotaxin 3 and in the case of IL-13 vs. TGF-β1 statistically significant). The concentration of TGF-β1 significantly correlated with the diagnosis of EoE and showed a weak positive correlation with the PEC. The opposite situation was observed for eotaxin 3, which significantly correlated with the PEC, without a significant positive correlation with the EoE diagnosis. The strongest statistically significant correlation was obtained for MBP, both with the PEC and diagnosis, which indicates the potential importance of this biomarker in diagnosing EoE.

Table 2. Spearman's rank correlation coefficients between biomarkers, diagnosis and PEC, before and after treatment.

Parameters	Before Treatment ($n = 16$)						After Treatment ($n = 7$)
	PEC	Diagnosis of EoE	IL-13	IL-5	TGF- β1	Eotaxin 3	PEC
IL-13	−0.12	−0.19					0.02
IL-5	−0.04	0.10	0.42				0.71
TGF-β1	0.10	0.27	−0.33	−0.12			0.53
Eotaxin 3	0.33	0.24	−0.46	−0.15	0.08		0.66
MBP	0.53	0.41	0.03	−0.13	0.06	−0.01	0.43

In addition to assessing the possible role of biomarkers in predicting the diagnosis of EoE, this study also attempts to assess their importance in predicting histological remission. For this purpose, the concentrations of biomarkers obtained from patients with EoE after 8 weeks of PPIs therapy were correlated with the PEC in samples collected from the esophageal mucosa during the control esophagogastroduodenoscopy. Due to the invasiveness and nuisance of the follow-up examination and limited endoscopic control during the COVID-19 pandemic, only 7 patients (i.e., 43.75% of project participants diagnosed with EoE) participated in the re-evaluation after two months of treatment. Among them, 5 patients (71.43%) achieved histopathological remission, defined as a reduction in the number of eosinophils found in esophageal mucosa biopsies below 15/HPF (median 10, range 0–70 eosinophils/HPF). The median (IQR) concentrations of the biomarkers after treatment are as follows: IL-5—5.8 (range 3.2–8.8) pg/mL, IL-13—4.1 (range 1.4–26.2) pg/mL, eotaxin 3—61.3 (range 34.9–120.7) pg/mL, MBP—577 (range 349–637) ng/mL, and TGF-β1—6690 (range 5670–15,024) pg/mL. Analysis of these values showed a strong positive but not

statistically significant correlation of TGF-β1, eotaxin 3, and MBP with PEC value (Table 2). The correlation of these markers with diagnosis and histopathological advancement was thus confirmed both at the diagnosis of EoE and after the first 8 weeks of treatment, but statistically significant values were obtained only in the first examination (Table 2).

3.3. Biomarkers in the Assessment of Endoscopic Advancement and Prognosis of Inflammatory or Fibrostenotic Course

In addition to the possible diagnostic potential in predicting histopathological advancement of EoE, the usefulness of the studied biomarkers in correlation with an endoscopic assessment of total, inflammatory and fibrostenotic EREFS was also checked (Table 3).

Table 3. Spearman's rank correlation coefficients between biomarkers and EREFS subscores, before and after treatment.

	Parameter	IL-13	IL-5	TGF-β1	Eotaxin 3	MBP
Before Treatment (n = 16)	Inflammatory EREFS	0.021	−0.037	0.145	−0.014	0.526
	Fibrostenotic EREFS	−0.134	−0.142	0.226	0.106	0.264
	EFERS	−0.063	−0.084	0.224	0.094	0.447
After Treatment (n = 7)	Inflammatory EREFS	0.144	0.000	−0.144	0.577	0.874
	Fibrostenotic EREFS	−0.722	0.289	0.289	0.289	−0.291
	EFERS	−0.535	0.267	0.134	0.802	0.539

MBP was a marker most strongly (statistically significantly) correlated with eosinophilic infiltration and endoscopic advancement in all EREFS subscores. Contrary to the results obtained before treatment, the correlation with fibrostenotic EREFS after treatment was weak negative (but statistically significant). IL-13 significantly correlated only with post-treatment fibrostenotic EREFS, and this correlation was strong negative. The moderate negative correlation between IL-13 and total EREFS after treatment is also noteworthy. A relationship pattern opposite to MBP after treatment was observed for TGF-β1 after treatment—there were significant weak correlations, negative with inflammatory EREFS and positive with fibrostenotic EREFS. After treatment, positive statistically significant correlations were obtained for eotaxin 3—moderate for inflammatory EREFS, weak in the fibrostenosis, and strong for a total score. In the case of IL-5, only weak and statistically insignificant correlations with EREFS were observed, which does not allow including this interleukin among the markers of prognostic importance in assessing endoscopic advancement.

3.4. Diagnostic Potential of the Studied Biomarkers

Biomarker concentrations before diagnosis and after 8 weeks of therapy in the group of patients with EoE are presented in Figure 2. After treatment with PPI, a statistically significant decrease in MBP concentration was observed (p = 0.05). The treatment also caused an increase in the IL-13 level (p = 0.03).

A graphical representation of the effectiveness of studied biomarkers in predicting EoE diagnosis is presented in Figure 3 as ROC curves. The calculated AUC values (area under the ROC curve) for all markers oscillated in the range of 0.593–0.742. The highest AUC value was obtained for the MPB, simultaneously with the lowest AUC error value.

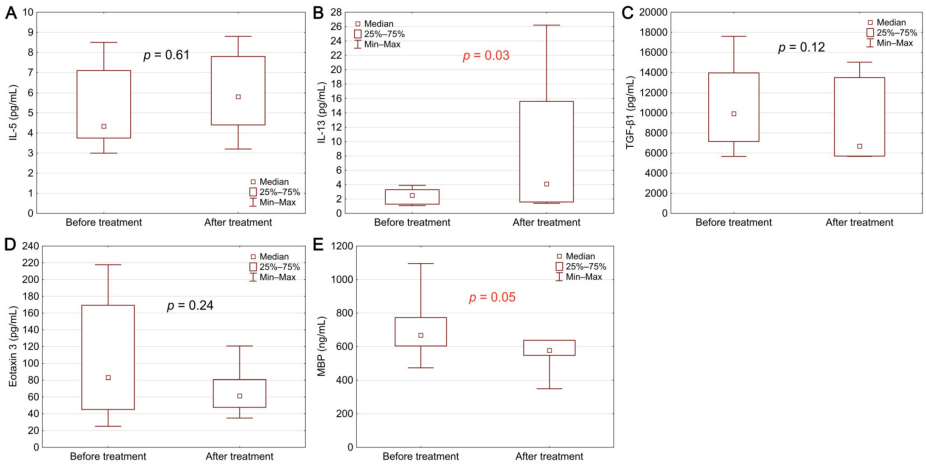

Figure 2. Biomarker levels in EoE patients before and after treatment: (**A**) IL-5, (**B**) IL-13, (**C**) TGF-β1, (**D**) eotaxin 3, (**E**) MBP. Statistical significance was evaluated with a Wilcoxon test.

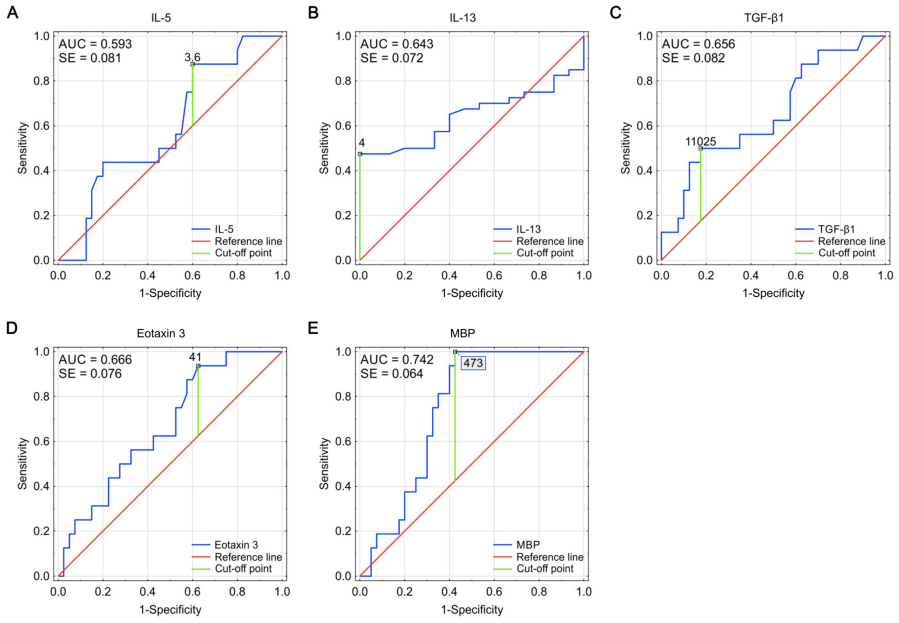

Figure 3. ROC curves for IL-5 (**A**), IL-13 (**B**), TGF-β1 (**C**), eotaxin 3 (**D**), and MBP (**E**), showing their effectiveness as markers predicting the diagnosis of EoE. Optimal cut-off points were determined using Youden index analysis (AUC—area under the curve, SE—standard error).

4. Discussion

So far, many studies have attempted to identify a tissue marker correlating with diagnosis [21], progression [22–25], and response to EoE treatment [22,26,27], allowing for the differentiation of esophageal diseases with accompanying dysphagia [28–30] and

being a trigger marker in disease development, and thus an effective target of biological therapies [31]. Due to the predicted low specificity of markers involved simultaneously in the pathomechanisms of numerous allergic diseases [32] and the ambiguous results of research on tissue markers in EoE, little attention was paid to assessing the significance of these markers' serum levels.

In this study, we looked for a minimally invasive marker, determined in venous blood serum, having a potential predictive value for the diagnosis, histopathological and endoscopic advancement of EoE, and correlated with the response to PPI treatment.

Based on the results of our study, it can be concluded that MBP was a serum marker most strongly (statistically significantly) correlated with both the diagnosis of EoE, as well as the peak number of eosinophils/HPF and endoscopic advancement (assessed at diagnosis by inflammatory, fibrostenotic, and total EREFS). The highest sensitivity and specificity also characterized this marker. Although the correlation between the level of MBP in blood serum [33] or saliva of patients [34] and the diagnosis or stage of EoE has not been proven so far, this marker's importance in the esophageal string test has been repeatedly emphasized [35,36], and above all in tissue tests. Positive correlations were found in predicting the diagnosis of EoE [28,29] and in assessing the response to treatment [26]. The advantage of MBP1 over the peak eosinophil count (PEC) in diagnosing the disease was also proven in two research studies based on the assessment of tissue markers [23,37]. This was justified pathophysiologically by the degranulation of eosinophils, which by releasing granular proteins, including MBP, into the tissues, lose their cellular morphological phenotype and therefore are not included in the histopathological examination result [23,37]. The correlation between MBP and the diagnosis of EoE found in our study, although strong and statistically significant, is weaker than the correlation between the diagnosis and PEC. This is probably the price of less invasive serological determinations, but in the face of a limited number of studies on this group of potential predictors of EoE diagnosis, it does not undermine the sense of the study.

A serum marker that was also strongly correlated with PEC in our study was eotaxin 3, while the level of TGF-β1 correlated with the diagnosis of EoE. For these three markers, i.e., MBP, eotaxin 3, and TGF-β1, there were also strong positive, but not statistically significant, correlations with the PEC after 8-week PPI therapy. Due to the small group of patients with EoE recruited to the project, low attendance (43.75%) in control studies after 8-week PPI therapy, as well as the lack of statistical significance of the correlations between markers and PEC after treatment, it is difficult in this study to select a serum marker predicting the histological remission of the disease.

The levels of MBP, TGF-β1, and eotaxin 3 were positively correlated with each other. In turn, a negative correlation occurred between these markers and IL-5 and IL-13 cytokines (with a positive correlation between them). It can be interpreted as a synergy of these proteins' actions at subsequent stages of developing the inflammatory reaction involving eosinophils. The pathophysiology of the disease confirms this. After the significant participation of IL-5 and IL 13 in the stimulation of the influx of eosinophils to the esophageal mucosa, with the development of inflammation, their importance and tissue concentration decrease, and secondarily also their concentration in the blood serum. They give way to induced eosinophil-activating chemokines, such as eotaxin 3, and products of eosinophil degranulation, including MBP and TGF-β [38].

Considering the small number of studies on blood serum markers to date, an attempt to predict the course and advancement of inflammatory and fibrostenotic EoE based on the correlation with the endoscopic assessment of EREFS seems innovative. Apart from the already discussed correlation with MBP, we also observed strong and moderate statistically significant correlations of eotaxin 3 with remission scores in each of the post-treatment EREFS subscores in our study. The inhibitory effect of treatment with conventional doses of PPIs on the expression of eotaxin 3, and secondarily on the development of the disease [39–41], would therefore be reflected in the results of this study and would settle the

hitherto ambiguous observations confirming [42] or denying [33,43,44] the importance of eotaxin 3 concentration in monitoring the course of EoE.

Based on the interpretation of the IL-13 and TGF-β1 concentrations, it seems possible to differentiate the course of the inflammatory and fibrostenotic EoE in the studied group of patients. The increase in the concentration of TGF-β1, with the simultaneous decrease in the concentration of IL-13 in the serum, may correspond to the development of fibrostenosis in the course of EoE. Conversely, a low concentration of TGF-β1 in the serum, with a simultaneous increase in the concentration of IL-13, may indicate less advanced disease and the predominance of inflammatory processes over fibrostenotic processes. These correlations were observed despite the apparent individual low specificity of both TGF-β1 and IL-13 in the diagnosis and monitoring of EoE [33]. TGF-β1 is considered the "main mediator of fibrosis" responsible for the activation of fibroblasts and the induction of epithelial-mesenchymal transformation in many fibrostenotic processes [45]. In turn, IL-13 is well-known for its role in many atopic diseases, where it contributes to eosinophil chemotaxis, goblet cell hyperplasia, collagen deposition and an increase in smooth muscle contractility [17].

Another investigated serum marker with a confirmed role in the pathomechanism of EoE is IL-5. Previous studies assessing the importance of this cytokine in diagnosing and monitoring EoE have not confirmed the correlation of its concentration with the diagnosis and course of the disease in the group of adult patients [34] and the pediatric population [46]. In another prospective study evaluating serum biomarker levels in EoE after PPIs therapy, a statistically significant negative correlation was found between IL-5 and esophageal eosinophilia and no prediction of the post-treatment tissue eosinophilia [44]. Similar conclusions can be drawn from this study, but the negative correlation with esophageal eosinophilia was not statistically significant. In the cited study, the described relationship was justified by the high accuracy of the ELISA test used [44], which may also be reflected in our study (at the detection threshold of 5 pg/mL, the median IL-5 concentration in the group of patients with EoE was 4.07 pg/mL and 4.30 pg/mL in the control group). A negative correlation with tissue eosinophilia can also be observed in the case of IL-13, the concentration of which, similarly to IL-5, significantly increases in the serum after treatment. The described observation is not entirely clear but may be due to the mediation of these interleukins in the remodeling process, leading to esophageal motility disorders, which may persist regardless of the active eosinophilic inflammation, even after its complete resolution [47–49].

This study's undoubted advantage is an attempt to minimize the invasiveness of diagnosis and monitoring of EoE by evaluating the so far rarely assessed or not assessed markers in blood serum with a confirmed pathophysiological relationship with EoE. Important aspects are also: the prospective nature of the study, the shortest possible time interval between taking serum samples and performing endoscopic examinations with biopsies, re-verification of all histopathological examinations of the specimens collected during the project, as well as the correct selection of the study population—homogeneous in terms of age and symptoms, allergic burden, and heterogeneous only in terms of gender. Male gender is a significant risk factor for EoE resulting from the suggested sex-dependent association between single nucleotide polymorphisms in the thymic stromal lymphopoietin gene and its receptor and the protective effect of estrogen hormone signaling in women [7]. The weakness of this study is the relatively small study group, the population limited to adults only, and the lack of a pH-metric assessment that would allow for objective classification of patients with possible gastroesophageal reflux, often coexisting with EoE or being an independent cause of dysphagia in the group of patients without EoE diagnosis. The limitations of this project suggest the need to continue research on noninvasive blood serum biomarkers and confirm the obtained results in the validation cohort, taking into account the possible effect of co-occurrence and overlapping of EoE and GERD, as well as depending on the pharmacotherapy used: PPI, local steroid therapy or elimination diet.

5. Conclusions

Based on the results of this study and the available literature data, it is not possible to select one serum biomarker with pleiotropic predictive and prognostic functions in EoE.

The observed trend, suggesting the importance of MBP in predicting the diagnosis and eotaxin 3 in predicting disease advancement, emphasizes the potential for improving the management and increasing the individualization of treatment. However, the necessary condition is to determine the markers several times, and not one parameter should be considered, but the whole group of them together, taking into account the pathophysiological role and interdependencies.

It can be predicted that this project, as well as the existing high-quality prospective studies correlating the concentration of individual markers in the blood serum with the diagnosis and progression of EoE, has developed a material for the creation of an automated algorithm that would provide intelligent analysis of the obtained data and could improve the precision of EoE diagnostics and therapy in the future.

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

Data Availability Statement: The data generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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

Trial Registration

This trial was registered with ClinicalTrials.gov (no. NCT04803162). Registered 17 March 2021—Retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT04803162 (accessed on 13 June 2021).

Abbreviations

AUC	area under the curve
ELISA	enzyme-linked immunosorbent assay
EoE	eosinophilic esophagitis
EREFS	Eosinophilic Esophagitis Endoscopic Reference Score
HPF	high power field
IL-5	interleukin 5
IL-13	interleukin 13
IQR	interquartile ranges
MBP	major basic protein
PEC	peak eosinophil count
PPI	proton pump inhibitor
ROC	receiver operating characteristic
TGF-β1	transforming growth factor β1

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5. Esophageal motility disorders in the natural history of acid-dependent causes of dysphagia and their influence on patients' quality of life – a prospective cohort study.

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Article

Esophageal Motility Disorders in the Natural History of Acid-Dependent Causes of Dysphagia and Their Influence on Patients' Quality of Life—A Prospective Cohort Study

Joanna Sarbinowska [1,*], Benita Wiatrak [2] and Dorota Waśko-Czopnik [1]

[1] Department of Gastroenterology and Hepatology, Wroclaw Medical University, Borowska 213, 50-556 Wroclaw, Poland; dorota.wasko-czopnik@umed.wroc.pl
[2] Department of Pharmacology, Wroclaw Medical University, Mikulicza-Radeckiego 2, 50-345 Wroclaw, Poland; benita.wiatrak@umed.wroc.pl
* Correspondence: sarbinowska.joanna@gmail.com; Tel.: +48-717-332-130

Abstract: Background: Esophageal dysmotility may be the cause or a secondary effect of gastric acid-dependent diseases: erosive reflux disease (ERD), Schatzki ring (SR) and eosinophilic esophagitis (EoE). Methods: This study aims to compare concomitant dysphagia with ERD, SR and EoE, considering manometric patterns, their role in the natural history and their impact on assessing quality of life. Fifty-eight patients with dysphagia underwent high-resolution manometry and esophago-gastro-duodenoscopy (EGD) with an assessment of SR, ERD and sampling for EoE, completed a questionnaire with the Eating Assessment Tool (EAT-10) and the Gastrointestinal Quality of Life Index. Based on endoscopic images and the histopathological criterion of EoE ($\geq$15 eosinophils/high-power field), patients were assigned to groups with ERD, EoE, SR and with normal endoscopic and histopathological images. In the data analysis, $p \leq 0.05$ was considered statistically significant. This trial was registered with ClinicalTrials.gov (no. NCT04803162). Results: Both EoE, SR and ERD correlate with ineffective motility. In ERD, normal peristalsis precedes the development of the disease, unlike EoE, which develops later and leads to absent contractility. The development of SR is associated with disorders of the upper esophageal sphincter (UES). In the group with SR and ERD, UES insufficiency significantly reduces the quality of life. Patients with normal esophagus in EGD scored the lowest quality of life and those with SR had the most severe dysphagia. Conclusion: The esophageal motility disorders co-occurring with endoscopic and histological anomalies do not significantly affect the severity of dysphagia, however, in the case of patients with ERD and SR and concomitant UES insufficiency, this motor dysfunction has a significant impact on the reduction in the patients' quality of life. Although no specific esophageal motility pattern typical of EoE, ERD and SR has been identified, comparative assessment of manometric features may have a potential role in differential diagnosis.

Keywords: diagnostic delay; esophageal motility disorders; gastrointestinal quality of life index; high-resolution manometry

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1. Introduction

Dysphagia, defined as a swallowing disorder consisting of difficulty in biting food and moving it towards the throat through the esophagus into the stomach, affects every 17th person in the world during their lifetime [1]. Complications related to dysphagia increase healthcare costs, and its chronic and recurrent nature, necessitating the need for invasive diagnostic tests, significantly reduces the quality of life of patients [2].

Both in Western Europe and North America, with the spread of proton pump inhibitors (PPIs), the incidence of GERD's complications as a cause of esophageal dysphagia is decreasing in favor of eosinophilic esophagitis (EoE), which is increasingly recognized as the main cause in children, as well as in adults [1].

Until recently, responses to food and inhalant allergens have been the main contributors to the pathophysiology of chronic EoE [3]. However, it is currently known that damage to the esophagus caused by hydrochloric acid, and the resulting discontinuance of the integrity of the epithelium, may favor the penetration of the allergen [4,5]. Thus, the relationship between GERD and EoE can be bidirectional and complex. Moreover, the coexistence of these two disease entities may constitute a specific perpetual motion machine in the activation of the chronic inflammatory response and the intensification of clinical symptoms.

Although dysphagia is sometimes associated with uncomplicated GERD, its presence raises the suspicion of endoscopic macroscopic changes in the form of erosive esophageal reflux disease (ERD) or stenosis, neoplastic infiltration or rings [6,7]. The pathophysiology of the formation of the lower esophageal ring, also known as the Schatzki ring (SR), is usually associated with chronic GERD and is intended to be a natural form of self-protective response against exposure to the acid reflux, thus minimizing symptoms and preventing the development of other complications such as Barrett's esophagus [8–10]. According to the available literature data, GERD justifies the development of SR in only two-thirds of patients [11]. The etiology in the remaining patients is unknown or hypothetically related to the advanced process of esophageal fibrosis and trachealization in the course of EoE [10].

In the etiology of dysphagia, which is a symptom of these diseases, esophageal motility disorders are also important, as it may be the cause and predictor or be a secondary effect and complication of acid-dependent esophageal diseases. However, the manometric findings in patients with EoE, ERD and SR are inconsistent and ambiguous [12–14]. Thus far, the significance of the possible coexistence of manometric disorders with the discussed esophageal diseases in the context of decreased quality of life of patients with these disorders has not been assessed.

Therefore, this study focuses on the comparative characteristics of dysphagia in a population of patients diagnosed with gastric acid-related esophageal diseases: ERD, EoE and SR, considering manometric patterns and the quality of life of patients assessed by the Gastrointestinal Quality of Life Index (GIQLI) questionnaire [15]. The study aimed to evaluate the significance of the coexistence of manometric disorders and acidic esophageal diseases and their role in shaping the assessment of patients' quality of life.

2. Materials and Methods

2.1. Study Population

Patients referred for the diagnosis of dysphagia to the Department of Gastroenterology and Hepatology and the Department of Otolaryngology, Head and Neck Surgery at the Medical University of Wroclaw were recruited to participate in a two-center prospective cohort study. From 1 November 2017 to 4 April 2020, 58 patients were enrolled in the project. The exclusion criterion from the survey was already diagnosed chronic diseases with possible eosinophilic infiltration of the gastrointestinal tract, rheumatological, dermatological and genetic disorders with possible peripheral eosinophilia and neoplastic infiltration of the esophagus. All project participants gave their written consent to participate in the study, and the ethical consent to implement the project was obtained by the Bioethics Committee of the Medical University of Wrocław on 17 August 2017 (KB No. 544/2017), with another extension on 6 December 2018 (KB No. 730/2018).

2.2. Endoscopy and Specimen Collection

All project patients underwent esophagogastroduodenoscopy (EGD) with an assessment of the presence of SR, defined as a peripheral thin membranous ring of the mucosa located above the gastroesophageal junction [16]. During the examination, the presence of endoscopic esophagitis was also monitored (the severity of ERD was assessed according to the Los Angeles classification) [7]. Regardless of the presence of macroscopic changes in the esophagus, six additional pieces of biopsies (two distal, medial and proximal) were taken to assess the maximum number of eosinophils per high-power field (HPF). Two independent

pathologists assessed all samples, while EGD was performed by one gastroenterologist using Olympus devices (GIF-Q180).

Based on endoscopic images and the histopathological criteria for the diagnosis of EoE, that is, the presence of $\geq$15 eosinophils/HPF, patients were assigned to the following groups: group 1, patients with ERD; group 2, patients with EoE; group 3, subjects with SR. The patients excluded from the above diagnoses constituted a group of patients with dysphagia without endoscopic and histopathological abnormalities.

2.3. High-Resolution Manometry

In order to avoid the therapeutic effect of the endoscope, and possible influence on manometric parameters and esophageal motility assessment in all patients, HRM was performed before esophagogastroduodenoscopy. HRM was performed, in patients who fasted for 8 h, in the supine position and after prior nasal anesthesia with lignocaine gel. After pre-calibration, a catheter with 36-solid state circumferential sensors spaced at 1 cm intervals (Sierra Scientific Instruments, Los Angeles, CA, USA) was inserted through the patient's nose into the stomach. After 3–5 min of rest, basal sphincter pressure (landmark ID frame) was assessed for another 30 s, followed by 10 sips of 10 mL water (swallow frames) and in the end, a Multi Rapid Swallow Test (MRS-test). High-resolution manometry data were recorded and analyzed using the ManoViewTM ESO 3.0 software (Sierra Scientific Instruments). Interpretation of the results was made according to the Chicago classification 3.0 [17].

2.4. Characteristics of Dysphagia and Gastrointestinal Quality of Life Index

In addition to the assessment of esophageal motility and the endoscopic and histopathological findings, the participants included in the project also completed a questionnaire on age, sex, marital status, place of residence, weight and height, smoking and alcohol consumption, possible chronic diseases—including atopic diseases—and diagnostic delay of dysphagia (interpreted as the time elapsed between the first episodes of dysphagia and the clinical diagnosis). In order to estimate the severity of dysphagia, the Eating Assessment Tool (EAT-10) questionnaire was used [18], consisting of 10 statements concerning problems with swallowing and subject to a 5-step assessment by the surveyed patients (where 0 points means no problem and 4 points—hard problem). Each of the individual statements and the total value made it possible to compare the severity of dysphagia in the studied groups of patients. To expand the EAT-10 questionnaire, patients in this study also reported the presence and severity of heartburn, regurgitation and globus according to the above criteria. However, the score obtained for the presence of these symptoms was considered separately and was not summed up with the total EAT-10. The standardized GIQLI questionnaire was used to assess the patients' quality of life, containing 36 questions about the symptoms of gastrointestinal diseases and related symptoms [15]. In terms of GIQLI, 5 measurement domains were distinguished: concerning symptoms (19 questions: No. 1–9 and 27–36), emotional state (5 questions: No. 10–14), physical functions (7 questions: No. 15–21), social functions (4 questions: No. 22–23 and 25–26) and treatment outcomes (1 question: No. 24). As the present study did not intend to intervene, the area of patient's treatment was not analyzed separately. For each of the 36 questions contained in the GIQLI, the respondent answered from "all of the time" to "never," with 0 to 4 points, respectively. Then, the results of individual questions were summarized, and the obtained result could reach a maximum of 144 points, which was interpreted as the best quality of life. The results for individual GIQLI domains were calculated as the arithmetic mean of points obtained in the questions included in domains [15].

2.5. Statistical Analysis

In statistical analysis, chi^2 tests were used to evaluate qualitative data. Quantitative data were used for Kruskal–Wallis ANOVA calculations with appropriate post-hoc. The correlation was calculated using Spearman ranks. The point of significance was $p \leq 0.05$.

3. Results

3.1. Characteristics of the Population

By applying the exclusion criteria, 58 patients with dysphagia were eventually enrolled in the study. Based on endoscopic examinations and histopathological evaluation of specimens taken from the esophageal mucosa, in as many as 58.62% of cases the cause of dysphagia reported by patients was not found. ERD was diagnosed in 7, SR in 8 and EoE in 16 patients. The disproportions in the size of the groups did not significantly affect the differences in demographic characteristics and disease burden, including atopic burden (Table 1). Among comorbid non-atopic diseases reported by project participants, the most common were hypothyroidism (10.34% of patients), hypertension (5.17%), insulin resistance (3.45%), polycystic ovary syndrome (3.45%) and tetany (3.45%), as well as in single patients: Wilson's disease, microscopic colitis, psoriasis, osteoarthritis, chronic sinusitis, paroxysmal tachycardia and irritable bowel syndrome. The lack of coexistence of many gastroenterological disorders, apart from the assessed swallowing disorders, made it possible to use the GIQLI questionnaire as a reliable tool for assessing and comparing the quality of life of the studied population.

Despite the demographic homogeneity of the project participants, statistically significant ($p < 0.001$) differences were observed in the manometric diagnoses included in the Chicago classification and in the assessment of the function of the upper esophageal sphincter (UES) and esophagogastric junction (EGJ). There were no cases of achalasia, distal esophageal spasm or jackhammer esophagus in the studied group of patients. In patients diagnosed with SR, absent contractility was diagnosed more often than in the other groups, and in the case of EoE, lower esophageal sphincter (LES) insufficiency. Patients with ERD in the study population more often than in the other groups of patients had ineffective motility (IEM), UES insufficiency and manometric features of hiatal hernia, while in patients with normal findings in endoscopic and histopathological examinations, EGJ outflow obstruction, UES spastic disorders and normal peristalsis were more often found (Table 1).

Statistically, differences between the patient groups were also found regarding the characteristics and severity of dysphagia assessed using the EAT-10 questionnaire (Table 1). Patients diagnosed with SR significantly more often reported difficulty in swallowing fluids ($p = 0.03$), odynophagia ($p = 0.04$) and rated the severity of dysphagia in summary EAT-10 assessment as the highest compared to other groups of patients ($p = 0, 05$). Swallowing stress was most commonly reported in patients without macroscopic and microscopic esophageal abnormalities ($p = 0.001$) and in patients diagnosed with ERD ($p = 0.04$). Heartburn was characteristic of the ERD group, while regurgitation and globus were more common in SR patients.

The subjective assessment of the quality of life of patients in terms of symptoms, physical and emotional well-being resulted as significantly statistically different between the groups of patients and it was assessed the worst in the group of patients with EoE (Table 1).

Table 1. Demographic and clinical characteristics of participants.

Parameters		All Patients	ERD	SR	EoE	Normal Esophagusin EGD and Histopathology	*p*
Patients [n (%)]		58 (100%)	7 (12.1%)	8 (13.8%)	16 (27.6%)	34 (58.6%)	$p = 0.00001$
Age median (range)		34 (20–68)	43 (20–60)	49 (24–68)	28.5 (20–50)	33.5 (24–63)	*NS*
Demography male gender [n (%)]		30 (51.7%)	0	2 (25.0%)	5 (31.3%)	24 (70.6%)	*NS*
Marital single status [n (%)]		11 (19.0%)	2 (28.6%)	1 (12.5%)	4 (25.0%)	8 (23.5%)	*NS*
Place of residence—a city with over 100,000 inhabitants [n (%)]		35 (60.3%)	4 (57.1%)	4 (50.0%)	9 (56.3%)	20 (58.8%)	*NS*
BMI median (range)		23.09 (16.26–33.3)	24.25 (22.39–26.57)	24.58 (21.09–29.41)	24.09 (19.13–28.08)	22.44 (16.26–33.30)	*NS*
Smoker [n (%)]		9 (15.52%)	2 (28.6%)	0	6 (37.5%)	4 (11.8%)	*NS*
Alcohol consumption [n (%)]		35 (60.3%)	6 (85.7%)	5 (62.5%)	11(68.8%)	21 (61.8%)	*NS*
Number of patients with additional non-atopic chronic diseases [n (%)]		17 (29.3%)	3 (42.9%)	1 (12.5%)	4 (25.0%)	9 (26.5%)	*NS*
Atopy [n (%)]		28 (48.3%)	2 (28.6%)	2 (25.0%)	8 (50.0%)	18 (52.9%)	*NS*
Atopy [n (%)]	inhalation allergies	14 (24.1%)	1 (14.3%)	2 (25.0%)	4 (25.0%)	9 (26.5%)	*NS*
	food allergies	9 (15.5%)	2 (28.6%)	0	3 (18.8%)	6 (17.6%)	*NS*
	bronchial asthma	5 (8.6%)	2 (28.6%)	1 (12.5%)	1 (6.3%)	2 (5.9%)	*NS*
	atopic dermatitis	5 (8.6%)	1 (14.3%)	1 (12.5%)	0	3 (8.8%)	*NS*
	allergic sinusitis	4 (6.9%)	0	1 (12.5%)	0	3 (8.8%)	*NS*
EAT-10 [mean ± SD]	1. My swallowing problem has caused me to lose weight.	0.33 (0.76)	0.43 (0.53)	0.25 (0.46)	0.25 (0.58)	0.38 (0.89)	*NS*
	2. My swallowing problem interferes with my ability to go out for meals.	1.19 (1.23)	0.71 (0.76)	1.50 (1.20)	1.56 (1.21)	1.18 (1.29)	*NS*
	3. Swallowing liquids takes extra effort.	0.60 (1.04)	0.71 (0.95)	1.12 (1.25) $p = 0.03$	0.44 (0.89)	0.62 (1.13)	*NS*
	4. Swallowing solids takes extra effort.	1.05 (1.25)	0.71 (0.95)	1.38 (1.41)	1.25 (1.34)	1.12 (1.30)	*NS*
	5. Swallowing pills takes extra effort.	1.21 (1.31)	0.86 (0.90)	1.63 (1.51)	1.50 (1.41)	1.32 (1.39)	*NS*
	6. Swallowing is painful.	0.79 (1.20)	0.86 (1.07)	1.00 (0.76) $p = 0.04$	0.56 (0.89)	0.65 (1.15)	*NS*
	7. The pleasure of eating is affected by my swallowing.	1.26 (1.04)	0.57 (0.79)	1.12 (1.36)	1.12 (1.09)	1.44 (1.05)	*NS*
	8. When I swallow, food sticks in my throat.	1.02 (1.08)	0.86 (0.90)	1.75 (0.89)	1.13 (1.09)	0.97 (1.14)	*NS*
	9. I cough when I eat.	0.97 (1.15)	0.86 (0.90)	1.25 (1.39)	1.06 (1.12)	0.91 (1.22)	*NS*
	10. Swallowing is stressful.	1.55 (0.82)	1.14 (1.07) $p = 0.04$	1.00 (0.53)	1.25 (0.86)	1.59 (0.57) $p = 0.001$	*NS*
	Total	9.97 (7.24)	7.71 (4.35)	12.00 (8.33) $p = 0.05$	10.12 (7.59)	10.18 (7.64)	*NS*
Additional symptoms [mean ± SD]	Heartburn	1.10 (1.22)	1.71 (1.50)	1.12 (1.55)	1.06 (1.34)	1.00 (1.15)	$p < 0.0001$
	Regurgitation	1.00 (1.04)	1.14 (1.35)	1.50 (1.20)	1.00 (0.97)	0.88 (1.01)	$p < 0.0001$
	Globus	1.50 (1.39)	1.43 (1.40)	2.12 (1.25)	1.38 (1.41)	1.41 (1.44)	$p < 0.0001$
Motility pattern defined per Chicago classification [n (%)]	Achalasia	0	0	0	0	0	-
	EGJ outflow obstruction	4 (6.9%)	0	0	1 (6.3%)	3 (8.8%)	$p < 0.001$
	DES	0	0	0	0	0	-
	Jackhammer esophagus	0	0	0	0	0	-
	Absent contractility	3 (5.2%)	0	1 (12.5%)	1 (6.3%)	2 (5.9%)	$p < 0.0001$
	IEM	28 (48.3%)	6 (85.7%)	6 (75.0%)	10 (62.5%)	12 (35.3%)	$p < 0.0001$
	Normal peristalsis	24 (41.4%)	1 (14.3%)	1 (12.5%)	5 (31.3%)	17 (50.0%)	$p < 0.0001$
Manometric features [n (%)]	LES insufficiency	15 (25.9%)	2 (28.6%)	1 (12.5%)	6 (37.5%)	8 (23.5%)	$p < 0.0001$
	UES insufficiency	9 (15.5%)	2 (28.6%)	2 (25.0%)	4 (25.0%)	3 (8.8%)	$p < 0.0001$
	UES spastic disorders	20 (34.5%)	2 (28.6%)	1 (12.5%)	3 (18.8%)	13 (38.2%)	$p < 0.0001$
	hiatal hernia	4 (6.9%)	2 (28.6%)	1 (12.5%)	1 (6.3%)	1 (2.9%)	$p < 0.0001$
GIQLI [mean ± SD]	Physical well-being mean points	17.00 (5.35)	18.14 (5.98)	19.50 (5.83)	19.44 (4.88)	16.47 (5.17)	$p < 0.001$
	Gastrointestinal symptoms mean points	53.72 (11.07)	56.14 (17.28)	58.50 (12.88)	58.69 (10.45)	52.88 (9.62)	$p < 0.0001$
	Social well-being mean points	12.76 (3.15)	13.71 (2.75)	13.75 (2.87)	13.81 (2.77)	12.29 (3.49)	*NS*
	Emotional well-being median points	12.10 (4.46)	15.29 (5.09)	14.37 (3.74)	14.50 (4.23)	11.18 (4.27)	$p < 0.0001$
	Total mean points	98.44 (21.02)	106.71 (30.73)	109.37 (23.63)	109.75 (18.44)	95.41 (19.30)	$p < 0.0001$

3.2. Manometric Patterns of Eosinophilic Esophagitis, Erosive Esophagitis, Schatzki Ring

Despite the evaluation of possible correlation between endoscopic, histopathological and manometric diagnoses, no unequivocal motor patterns differentiating esophageal acid-dependent disorders have been established. The moderate positive correlations with IEM were observed for EoE, SR and ERD, although statistical significance was obtained only for ERD ($p = 0.04$). Additionally, the weak, negative, statistically significant correlation of IEM with the normal endoscopic and histopathological image of the esophagus allows classifying this manometric diagnosis as a predictive factor in differentiating the coexistence of other macro- and microscopic disorders (Table 2).

Table 2. Spearman's rank correlation coefficients between diseases diagnosed based on endoscopic and histopathological examination and manometric patterns and features. Statistically significant—in red.

Esophageal Motility Disorders		ERD	SR	EoE	Normal Esophagus in EGD and Histopathology
Motility pattern defined per Chicago classification	EGJ outflow obstruction	−0.100832	−0.108866	−0.015749	0.090513
	Absent contractility	−0.086525	0.132345	0.030031	0.038154
	IEM	0.277568	0.213920	0.175691	−0.309211
	Normal peristalsis	−0.203804	−0.234547	−0.126940	0.208333
Manometric features	LES insufficiency	0.022923	−0.122062	0.164044	−0.063406
	UES insufficiency	0.133574	0.104762	0.161651	−0.220044
	UES spastic disorders	−0.046076	−0.184996	−0.204297	0.093968
	Hiatal hernia	0.316900	0.088454	−0.015749	−0.185790

Among the diseases of the esophageal sphincters and the EGJ, the presence of a hiatal hernia weakly, positively, but statistically significantly correlated with ERD diagnosis and weakly positively, though slightly, with SR. Based on the obtained, although statistically insignificant, correlation systems of UES pressure disorders, it can be assumed that spasticity is associated with a normal endoscopic image and insufficiency with other accompanying diseases of the esophagus (Table 2).

3.3. Diagnostic Delay and the Occurrence of Manometric Disorders—"What Was Earlier—An Egg or a Hen?"

Among study participants, 51.72% of people stated that the diagnostic delay, defined as the time elapsed from the onset of the first episodes of dysphagia to the time of first diagnosis, was over six months, of which 43.75% were in the group of patients with EoE, 71.43% of patients with ERD and 87.50% of patients with SR (Table 3).

Table 3. Diagnostic delay of dysphagia patients with ERD, SR, EoE, normal esophagus in EGD and histopathology examination.

Diagnostic Delay of Dysphagia	All Patients (n = 58)	ERD (n = 7)	SR (n = 8)	EoE (n = 16)	Normal Esophagus in EGD and Histopathology (n = 34)
I don't know/I don't remember [n (%)]	15 (25.86)	2 (28,57)	0	5 (31.25)	9 (26.47)
less than 1 week [n (%)]	7 (12.07)	0	1 (12.5)	2 (12.50)	5 (14.71)
from 1 week to 1 month [n (%)]	1 (1.72)	0	0	0	1 (2.94)
from 1 month to 6 months [n (%)]	5 (8.62)	0	0	2 (12.50)	3 (8.82)
over half a year [n (%)]	30 (51.72)	5 (71,43)	7 (87.50)	7 (43.75)	16 (47.06)

The length of the diagnostic delay of dysphagia correlates positively with the diagnosis of SR and ERD. Still, in the case of SR, this correlation is stronger and statistically significant ($p = 0.03$)—Table 4.

Table 4. Spearman's rank correlation coefficients between diagnosis of ERD, SR, EoE, normal esophagus in EGD and histopathology examination and diagnostic delay of dysphagia. Statistically significant—in red.

Parameter	ERD	SR	EoE	Normal Esophagus in EGD and Histopathology
Diagnostic delay of dysphagia	0.093016	0.284778	−0.094162	−0.074057

Based on the direction of Spearman's correlation coefficient, the potential significance of esophageal manometric disturbances in the natural course of gastro-related diseases was assessed (Table 5). In ERD, normal esophageal peristalsis seems to significantly precede the development of the disease ($p = 0.05$), while the consequence is significantly more often IEM ($p = 0.001$). LES and UES insufficiency and hiatal hernia are potential predictors of the development of both ERD and EoE, with the two disorders appearing to differ in the motility of the esophageal body. Already existing motility disorders much more often precede the development of EoE: IEM ($p = 0.02$) and EGJ outflow obstruction ($p = 0.04$), and the absent contractility ($p = 0.02$) develops with time as the disease progresses. In the development of SR, esophageal sphincters dysfunction appears to be significant—LES insufficiency ($p = 0.04$) and UES spastic disorders ($p = 0.04$), while UES insufficiency is secondary ($p = 0.03$). In the group of patients without diagnosis on endoscopic and histopathological examinations, no significant strong relationships between diagnostic delay and manometric disorders were observed (Table 5).

Table 5. Spearman's rank correlation coefficients between the diagnostic delay of dysphagia in ERD, SR, EoE, normal esophagus and manometric patterns and features. Significant—in red.

Esophageal Motility Disorders		Diagnostic Delay of Dysphagia in ERD	Diagnostic Delay of Dysphagia in SR	Diagnostic Delay of Dysphagia in EoE	Diagnostic Delay of Dysphagia in the Normal Esophagus in EGD and Histopathology
Motility pattern defined per Chicago classification	EGJ outflow obstruction	-	-	−0.327411	−0.177861
	Absent contractility	-	0.14286	0.267882	0.082995
	IEM	0.645497	−0.21822	−0.372058	−0.037459
	Normal peristalsis	−0.645497	0.14286	0.248705	0.097641
Manometric features	LES insufficiency	−0.300000	−1.00000	−0.714352	0.176476
	UES insufficiency	−0.300000	0.21822	−0.732114	0.183598
	UES spastic disorders	0.400000	−1.00000	−0.073837	0.036836
	Hiatal hernia	−0.300000	0.14286	−0.327411	−0.231161

3.4. The Coexistence of Manometric Disorders Exacerbates Dysphagia and Reduces the Quality of Life in Patients with Erosive Esophagitis, EoE and SR

In the Spearman's rank correlation analysis, no statistically significant associations were found between the dysphagia assessment measured with EAT-10 and the coexistence of esophageal motility disorders.

Moreover, there was no significant reduction in quality of life measured as total GIQLI due to the coexistence of esophageal body motility disorders in any of the patient groups studied (Table 6).

Statistically important values were obtained only regarding the coexistence of UES insufficiency with SR ($p = 0.01$) and ERD ($p = 0.01$). To deepen the analysis of the impact of UES insufficiency on the worsening of quality of life in these patient groups, the coexistence of esophageal body manometric abnormalities assessed by Chicago classification correlated with this disturbance in the proximal part of the esophagus. There was a statistically significant correlation between UES insufficiency, abnormal motility ($p = 0.01$) and IEM ($p = 0.03$) in the group of SR patients. However, this observation was not confirmed in ERD patients without the presence of SR (Table 7).

Table 6. Spearman's rank correlation coefficients between diagnosis of ERD, SR, EoE, normal esophagus and total GIQLI mean points in patients with or without esophageal motility disorders. Significant—in red.

	Esophageal Motility Disorders	ERD	SR	EoE	Normal Esophagus in EGD and Histopathology
Total GIQLI Mean Points in Patients	without EGJ outflow obstruction	0.169862	0.215819	0.352961	−0.182693
	with EGJ outflow obstruction	-	-	0.25820	−0.25820
	without absentcontractility	0.151276	0.163310	0.325464	−0.144026
	with absentcontractility	-	0.86603	0.86603	−0.86603
	without IEM	0.311293	0.162217	0.433543	−0.148147
	with IEM	0.086272	0.183328	0.277046	−0.165420
	without normalperistalsis	0.066890	0.200323	0.256506	−0.140999
	with normalperistalsis	0.346712	0.165819	0.519207	−0.192189
	without LESinsufficiency	0.125747	0.271700	0.421602	−0.161055
	with LESinsufficiency	0.295312	−0.247657	0.267964	−0.294093
	without UESinsufficiency	−0.007154	0.068268	0.392817	−0.040431
	with UESinsufficiency	0.675635	0.675635	−0.043483	−0.550019
	without UES spastic disorders	0.319678	0.204422	0.366977	−0.275286
	with UES spastic disorders	−0.202478	−0.218986	−0.085058	0.145546
	without hiatalhernia	0.155825	0.270708	0.303854	−0.207247
	with hiatalhernia	0.235702	−0.544331	0.816497	0.272166

Table 7. Spearman's rank correlation coefficients between UES insufficiency and motility pattern defined per Chicago classification in patients with ERD, SR, EoE and normal esophagus in EGD and histopathology. Significant—in red.

Motility Pattern Defined per Chicago Classification	All Patients with UES Insufficiency	ERD with UES Insufficiency	SR with UES Insufficiency	EoE with UES Insufficiency	Normal Esophagus with UES Insufficiency
EGJ outflow obstruction	−0.063564	−0.188982	−0.188982	0.395285	−0.250000
Absent contractility	-	-	-	-	-
IEM	−0.225630	−0.357143	0.285714	−0.059761	−0.188982
Presence of any esophageal motility disorder defined per Chicago classification	−0.250000	0.357143	0.285714	−0.059761	−0.188982
Normal peristalsis	0.196221	0.357143	−0.285714	0.059761	0.188982

4. Discussion

Although the first published description of the lower esophageal ring by Richard Schatzki dates back to 1953 [19], in the early 1990s EoE was defined as a distinct clinicopathological syndrome [4], and about 15 years ago the current Montreal definition and classification of GERD was established [20], acid-related esophageal diseases continue to pose both a scientific and clinical challenge. This work, through a comparative assessment of esophageal motility disorders accompanying acid-dependent causes of dysphagia and their location in the natural history of these diseases, allows for a broader understanding of pathophysiology and improvement of management by indicating a new path of differential diagnosis.

Thus far, no specific motility patterns of acid-dependent diseases have been identified, but HRM invariably remains an integral part of the diagnosis of dysphagia [21]. In the case of GERD with symptoms resistant to treatment, it is performed to qualify for anti-reflux surgery—so it can not only explain the cause of symptoms by assessing the function of the EGJ, the presence of a hiatal hernia or LES incontinence but it may condition making therapeutic decisions [13]. It is estimated that in approximately 5.7% of cases, underdiagnosed SR or EoE may be responsible for the presence of EGJ outflow obstruction. Still, the exact influence of SR on the HRM picture has not been studied thus far [14]. The main topic of interest of manometrists in recent years is assessing the importance of HRM in the

diagnosis and monitoring of EoE, a less invasive method if compared to EGD with the collection of specimens for histopathological evaluation [12]. In EoE, dissociation occurs in the contraction of the longitudinal and circular muscles during primary peristalsis, most likely in response to eosinophilic infiltration and tissue remodeling leading to fibrosis [22]. Thus far, eight studies have been published regarding a possible correlation between different GERDs manifestations and specific manometric patterns. In short, they have demonstrated that: the manometric pattern of patients with PPI-responsive esophageal eosinophilia was similar to the motility of patients with GERD [23]; inflammatory and fibrostenotic EoE phenotypes were differentiated based on intrabolus pressure (values significantly higher in the case of the fibrostenotic phenotype) [24]; the resolution of manometric disturbances was confirmed after topical treatment with budesonide [25]; there is a correlation between increased distal contractile integral (DCI) and the severity of symptoms [26]. Moreover, EoE was associated with increased pan-esophageal pressurization [27], weak and failed peristaltic integrity worsening with the duration of the disease [28], as well as with achalasia and obstructive motor disorders [29]. Two studies did not identify an EoE-specific esophageal motility disorder correlating with the endoscopic picture and the severity of dysphagia [25,30]. The obtained ambiguous and inconsistent test results have not allowed HRM in diagnostic guidelines for any of the causes of acid-related esophageal dysphagia.

Similarly, this project failed to define esophageal motility patterns that would be unambiguous for the diseases studied. On the other hand, a significant relationship has been demonstrated between ERD and IEM development secondary to the advancement of erosive lesions in the duration of the disease. The opposite situation to ERD arising in the esophagus with normal motility of the body was observed in EoE, where IEM and EGJ outflow obstruction frequently preceded the development of the disease, while the disorders correlated with the duration of esophageal diseases consisted of absent contractility. It can be assumed that the presence of manometric disturbances preceding the result of the inflammatory reaction somehow programs the further path of its development. In the case of ERD, it depends on the action of acid. However in EoE, it also depends on food allergens, which due to motility disorders have prolonged contact with the esophageal mucosa [31]. Hiatal hernia was significantly associated with ERD, which was not found in SR. The development of SR was significantly preceded by LES insufficiency. This is justified by the very pathophysiology of the ring, which is a kind of mechanical protective barrier against exposure to further esophageal reflux of irritating gastric contents, exacerbated by LES insufficiency [8]. However, a previously unknown observation is the coexistence of UES disorders in the natural history of SR—the primary role of spastic disorders and secondary UES insufficiency. Hypothetically, spastic disorders may constitute an attempt at self-protective synergy with SR, against the action of irritating gastric contents, while UES insufficiency secondary to the development of the SR and arising with the duration of the disease may result from a decrease in resting pressure due to sphincter fatigue or from a reduction in the role of the sphincter secondary to a significant narrowing of the esophageal lumen by the ring.

When assessing the impact of manometric disturbances on the quality of life of patients with endoscopic causes of dysphagia, it was the UES insufficiency that had a significant bearing on the subjective assessment of GIQLI in patients with ERD and SR. The hypothesis that the effectiveness of UES affects the efficacy of peristaltic wave propagation, and thus the act of swallowing itself, which may affect the assessment of the quality of life, was confirmed only in the group of SR patients. In this group the correlation of UES disorder with the presence of other manometric abnormalities of the esophageal body was confirmed, including the development of IEM.

Undoubtedly, more than half of the project participants who did not have the cause of dysphagia identified in the endoscopic and histopathological examination, and especially the patients without manometric abnormalities (over a quarter of this group of respondents), definitely require a more in-depth analysis. Patients with no justification for dysphagia on endoscopic and histopathological examinations reported stress associated

with swallowing in the EAT-10 questionnaire more often than in other patients. They also assessed the quality of life the lowest in the GIQLI questionnaire. Moreover, in the comparative assessment, although without statistical significance, UES spastic disorders were found in this group of patients on the manometric test. Such disorders, despite the lack of unambiguous literature data, are associated with the globus symptom and increased mental tension [32,33]. The lack of justification for dysphagia in this group of patients may also result from a limitation of the project, such as the lack of pH-metry with impedance in the diagnosis of symptoms reported by patients, which would probably allow us to objectively select a group of patients with non-erosive esophageal reflux disease (NERD) [6].

5. Conclusions

In the light of the literature reports to date and the results of this project, it seems impossible to identify specific esophageal motility patterns typical for EoE, ERD and SR. In this project, the esophageal motility disorders co-occurring with endoscopic and histological diagnoses have been shown not to significantly affect the severity of dysphagia. However, in the case of patients with ERD or SR and concomitant UES insufficiency, this motor dysfunction had a significant impact on the worsening of the patients' quality of life.

Specific comparative assessment of manometric patterns and features, considering the impact of diagnostic delay and participation in shaping the quality of life of patients, can help to improve the differentiation of coexisting and often overlapping causes of acid-dependent dysphagia.

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Abbreviations

BMI	body mass index;
DES	distal esophageal spasm;
EAT-10	Eating Assessment Tool-10;
EGD	esophagogastroduodenoscopy;
EGJ	esophagogastric junction;
EoE	eosinophilic esophagitis;
ERD	erosive reflux disease;
GIQLI	Gastrointestinal Quality of Life Index;
IEM	ineffective motility;
LES	lower esophageal sphincter;
NS	statistically non-significant;
SD	standard deviation;
SR	Schatzki ring;
UES	upper esophageal sphincter

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PODSUMOWANIE I WNIOSKI

Światowe trendy promujące minimalizację kosztochłonności i inwazyjności, a przez to zmniejszenie ilości działań niepożądanych procedur medycznych, równoważone tendencją do zachowania czułości i swoistości testów, porównywalnej do metod obecnie stosowanych, nieustannie dążą do wyszukiwania nowych zastosowań znanych procedur i tworzenia nieznanych dotychczas innowacyjnych metod diagnostycznych, opartych na patomechanizmach chorób, szczególnie przewlekłych, stanowiących istotny problem kliniczny.

W przypadku EoE celem poszukiwań jest mniej inwazyjne badanie pozwalające cyklicznie monitorować aktywność choroby, unikając jednocześnie powtarzania endoskopii z biopsjami, które są badaniami inwazyjnymi, niepozbawionymi powikłań, a dodatkowo źle tolerowanymi przez chorych. Zaznaczyć należy, że z powodu konieczności wykluczenia ewentualnych zmian nowotworowych przełyku u pacjentów z dysfagią diagnozowanych w kierunku EoE, obecność EGD w algorytmie diagnostycznym choroby na chwilę obecną wydaje się niezaprzeczalne, a ewentualne metody alternatywne mogłyby posłużyć monitorowaniu przebiegu i skuteczności terapii. Według aktualnych danych, nie zidentyfikowano dotychczas metody o dostatecznie udowodnionej użyteczności w korelacji z zaawansowaniem histologicznym [16]. W niniejszej rozprawie doktorskiej zaproponowano zatem ocenę zastosowania stężeń biomarkerów reakcji zapalnej z udziałem eozynofilii: IL-5 i IL-13, eotaksyny 3, MBP i TGF-β1 oraz HRM, jako metodach opartych na przesłankach patofizjologicznych schorzenia.

Na podstawie wyników niniejszego projektu nie wyłoniono jednego biomarkera surowicy o jednoczesnej funkcji predykcyjnej i rokowniczej w EoE, jednak ocena układu biomarkerów oraz ich wzajemnych zależności pozwoliła potwierdzić znaczenie MBP w prognozowaniu rozpoznania choroby, eotaksyny 3 w przewidywaniu zaawansowania histologicznego i endoskopowego oraz korelacji IL-13 i TGF-β1 w różnicowaniu przebiegu zapalnego i włókniejącego EoE (zwyżka stężenia TGF-β1, przy jednoczesnym malejącym stężeniu IL-13 w surowicy, może odpowiadać rozwojowi fibrostenozy, natomiast odwrotny układ biomarkerów może świadczyć o przewadze procesów zapalnych nad fibrostenotycznymi).

W związku z najwyższą oceną nasilenia dysfagii w grupie chorych z SR w projekcie, posłużono się biomarkerami reakcji zapalnej z udziałem eozynofilii celem weryfikacji hipotezy

o związku SR i EoE, jako konsekwencji zaawansowanego procesu zapalnego prowadzącego do fibrostenozy. Na podstawie wyników obserwacji klinicznych i biochemicznych, nie można jednoznacznie zakwalifikować SR jako wyłącznego powikłania EoE ani GERD. Brak istotnego związku pomiędzy występowaniem SR i EoE w populacji pacjentów dorosłych, stwierdzono również w wykonanym przeglądzie systematycznym z metaanalizą, a obserwowane przypadki współwystępowania SR u chorych z EoE w niniejszym projekcie wskazywać mogą na nakładanie się EoE i postaci nieerozyjnej choroby refluksowej przełyku (NERD).

Podobnie jak w przypadku biomarkerów, na podstawie analiz będących podstawą rozprawy, nie udało się sprecyzować jednoznacznego wzorca manometrycznego przełyku, różnicującego EoE od innych przyczyn dysfagii. Zarówno w przypadku EoE, jak i innych schorzeń kwasozależnych: SR i ERD, obserwowano najsilniejsze dodatnie korelacje z nieefektywną motoryką (ineffective motility – IEM), choć istotność statystyczną uzyskano tylko dla ERD. Na podstawie kierunku oddziaływania współczynnika korelacji oceniono jednak potencjalne znaczenie zaburzeń manometrycznych przełyku w historii naturalnej rozwoju schorzeń zależnych od działania kwasu żołądkowego. Wykazano, że w przypadku EoE, IEM i utrudnienie przepływu żołądkowo-przełykowego (EGJ outfow obstraction) istotnie poprzedza rozwój schorzenia, natomiast wraz z czasem trwania choroby rozwijał się brak kurczliwości (absent contractility). Przeciwny związek niż w przypadku EoE obserwowano w przypadku ERD - IEM rozwijał się wtórnie do zaawansowania zmian erozyjnych. Obserwacje te mogą sugerować, że obecność zaburzeń manometrycznych poprzedzających rozwój schorzenia niejako determinuje dalszy szlak patogenezy - w przypadku ERD zależnej od działania kwasu, natomiast w EoE także od alergenów pokarmowych, które na skutek zaburzeń motoryki mają przedłużony kontakt z błoną śluzową przełyku zalegając w jego części nadwpustowej.

Wbrew przypuszczeniom, na podstawie wyników projektu nie stwierdzono istotnego obniżenia jakości życia mierzonego jako GIQLI całkowite na skutek współwystępowania zaburzeń motoryki trzonu w żadnej z badanych grup pacjentów. Natomiast najniższą ocenę jakości życia w porównaniu z pozostałymi grupami chorych z dysfagią uzyskali pacjenci z prawidłowym obrazem endoskopowym i histopatologicznym przełyku. Brak uzasadnienia dysfagii w tej grupie osób wynikać może z pominięcia obiektywizacji rozpoznania NERD, poprzez wykonanie u uczestników projektu również pH-metrii z impedancją. Niewątpliwym ograniczeniem projektu warunkowanym możliwościami finansowymi oraz organizacyjnymi jest również ograniczenie populacji wyłącznie do osób dorosłych oraz relatywnie mała grupa badana. Inwazyjne i obciążające badania uwzględnione w projekcie oraz sam wybuch pandemii

COVID-19, wpłynął na ograniczenie możliwości kontrolnego monitorowania endoskopowego oraz zgłaszalność uczestników do udziału w projekcie (do ponownej oceny po dwóch miesiącach terapii zgłosiło się jedynie 7 pacjentów tj. 43,75% badanych z rozpoznaniem EoE). To z kolei uniemożliwiło jednoznaczne określenie markera surowicy przewidującego remisję choroby po terapii.

Przewagą niniejszego projektu nad dotychczasowymi danymi literaturowymi podejmującymi tematykę zastosowania biomarkerów surowicy krwi oraz wzorców motorycznych przełyku w diagnostyce i monitorowaniu przebiegu EoE był dobór dotychczas rzadko ocenianych lub nieocenianych markerów o potwierdzonym patofizjologicznie związku z EoE. Niewątpliwie istotny jest również prospektywny charakter badania, maksymalnie skrócony odstęp czasowy pobranych próbek surowicy w odniesieniu do wykonanych badań endoskopowych z biopsjami, dwukrotna weryfikacja histopatologiczna wycinków pobranych podczas EGD, a także właściwy dobór badanej populacji jednorodnej pod względem wieku, podawanych objawów, obciążeń alergicznych, a zróżnicowany jedynie w zakresie płci (płeć męska jest istotnym czynnikiem ryzyka EoE uwarunkowanym genetycznie i hormonalnie [23-24]). Niewątpliwie nie bez znaczenia jest także dobór do projektu techniki HRM, w porównaniu do wcześniej stosowanych technik tradycyjnych oraz uwzględnienie w rozpoznaniu EoE najnowszych algorytmów diagnostycznych zawartych w aktualizacji wytycznych [4, 9].

Podsumowując, na podstawie wyników niniejszej pracy ani dotychczasowych badań nie można wyłonić jednego biomarkera surowicy o plejotropowej funkcji w EoE, ani zidentyfikować swoistego wzorca motorycznego. Można natomiast rokować, że niniejsza rozprawa, jak również dotychczasowe doniesienia naukowe posłużą w przyszłości do stworzenia zautomatyzowanego algorytmu obejmującego nie tylko stężenie pojedynczego markera, ale wielokrotne oznaczenia kilku biomarkerów z uwzględnieniem ich wzajemnych zależności, a być może jednocześnie z uwzględnieniem wzorców, cech i parametrów manometrycznych, celem poprawy precyzji i indywidualizacji terapii EoE.

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ZAŁĄCZNIKI

1. OPINIE KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 544/2017

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 78/XV R/2014 z dnia 26 listopada 2014 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

prof. dr hab. Maciej Bagłaj (chirurgia, pediatria)
prof. dr hab. Karol Bal (filozofia)
dr hab. Jacek Daroszewski (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód (duchowny)
mgr Luiza Müller (prawo)
prof. dr hab. Krystyna Orzechowska-Juzwenko (farmakologia kliniczna, choroby wewnętrzne)
prof. dr hab. Zbigniew Rudkowski (pediatria)
dr hab. Sławomir Sidorowicz (psychiatria)
Danuta Tarkowska (położnictwo)
dr hab. Andrzej Wojnar (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)

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

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

„Rola manometrii przełykowej wysokiej rozdzielczości i specyficznych biomarkerów zapalenia w diagnostyce pacjentów z dysfagią i podejrzeniem eozynofilowego zapalenia przełyku"

zgłoszonym przez **dr hab. Dorotę Waśko-Czopnik** zatrudnioną w Katedrze i Klinice Gastroenterologii i Hepatologii Wydziału Lekarskiego Kształcenia Podyplomowego Uniwersytetu Medycznego we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania przez uczestniczkę studiów doktoranckich **lek. Joannę Sarbinowską**, pod nadzorem dr hab. Doroty Waśko-Czopnik w Katedrze i Klinice Gastroenterologii i Hepatologii UMW **pod warunkiem zachowania anonimowości uzyskanych danych**.

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

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

Opinia powyższa dotyczy projektu badawczego realizowanego w ramach grantu dla młodych naukowców.

Wrocław, dnia 17 sierpnia 2017r.

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

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 730/2018

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

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

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

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z wnioskiem zgłoszonym przez **dr hab. Dorotę Waśko-Czopnik** zatrudnioną w Katedrze i Klinice Gastroenterologii i Hepatologii Uniwersytetu Medycznego we Wrocławiu do projektu badawczego pt.:

„Rola manometrii przełykowej wysokiej rozdzielczości i specyficznych biomarkerów zapalenia w diagnostyce pacjentów z dysfagią i podejrzeniem eozynofilowego zapalenia przełyku"

w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie przez uczestniczkę studiów doktoranckich **lek. Joannę Sarbinowską**, pod nadzorem dr hab. Doroty Waśko-Czopnik badania dodatkowo w:

- Klinice Otolaryngologii, Chirurgii Głowy i Szyi Uniwersyteckiego Szpitala Klinicznego im. Jana Mikulicza-Radeckiego we Wrocławiu

pod warunkiem zachowania anonimowości uzyskanych danych.

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

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

Opinia powyższa dotyczy projektu badawczego będącego podstawą działalności statutowej zarejestrowanego pod nr STM.C130.17.045.
Badanie otrzymało pozytywną opinię Komisji Bioetycznej nr KB-544/2017 z dnia 17 sierpnia 2017 r.

Wrocław, dnia 6 grudnia 2018 r.

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

2. OŚWIADCZENIA AUTORÓW

Wrocław, 4.11.2021 r.

Lek. Joanna Sarbinowska
Katedra i Klinika Gastroenterologii i Hepatologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIA

1. Oświadczam, że w pracy: Sarbinowska J., Waśko-Czopnik D. (2020) High-resolution manometry in diagnostics and evaluation of therapy effectiveness in patients with eosinophilic esophagitis - underestimated breakthrough or dead end? *Gastroenterology Review,* 15(1):22-26, mój wkład polegał na pracy nad koncepcją artykułu, przeglądzie literatury oraz pisaniu i korekcie manuskryptu.

Joanna Sarbinowska
LEKARZ
3132447
Podpis

2. Oświadczam, że w pracy: Sarbinowska J., Wiatrak B., Waśko-Czopnik D. (2021) Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis. *European Journal of Gastroenterology & Hepatology,* 33(9):1167-1173, mój wkład polegał na pracy nad koncepcją artykułu, przeglądzie systematycznym, analizie i interpretacji uzyskanych wyników oraz pisaniu i korekcie manuskryptu.

Joanna Sarbinowska
LEKARZ
3132447
Podpis

3. Oświadczam, że w pracy: Sarbinowska J, Wiatrak B, Waśko-Czopnik D. (2021) Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring. *Advances in Medical* Sciences, 66(2):279-283, mój wkład polegał na pracy nad koncepcją artykułu, przeglądzie literatury, zbieraniu materiału badawczego, analizie i interpretacji uzyskanych wyników oraz pisaniu i korekcie manuskryptu.

Joanna Sarbinowska
LEKARZ
3132447
Podpis

4. Oświadczam, że w pracy: Sarbinowska J., Wiatrak B., Waśko-Czopnik D. (2021) Searching for noninvasive predictors of the diagnosis and monitoring of eosinophilic esophagitis – the importance of biomarkers of the inflammatory reaction involving eosinophils. *Biomolecules*, 11(6):890, mój wkład polegał na pracy nad koncepcją artykułu, przeglądzie literatury, zbieraniu materiału badawczego, analizie i interpretacji uzyskanych wyników oraz pisaniu i korekcie manuskryptu.

Joanna Sarbinowska
LEKARZ
3132447
Podpis

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Joanna Sarbinowska
LEKARZ
3132447
Podpis

Wrocław, 4.11.2021 r.

Dr hab. n. med. Dorota Waśko-Czopnik
Katedra i Klinika Gastroenterologii i Hepatologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIA

1. Oświadczam, że w pracy: Sarbinowska J., Waśko-Czopnik D. (2020) High-resolution manometry in diagnostics and evaluation of therapy effectiveness in patients with eosinophilic esophagitis - underestimated breakthrough or dead end? *Gastroenterology Review,* 15(1):22-26, mój wkład polegał na pracy nad koncepcją artykułu, korekcie merytorycznej i ostatecznej akceptacji manuskryptu.

Dr hab n. med. Dorota Waśko-Czopnik
specjalista chorób wewnętrznych
dyplomowany konsultant żywieniowy
GASTROENTEROLOG
tel. kom. 0 602 621 780
9713806

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specjalista chorób wewnętrznych
dyplomowany konsultant żywieniowy
GASTROENTEROLOG
tel. kom. 0 602 621 780
9713806

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Dr hab n. med. Dorota Waśko-Czopnik
specjalista chorób wewnętrznych
dyplomowany konsultant żywieniowy
GASTROENTEROLOG
tel. kom. 0 602 621 780
9713806

Wrocław, 4.11.2021 r.

Dr inż. Benita Wiatrak
Katedra i Zakład Farmakologii
Uniwersytet Medyczny im. Piastów Śląskich we Wrocławiu

OŚWIADCZENIA

1. Oświadczam, że w pracy: Sarbinowska J., Wiatrak B., Waśko-Czopnik D. (2021) Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis. *European Journal of Gastroenterology & Hepatology,* 33(9):1167-1173, mój wkład polegał na analizie statystycznej oraz współtworzeniu i tłumaczeniu pracy.

Podpis

2. Oświadczam, że w pracy: Sarbinowska J, Wiatrak B, Waśko-Czopnik D. (2021) Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring. *Advances in Medical* Sciences, 66(2):279-283, mój wkład polegał na analizie statystycznej oraz współtworzeniu i tłumaczeniu pracy.

Podpis

3. Oświadczam, że w pracy: Sarbinowska J., Wiatrak B., Waśko-Czopnik D. (2021) Searching for noninvasive predictors of the diagnosis and monitoring of eosinophilic esophagitis – the importance of biomarkers of the inflammatory reaction involving eosinophils. *Biomolecules*, 11(6):890, mój wkład polegał na analizie statystycznej oraz współtworzeniu i tłumaczeniu pracy.

Podpis

4. Oświadczam, że w pracy: Sarbinowska J., Wiatrak B., Waśko-Czopnik D. (2021) Esophageal motility disorders in the natural history of acid-dependent causes of dysphagia and their influence on patients' quality of life – a prospective cohort study. *International Journal of Environmental Research and Public Health*, 18:11138, mój wkład polegał na analizie statystycznej, współtworzeniu i tłumaczeniu pracy.

Podpis

3. DOROBEK NAUKOWY

3.1. Publikacje w czasopismach naukowych

3.1.1 Publikacje w czasopiśmie naukowym posiadającym Impact Factor

Lp	Opis bibliograficzny	Rok	IF	PK	Typ KBN
1.	**Association between Schatzki ring and eosinophilic esophagitis: a systematic review and meta-analysis.** [AUT. KORESP.] JOANNA SARBINOWSKA, [AUT.] BENITA WIATRAK, DOROTA WAŚKO-CZOPNIK. *Eur.J.Gastroenterol.Hepatol.* 2021 Vol.33 no.9 s.1167-1173, ryc. tab. bibliogr. 25 poz. summ. DOI: 10.1097/MEG.0000000000002067	2021	2,566	40,00	praca oryginalna
2.	**Association of eosinophil-mediated inflammatory biomarkers with the presence of the Schatzki ring.** [AUT. KORESP.] JOANNA SARBINOWSKA, [AUT.] BENITA WIATRAK, DOROTA WAŚKO-CZOPNIK. *Adv.Med.Sci.* 2021 Vol.66 no.2 s.279-283, ryc. tab. bibliogr. 30 poz. summ. DOI: 10.1016/j.advms.2021.05.004	2021	3,287	100,00	praca oryginalna
3.	**Esophageal motility disorders in the natural history of acid-dependent causes of dysphagia and their influence on patients' quality of life - a prospective cohort study.** [AUT. KORESP.] JOANNA SARBINOWSKA, [AUT.] BENITA WIATRAK, DOROTA WAŚKO-CZOPNIK. *Int.J.Environ.Res.Public Health* 2021 Vol.18 no.21 art.11138 [12 s.], tab. bibliogr. 33 poz. summ. DOI: 10.3390/ijerph182111138	2021	3,390	70,00	praca oryginalna
4.	**Searching for noninvasive predictors of the diagnosis and monitoring of eosinophilic esophagitis - the importance of biomarkers of the inflammatory reaction involving eosinophils.** [AUT.] JOANNA SARBINOWSKA, [AUT. KORESP.] BENITA WIATRAK, [AUT.] DOROTA WAŚKO-CZOPNIK. *Biomolecules* 2021 Vol.11 no.6 art.890 [14 s.], ryc. tab. bibliogr. 49 poz. summ. DOI: 10.3390/biom11060890	2021	4,879	100,00	praca oryginalna
			14,122	310,00	

3.1.2 Publikacje w czasopiśmie naukowym nieposiadającym Impact Factor

Lp.	Opis bibliograficzny	Rok	IF	PK	Typ KBN
1.	**Warsztaty edukacyjne dla pacjentów chorych na cukrzycę - między teorią a praktyką promocji zdrowia(Educational workshop for patients with diabetes - between theory and practice of health promotion).** [AUT.] MICHAŁ JĘDRZEJEK, JOANNA SARBINOWSKA. *Pielęg.Zdr.Publ.* 2012 Vol.2 nr 3 s.213-220, bibliogr. 19 poz. streszcz. summ.	2012	0,000	0,00	praca przeglądowa
2.	**Idea upodmiotowienia na rzecz zdrowia na przykładzie wybranych europejskich kampanii społecznych(Idea of empowerment for health based on selected European social campaigns).** [AUT.] JOANNA SARBINOWSKA, MICHAŁ JĘDRZEJEK, MAŁGORZATA SYNOWIEC-PIŁAT. *Hygeia Publ.Health* 2013 T.48 nr 4 s.424-431, ryc. bibliogr. 31 poz. streszcz. summ.	2013	0,000	7,00	praca przeglądowa
3.	**Medical knowledge level and health behaviours of patients after coronary artery bypass grafting.** [AUT.] MAŁGORZATA SYNOWIEC-PIŁAT, MICHAŁ JĘDRZEJEK, JOANNA SARBINOWSKA. *Folia Cardiol.* 2014 T.9 nr 2 s.105-112, ryc. tab. bibliogr. 25 poz. summ.	2014	0,000	4,00	praca oryginalna
4.	**Przetrwały przewód tętniczy - zagadnienie nie tylko dla pediatrów(Patent ductus arteriosus - not only apaediatric issue).** [AUT.] MICHAŁ JĘDRZEJEK, JOANNA SARBINOWSKA, KATARZYNA WIŚLIŃSKA, WITOLD BŁAŻ. *Pediatr.Med.Rodz.* 2014 T.10 nr 3 s.291-305, ryc. bibliogr. 28 poz. streszcz. summ. DOI: 10.15557/PiMR.2014.0032	2014	0,000	4,00	praca przeglądowa
5.	**High-resolution manometry in diagnostics and evaluation of therapy effectiveness in patients with eosinophilic esophagitis - underestimated breakthrough or dead end?.** [AUT.] JOANNA SARBINOWSKA, DOROTA WAŚKO-CZOPNIK. *Przegl.Gastroenterol.* 2020 Vol.15 nr 1 s.22-26, bibliogr. 30 poz. summ. DOI: 10.5114/pg.2019.83793	2020	0,000	40,00	praca przeglądowa
			0,000	55,00	

3.2 Streszczenia zjazdowe

Lp.	Opis bibliograficzny	Rok	IF	PK	Typ KBN
1.	**Rethoracotomy in an early postoperative period - causes and costs.** [AUT.] JOANNA SARBINOWSKA, MICHAŁ JĘDRZEJEK, ŁUKASZ KOTYRA. W: II International Students' Conference of Young Medical Researchers; XVII Ogólnopolska Konferencja Studenckich Kół Naukowych Uczelni Medycznych. Wrocław, 13-14 kwietnia 2012 Wrocław 2012, Akademia Medyczna im. Piastów Śląskich, s.164 poz.4, 978-83-7055-421-7.	2012	0,00	0,00	inne
2.	**Warsztaty edukacyjne dla pacjentów chorych na cukrzycę.** [AUT.] MICHAŁ JĘDRZEJEK, JOANNA SARBINOWSKA. W: V Studenckie Sympozjum Naukowe "Wrocławskie Dni Zdrowia Publicznego". Wrocław, 14-15 maja 2012 r. Abstrakty, s.16-17.	2012	0,00	0,00	inne
3.	**Idea upodmiotowienia na rzecz zdrowia na przykładzie wybranych europejskich kampanii społecznych.** [AUT.] JOANNA SARBINOWSKA, MICHAŁ JĘDRZEJEK. W: VI Studenckie Sympozjum Naukowe "Wrocławskie Dni Zdrowia Publicznego" - "Europejskie zdrowie - aktywność Unii Europejskiej w dziedzinie Zdrowia Publicznego". Wrocław, 13-14 maja 2013 r, s.12.	2013	0,00	0,00	inne
4.	**Przygotowanie bloku operacyjnego do zabiegu zamknięcia przetrwałego przewodu tętniczego (PDA) metodą wideotorakoskopową.** [AUT.] BOGUMIŁA CIARACH, JOANNA SARBINOWSKA, MICHAŁ JĘDRZEJEK, ROMUALD CICHOŃ. W: VII Kongres Polskiego Towarzystwa Kardio-Torakochirurgów. Warszawa, 5-7 czerwca 2014. Program szczegółowy, s.59-60.	2014	0,00	0,00	inne
5.	**Motywacje i oczekiwania zawodowe studentów Uniwersytetu Medycznego we Wrocławiu(Motivations and professional students expectations of Wroclaw Medical University).** [AUT.] A. ŻARCZYŃSKA-DOBIESZ, I. JANIAK-REJNO, B. CHOMĄTOWSKA, D[OROTA] KIEDIK, A[NNA] FELIŃCZAK, J[OANNA] SARBINOWSKA, J[OLANTA] GRZEBIELUCH. *Public Health Forum* 2017 Vol.3 no.3 s.236, III Międzynarodowy Kongres Polskiego Towarzystwa Zdrowia Publicznego.	2017	0,00	0,00	inne

	Wrocław, 30.11-01.12.2017. Toż w: Public Health Forum 2017 Vol.3 no.4 s.295.				
6.	**The role of high-resolution esophageal manometry in the diagnosis of patients with dysphagia and suspected eosinophilic esophagitis - preliminary report.** [AUT.] DOROTA WAŚKO-CZOPNIK, JOANNA SARBINOWSKA. *Przegl.Gastroenterol.* 2018 Vol.13 supl.1 s.s42, XVIII Kongres Polskiego Towarzystwa Gastroenetrologii. Warszawa, 20-22 września 2018 r. Streszczenia.	2018	0,000	0,00	inne
7.	**Znaczenie współwystępowania alergii pokarmowych i wziewnych w diagnostyce pacjentów z dysfagią i podejrzeniem eozynofilowego zapalenia przełyku - doniesienia wstępne.** [AUT.] JOANNA SARBINOWSKA, DOROTA WAŚKO-CZOPNIK. *Alergol.Pol.* 2018 Vol.5 spec.iss. art.33700-10, XIII Międzynarodowy Kongres PTA. Mikołajki, 26-29 września 2018 r. Streszczenia.	2018	0,000	0,00	inne
8.	**W poszukiwaniu predykatorów eozynofilowej ziarniniakowatości z zapaleniem naczyń. Opis przypadku pacjentki z eozynofilowym zapaleniem żołądka i jelit.** [AUT.] JOANNA SARBINOWSKA, DOROTA WAŚKO-CZOPNIK. W: VII Krajowe Spotkania Reumatologiczne 2019. Toruń 20-21 września 2019 roku. Streszczenia, s.34 poz.P45, [Dostęp 23.09.2019]. Dostępny w: http://www.ksr.viamedica.pl/files/ksr/2019/Krajowe_Spotkania_Reumatologiczne_2019_streszczenia.pdf.	2019	0,000	0,00	inne
9.	**Czy biomarkery surowicy krwi mogą zastąpić endoskopową ocenę zaawansowania eozynofilowego zapalenia przełyku w dobie pandemii COVID-19?(Could serum biomarkers replace the endoscopic assessment of the intensity of eosinophilic esophagitis in the age of the COVID-19 pandemic?).** [AUT.] J[OANNA] SARBINOWSKA, B[ENITA] WIATRAK, D[OROTA] WAŚKO-CZOPNIK. W: I Ogólnopolska Doktorancka Konferencja Interdyscyplinarna. Wrocław, 19.09.2020 r. Księga abstraktów Wrocław 2020, s.10 poz.5.	2020	0,000	0,00	inne
10.	**Serum biomarkers in the diagnostics of patients with suspected eosinophilic esophagitis - preliminary report.** [AUT.] J[OANNA] SARBINOWSKA, D[OROTA]	2020	0,000	0,00	inne

WAŚKO-CZOPNIK, B[ENITA] WIATRAK. *Unit.Eur.Gastroenterol.J.* 2020 Vol.8 no.8 suppl. s.189 poz.P0095, bibliogr. 8 poz, 28th United European Gastroenterology Week Virtual 2020. DOI: 10.1177/2050640620927345

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