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New approach to diagnosis and treatment of *Acanthamoeba* keratitis – systematic review of literature

Nowe podejście do diagnostyki i leczenia zapaleń rogówki wywołanych przez Acanthamoeba sp. – przegląd literatury

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Streszczenie:
Cel: w pracy zaprezentowano nowe metody w diagnostyce I leczeniu zapaleń rogówki wywołanych przez *Acanthamoeba spp.*
Metoda: korzystano z bazy Medline w celu wyszukania artykułów w języku angielskim opublikowanych od 2006 do początku 2011 roku i opisujących sposoby diagnostyki i leczenia pełzakowego zapalenia rogówki wraz z ich wynikami. W pracy wykorzystano badania kliniczne jak również prace przeglądowe i opisy przypadków dotyczące powyższego zagadnienia.
Wyniki: w pracy przytoczono 14 publikacji opisujących obecny stan wiedzy odnośnie diagnostyki zapaleń rogówki wywołanych przez *Acanthamoeba* wraz z oceną ich swoistości i czułości. Szczególną uwagę zwrócono na nowe możliwości barwienia wymazów z zeskrobin rogówkowych oraz ich skuteczność. Tradycyjne metody laboratoryjne porównano z coraz częściej wykorzystywanymi metodami molekularnymi, czyli polimerazową reakcją łańcuchową (PCR), wskazując na jej zalety i ograniczenia. Cytowane są także bieżące informacje na temat badań obrazowych wykorzystywanych w diagnostyce patologii rogówki, szczególnie mikroskopii konfokalnej mającej coraz szersze zastosowanie w identyfikacji etiologii pełzakowego zapalenia rogówki. Następnie przytoczono 17 artykułów dotyczących sposobów i efektów terapii. Obok leczenia farmakologicznego pokazano wyniki naświetlań UVA połączonych z aplikacją ryboflawiny na rogówkę – metody znanej w terapii ektazji rogówki, a dającej również efekty u pacjentów z zapaleniem rogówki wywołanym przez *Acanthamoeba*. Fotokeratektomia znalazła także zastosowanie na początkowych etapach leczenia infekcji. Ze względu na ciągłe udoskonalanie sprzętu i technik operacyjnych szczególną uwagę poświęcono keratoplastyce zarówno drążącej, jak i warstwowej w leczeniu zapaleń rogówki o dużym zaawansowaniu. W pracy przedstawiono także doniesienia o eksperymentalnych metodach terapii tego schorzenia.
Wnioski: brak jednolitych standardów postępowania diagnostycznego i leczniczego w przypadku zapaleń rogówki wywołanych przez *Acanthamoeba* wymusza poszukiwanie nowych metod terapii. Taki stan wiedzy potwierdza badanie ankietowe przeprowadzone wśród specjalistów w 2011 roku, wskazując przede wszystkim na duży stopień niepewności związanej z leczeniem tego schorzenia. Problemem pozostaje także brak dostępności do leków przeciwpełzakowych oraz ich toksyczność. Większość przytaczanych publikacji wskazuje na konieczność szybkiej diagnozy jako najważniejszego czynnika warunkującego dobre efekty leczenia. Dlatego pomimo dużego postępu w rozpoznawaniu tej infekcji najwłaściwsze wydaje się rozpoczynanie terapii u pacjentów, u których istnieje duże prawdopodobieństwo choroby nawet bez potwierdzenia diagnostycznego.
Słowa kluczowe: pełzakowe zapalenie rogówki, mikroskopia konfokalna, keratoplastyka.
Summary:
Purpose: To present new methods of diagnosis and treatment of *Acanthamoeba* keratitis.
Material and methods: Searching Medline base for articles in English publicated since 2006 till the beginning of 2011, describing the way and results of diagnosis and treatment *Acanthamoeba* keratitis. Publications include studies, reviews as well as case reports concerning discussed subject.
Results: 14 publications were found and used to present miscellaneous methods utilized nowadays to identify the reason of *Acanthamoeba* keratitis with simultaneous evaluations of its' sensitivity and specificity. The review comprises improvements of laboratory diagnosis as well as corneal imaging systems helpful to diagnose infectious keratitis. 17 publications were quoted to elaborate results of pharmacological protocols as well as surgical procedures of *Acanthamoeba* keratitis treatment. Outcomes of experimental investigations were also cited.
Conclusions: As none of presented methods of diagnosis and treatment of *Acanthamoeba* keratitis have become a standardized guideline jet, still there is a great need to look for new ones.
Key words: *Acanthamoeba* keratitis, confocal microscopy, keratoplasty.

Introduction

Acanthamoeba is an ubiquitous protozoa living in aquatic environment as well as in sewage systems and swimming pools. Some people are carriers of the cysts in the upper respiratory tract. In favorable circumstances cysts convert into trophozoites which produce enzymes able to penetrate and destruct tissues. *Acanthamoeba* keratitis is a severe infection of cornea, which can cause significant loss of vision. The majority of affected patients are soft contact wearers, which is the most common risk factor of this disease (1). However, in developing countries corneal injury is an overwhelming reason responsible for *Acanthamoeba* infection (2). In the early stage of keratitis corneal symptoms are not specific and can be confused with herpetic infections. A discriminating feature can be concomitant pain which is disproportionate to clinical signs. More pathognomonic are radial keratoneuritis which appears in the first weeks of infection. In advanced stage ring infiltrates appear (Fig. 1a.), converting into corneal disciform structures (Fig. 1b.) which can lead to corneal thinning resulting in perforation and mimic fungal infections. Probably, due to the increasing number of disposable contact lens users *Acanthamoeba* keratitis has recently become more frequent reason of corneal infection and should be taken into consideration especially in this group of patients.

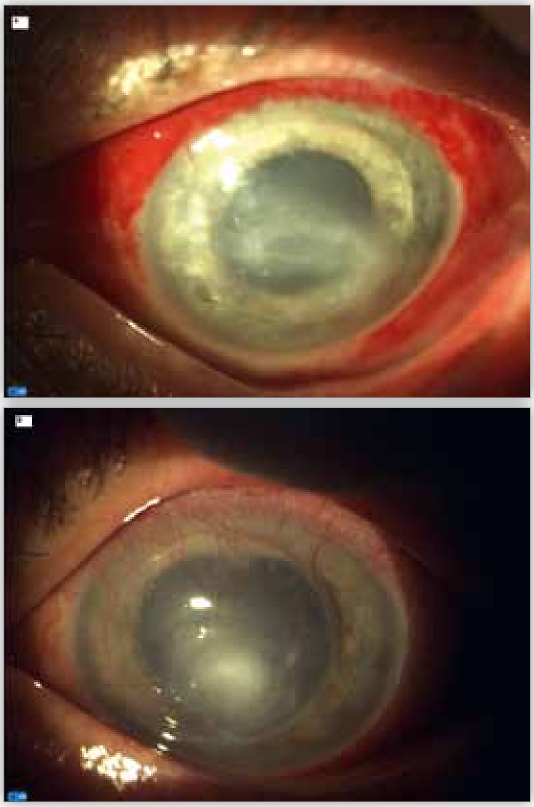

Fig. 1. Patient after successful penetrating keratoplasty due to keratoconus in 2001, using soft contact lenses after surgery. *Acanthamoeba* keratitis has progressed since last year (ring infiltrates in corneal stroma – a., deep stromal involvement – b.).

Ryc. 1. Pacjent po przeszczepie drążącym rogówki z powodu stożka rogówki przeprowadzonym w 2001 r., używający soczewek kontaktowych po zabiegu. Zapalenie rogówki wywołane przez *Acanthamoeba sp*. rozwijające się od zeszłego roku (pierścieniowate nacieki w miąższu rogówki – a., rozległe zajęcie miąższu rogówki – b.).

Material and methods

We reviewed Medline base for articles in English inserted there since 2006 till the beginning of 2011, searching for studies, reviews and case reports describing new modalities of diagnosis and treatment of *Acanthamoeba* keratitis. 31 publications were found, in which 14 articles concerned new methods of indentifying the protozoan etiology of keratitis including laboratory techniques as well as new corneal imaging systems. 17 publications were cited to present pharmacological protocols and surgical treatment of *Acanthamoeba* keratitis. We also mentioned experimental studies found in Medline base.

Diagnosis

The conventional method of microbiological identification includes corneal scrapings for smears and cultures. Recently, efforts to find the most appropriate staining have been made. Bharathi et al. stated that the sensitivity of smears mounted on 10% potassium hydroxide (KOH) (91.45%) was higher than that of Gram-stained and Giemsa-stained smears (60%, 45.71%) in the detection of *Acanthamoeba*, due to the fact that KOH wet-mount preparation gave much more remarkable clarity of the cysts (3). Shah et al. judged that staining using Fungiflora Y (FFY) and Calcofluor white (CFW) are techniques with similar sensitivity as well as specificity as they both stain the cellulose in *Acanthamoeba* cysts. Although the disadvantage of using CFW is the necessity of quick evaluation in a wet mount, staining with FFY requires expensive machinery to obtain frozen samples (4). As non of these conventional techniques present satisfactory specificity as well as sensitivity as a fundamental diagnostic tool, Polymerase Chain Reaction (PCR) was used as a new diagnostic method based on pathogen's DNA analysis. The greatest advantage of this method is the need of only a small sample of microbiological material without necessity to know all analyzed genom. Boggild et al. assessed that PCR is the most sensitive method (90.0% using Nelson primers and 65% using JDP primers), comparing to direct microscopy and culture (55.0 and 73.7% respectively), especially with specimens such as contact lens solutions, where dilution effect can appeared. Nevertheless, high false-positivity rate should be taken into consideration (5). Year et al. also confirmed the highest sensitivity of PCR method among other diagnostic tools also in patients already treated with amoebicidal antibiotics. It can be increased (from 81% to 94%) by the use of combination of primers pairs (especially the Nelson and JDP primers) (6). Kandori et al. indicated the application of PCR particularly in the early stages of AK, as the only presence of trophozoites cannot be detected by use of direct microscopy with staining and cultures. This is the reason why the real-time PCR can be a valuable method of detecting *Acanthamoeba* in patients, whose symptoms as well as clinical signs suggest this etiology (7). Prashanth et al. presented a fluorescence amplified fragment length polymorphism (FAFLP) method as a useful tool for distinguishing between *Acanthamoeba* strains and their subsets, what can succeeded in easier tracing the potential paths of AK infections. In authors' opinion FAFLP can be a cheaper and quicker option for sequence analysis, thus it demands previous refining from bacteria, fungi and virus DNA contamination (8). Among corneal imaging systems confocal microscopy ap-

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pears to be the most promising diagnostic tool, due to its non-invasive quality (Fig. 2a., Fig. 2b.) Vaddavalli et al. highlighted that confocal microscopy is a rapid diagnostic modality in the etiologic diagnosis of *Acanthamoeba* keratitis with the sensitivity (88.3%) and specificity (91.1%) comparable with standard microbiological methods, especially in cases where infiltrates are too deep to obtain applicable specimen. However, due to its cost and limited availability it may not be used as a primary diagnostic tool (9). Nevertheless Tu et al. claimed that simultaneous to confocal microscopy corneal scrapings for smears and culture can improve the correct and rapid diagnosis. He achieved sensitivity of confocal microscopy exceeding 90% and specificity ranging from 77 to 100% depending on estimated AK definition no matter of affected corneal layer (10). Shiraishi et al. indicated that newly developed confocal microscope HRT II-RCM is a noninvasive diagnostic tool that can show highly-resolution images of *Acanthamoeba* cysts and trophozoites as well as inflammatory cells and their changes in quantity and morphology during clinical course, what can determine the way of treatment at any time (11). Matsomoto et al. claimed that in vivo new generation diod-laser confocal microscope with a wavelength of 670 nm – HRTII-RCM can provide images with more details compared to white-light confocal microscopy, making diagnosis of *Acanthamoeba* trophozoites easier and set the end-point of treatment, avoiding prolonged mediacal therapy. In authors' experience the final clinical outcome depends more on the depth in which the organisms are located rather than the numbers (12). Chen et al. used HRT III-RCM, which is similar to HRT II-RCM except with a reduced number of components, to evaluate the utility of this diagnostic tool in patient and rabbits with *Acanthamoeba* keratitis. He obtained smaller size of cysts and characteristic images of trophozoites, not observed in previous reports, using HRT II-RCM (13). Although great development has been made recently in a way of *Acanthamoeba* diagnosis it still seems to be the most appropriate way to start initiating treatment in cases with strong suspicion of *Acanthamoeba* keratitis when the diagnosis cannot be confirmed (1,14) (Fig. 3a., Fig. 3b.).

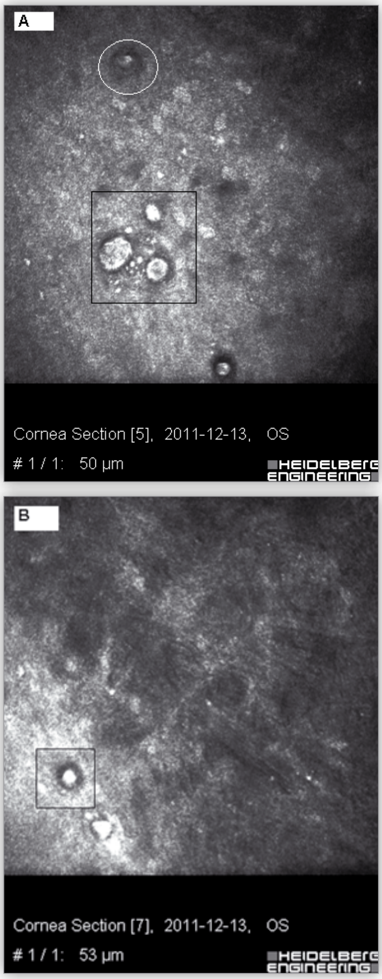

Fig. 2a., b. The same patient as in figure 1 with confirmed *Acanthamoeba* karatitis – images of *Acanthamoeba* cysts (black square) and forming trophozoite (white circle) in a condense subepithelial layer.

Ryc. 2a., b. Ten sam pacjent co na ryc. 1. z potwierdzonym zapaleniem rogówki wywołanym przez *Acanthamoeba sp*. – na skanach cysty *Acanthamoeba sp*. (w czarnym kwadracie) i formujące się trofozoity (w białym kole) w zagęszczonym zrębie warstwy podnabłonkowej.

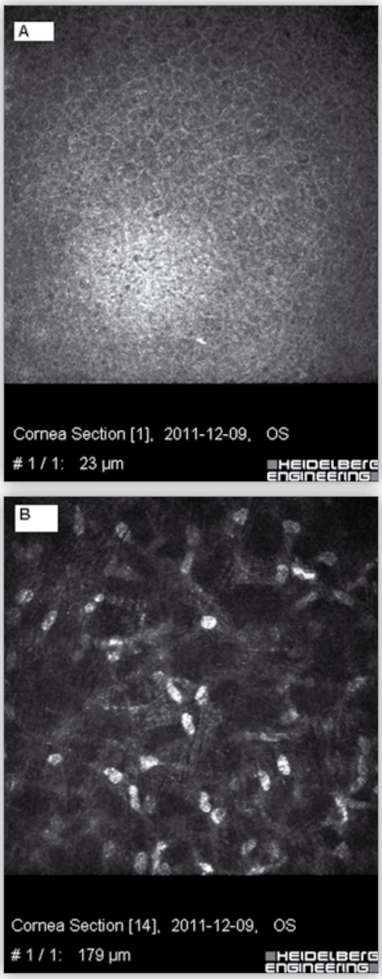

Fig. 3a., b. Patient with a strong suspicion of *Acanthamoeba* karatitis, not--confirmed in confocal microscopy (clear epithelium layer with fragmentarily thickened outlines of cells – a, layer of corneal stroma with activated keratocytes and no evidence of AK – b).

Ryc. 3a., b. Pacjent z podejrzeniem zapalenia rogówki wywołanym przez *Acanthamoeba sp*., niepotwierdzonym w mikroskopii konfokalnej (warstwa nabłonka z fragmentarycznie pogrubionymi konturami komórek – a., warstwa miąższu rogówki z aktywnymi keratocytami bez cech zakażenia *Acanthamoeba sp*. – b.)

Treatment

In almost all reported studies in recent years authors highlighted that early diagnosis with quick appropriate treatment is the major factor influencing final outcomes. Although availability of medications differs in respective countries, combination of corneal scraping, antifungal drugs and antibiotics was reported to be very successful, when *Acanthamoeba* keratitis is diagnosed in early stage (15). Mutoh et al. stated that surgical, repeated debridement might be useful as an additional treatment to medical therapy, resulting in better final results (14). Gooi et al. in presented case report confirmed adventage of combination amebicidal therapy over chlorhexidine monotherapy, which can be insufficient to eradicate pathogens from deeper parts of corneal stroma. Authors suggested a static effect of chlorhexidine therapy on *Acanthamoeba* organisms, resulted in reducing clinical evidence of inflammation but not enough to discard all pathogens (16). Voriconazol is a triazole antifungal medication that is generally used to treat fungal infections but also with additional antiamebicidal efficacy. Tu et al. described two cases of chronic *Acanthamoeba* keratitis successfully treated with oral voriconzole initially in conjunction with topical amebicidal drugs. However, effective sole application of oral voriconazole in disease exacerbation indicates the large role of voriconazole monotherapy especially in patient non-responsive to traditional therapy (17). Bang et al. also presented cases of culture-proven *Acantamoeba* keratitis successfully treated with topical and intrastromal voriconazole in combination with standard treatment, which appeared to be insufficient in patients resistant to chlorhexidine and hexamidine. Summing up, voriconazole might be a therapeutic option as an adjuvant or alternative especially in these patients (18). Surprisingly, in literature there are presented cases of patients treated successfully only with non-steroidal eye drops with no recurrence observed during the follow-up period. Agahan et al. indicated that treatment focusing on inflammatory process's control might be sufficient in patient with verified *Acanthamoeba* keratitis (19). Researches exploring new methods of pharmacological treatment of *Acanthamoeba* keratitis are still sought, especially due to the fact of corneal toxicity during prolong usage and poor access to amoebacidal medications (20). Furnkranz et al. reported that N-Chlorotautine is active against *Acanthamoeba spp*. having at least amoebostatic effect. As both trophozoites and cysts are susceptible to NCT at concentrations well tolerated by human tissues it might be useful as a drug for medical treatment in a future (21). Topical application of riboflavin (B2) solution combined with ultraviolet light A(UVA) irradiation is well known therapy in patients with corneal ectasias such as keratoconus. However, Khan et al. presented such successful treatment in patients suffering from *Acanthamoeba* keratitis at the stage of superficial lesions resulting in complete absence of *Acanthamoeba* organisms after two sessions of this therapy. Although, a dramatic resolution of symptoms as well as a decrease in ulcer size was observed after this therapy, some patient needed additional penetrating keratoplasty because of corneal scars obstructing vision. Due to the fact that all patients continued their medical therapy concurrently with the UVA and B2 therapy, no statement can be made about the efficacy of such procedure as an independent treatment of AK (22). Favourable outcomes were also achieved after only one session of standard protocol for UV corneal therapy in patient non-responsive to topical antimicrobial agents in a case report presented by Garduno-Vieyra et al. (23). Excimer laser phototherapetic keratectomy (PTK) is a procedure, which allows to ablate corneal surface in order to remove pathological material from affected surface. Kandori et al. indicated that phototherapeutic keratectomy performed in patients with *Acanthamoeba* keratitis resistant to medical amoebic treatment combined with mechanical corneal debridement can be very beneficial, when lesions are limited to the superficial stroma as it allows to remove directly amoebic cysts, inflammatory cells and necrotic tissues as well as to facilitate medical treatment and suppress tissue scarring. However, the optimal period to perform PTK is limited and corneal thinning is unavoidable (24). In patients resistant to traditional therapy corneal transplantation is the last chance to regain vision. The efficiency of this treatment in medical literature have been unequal and due to rapid development of new techniques of these procedures and more effective medical treatment are still surprising. Sharma et al. going by peer-reviewed literature emphasized the role of therapeutic keratoplasty in the treatment of microbial keratitis, including *Acanthamoeba* infections, quoting indications such as refractory to maximal medical therapy, extreme thinning or perforation of cornea as well as scleral involvement (25). Kashiwabuchi et al. indicated that penetrating keratoplasty can be an alternative to medical treatment in patients with *Acanthamoeba* keratitis, though graft survival rate is low (55% at 1 year), especially in patients with postoperative glaucoma and second PK procedures are needed (26). However, Nguyen et al. achieved good visual outcome (20/50 or better) as well as no recurrence during the follow-up period (8-34 months) performing PK procedure in patients with *Acanthamoeba* keratitis in acute stage but before scleral and peripheral corneal extension or impending perforation, what is believed to minimize poor results of this surgical treatment. He also indicated the insufficiency of confocal microscopy to outline the extent of involvement or the size of trephination at the time of surgery (27). Report by Kitzmann et al. showed much better graft survival as well as visual outcomes of optical keratoplasty (100%, 100%, 66,7% at 1,5,10 years with median VA 20/25), rather than therapeutic keratoplasty (45.5%, 45.5%, 37.5% respectively with median VA 20/40), in patient with *Acanthamoeba* keratitis. Nevertheless, endothelial rejection episodes are more frequent in this group, probably due to the longer follow-up period (28). Anshu et al. achieved therapeutic success in 88.9% (8 of 9 eyes) therapeutic deep anterior lamellar keratoplasty (TDALK) cases of patients with *Acanthamoeba* keratitis at the first attempt and a repeat TDALK procedure in the remaining eye with recurrence of primary disease was also successful. In Anshu's report patients with *Acanthamoeba* keratitis who underwent TDALK comprised most of the group with epithelial problems, which can be related to the toxicity of topical amebicidal drugs (20). The use of steroids in preoperative as well as postoperative period is still controversial. Ueki et al. highlighted that topical steroids ought to be recommended against inflammatory processes but only with exclusion of the presence of *Acanthamoeba* organisms (15). Shi et al. also confirmed the earlier suggestions that using steroids in the early postoperative period can compound

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the prognosis and should be utilized only in cases with serious anterior chamber inflammation (29). Artificially synthesized, small interfering RNAs are used for silencing gens expression in parasitic organisms and are promising therapeutic tools against *Acanthamoeba* spp. Lorenzo-Morales et al. in his study analyzed the use of combination of siRNAs against genes of two *Acanthamoeba's* enzymes: serine protease and glycogen phosphorylase, which appeared to block the natural evolution and division of these pathogens and may appeared to be successful treatment in the future (30).

Conclusions

Inability to create standardized guidelines describing the optimal protocol of Acanthamoeba keratitis treatment forces us to improve and look for new therapeutic methods. An online survey developed by Oldenburg et al. and published in December 2011 confirms a high level of uncertainty about adequate treatment among corneal specialists. In accordance with presented literature most of responders preferred combination therapy with biguanide and diamide as an ideal choice rather than monotherapy and introduced surgical techniques in patients not responding to medical treatment. Confocal microscopy was thought to be the most appropriate corneal imaging system but mostly used in addition to culture and smears. We sustain authors observation that due to the rarity of *Acanthaoeba* keratitis evidence-based decision-making will be very difficult for this disease (31).

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