Universidade do Minho
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Acanthamoeba and Contact Lenses

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Acanthamoeba and Contact Lenses

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Professora Doutora Maria Madalena Cunha Faria Lira

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A todos o meu Muito Obrigada!

Statement of Integrity

I hereby declare having conducted this academic work with integrity. I confirm that I have not used plagiarism or any form of undue use of information or falsification of results along the process leading to its elaboration.

I further declare that I have fully acknowledged the Code of Ethical Conduct of the University of Minho.

Resumo

Acanthamoeba e Lentes de Contacto

A Queratite por *Acanthamoeba* é uma infeção causada por um protozoário unicelular do género *Acanthamoeba* que está universalmente distribuído. Desta forma, e porque o contacto com o ser humano acontece diariamente, o risco de exposição a esta infeção pode ser elevado. Até aos dias de hoje, a maioria dos casos são reportados em usuários de lentes de contacto, porém é também uma realidade em não usuários, mais associados ao trauma corneal. Para além desta diferença entre usuários e não usuários verifica-se também uma variação na incidência entre países desenvolvidos e em desenvolvimento, no que toca à etiologia da infeção.

O trauma corneal, que pode ter variadas causas tal como o uso de lentes de contacto, facilita a invasão deixando uma porta aberta para os micro-organismos penetrarem na córnea. Para além do trauma, os fatores de risco estão maioritariamente associados aos comportamentos do usuário, como o contacto da lente com água contaminada no banho, na piscina, na praia, etc., a falta de cuidados de higiene e de limpeza das lentes de contacto e dos estojos, e o uso de soluções de manutenção ineficazes contra a *Acanthamoeba*. O facto de uma solução de manutenção não ser completamente eficaz contra quistos e/ou trofozoitos, as duas formas do ciclo de vida da *Acanthamoeba*, pode fazer com que haja um reaparecimento da infeção, pois apenas um quisto leva ao aparecimento de uma nova população de *Acanthamoeba*.

Este trabalho baseia-se numa revisão bibliográfica e tem como principal objetivo aprofundar o conhecimento sobre a Queratite por *Acanthamoeba*, apresentando os principais fatores de risco e salientar as medidas de prevenção para este tipo de infeções corneais, uma vez que os tratamentos nem sempre são eficazes. Tem como público alvo os especialistas na área da saúde visual, reforçando os seus conhecimentos nesta área, para que possam informar melhor os seus pacientes sobre cuidados de higiene, medidas de desinfeção apropriadas e formas de minimizar o risco de infeção. Nesta fase, é importante realçar o papel essencial que os especialistas têm no momento da adaptação, monitorização e acompanhamento dos pacientes para minimizar o perigo de infeção.

"An ounce of prevention is worth a pound of cure" (Benjamin Franklin)

Palavras-chave: *Acanthamoeba*, Queratite por *Acanthamoeba*, lentes de contacto, soluções de manutenção e prevenção.

Abstract

Acanthamoeba and Contact Lenses

Acanthamoeba keratitis is an infection caused by a unicellular protozoan of the genus *Acanthamoeba* which is universally widespread. Thus, and because contact with humans happens daily, the risk of exposure to this infection is very high. Until now, the majority of cases are reported in contact lens wearers although it's also a reality in non-wearers, mostly connected with corneal trauma. In addition to this difference between wearers and non-wearers, there was also a variation in incidence, regarding the etiology of the, between develop and developing countries.

Corneal trauma, which can be caused by varied causes and the use of contact lenses, facilitates invasion by leaving an open door to microorganisms to penetrate the cornea. In addition to trauma, risk factors are mostly associated with patients' behaviors, such as contact of lenses with contaminated water in the shower, swimming pools and beach, etc., lack of hygiene habits with contact lenses and respective cases and the use of ineffective disinfecting solutions against *Acanthamoeba.* The fact that a disinfecting solution isn't completely effective against trophozoites and/or cysts, both forms of *Acanthamoeba*'s lifecycle, can cause the infection to reappear as only one cyst leads to the emergence of a new population of *Acanthamoeba*.

This work is based on a literature review and the main goal is to deepen the knowledge about *Acanthamoeba* keratitis, presenting the main risk factors and focusing on prevention actions for this type of corneal infection, since the treatments are not always effective. It targets specialists in visual health to strengthening their knowledge in this area as well as that they can better inform their patients about hygiene care, appropriate measures of disinfection and ways to minimize the risk of infection. At this stage it's important to highlight the essential role that specialists play in fitting, monitoring and follow-up of patients to minimize the danger of infection.

"An ounce of prevention is worth a pound of cure" (Benjamin Franklin)

Key words: *Acanthamoeba*, *Acanthamoeba* keratitis, contact lenses, maintenance solutions and prevention.

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Acronyms and Abbreviations

AK: *Acanthamoeba* keratitis

BAK: Benzalkonium chloride

CL: Contact lens

DALK: Deep Anterior Lamellar Keratoplasty

DMSO: Dimethyl sulfoxide

E. Coli: Escherichia coli

EDTA: Ethylenediaminetetraacetic acid

FDA: Food and Drug Administration

GAE: Granulomatous amoebic encephalitis

IgA: Immunoglobulin A

IgG: Immunoglobulin G

ISO: International Organization for Standardization

LC: Langerhans cell

MAPD: Myristamidopropyl dimethylamine

MBP: Mannose-binding protein

MPS: Multipurpose solution

μm: Micrometers

PAPB: Polyaminopropyl biguanide

PCR: Polymerase Chain Reaction

PHMB: Polyhexamethylene Biguanide

PK: Penetrating keratoplasty

R: Riboflavin

RGP: Rigid gas permeable

SEM: Scanning Electron Microscope

sIgA: Secretory immunoglobulin A

TEM: Transmission Electron Microscope

UV: Ultraviolet radiation

UVA: Ultraviolet-A radiation

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1. Introduction

The continued growth of contact lens (CL) wearers associated with a lack of information on hygiene habits, risk factors and handling procedures can lead to an increase risk of infections, in which *Acanthamoeba* keratitis is inserted.

This revision work intends to minimize this information gap through gathering as much information as possible about *Acanthamoeba* keratitis in order to understand the disease mechanism, risk behaviors, diagnostic and treatment procedures so that eye care professionals can be better informed about this condition when a patient recurs to them with the characteristic signs and symptoms. If the eye specialist is well informed about this condition, he will be able to convey a correct message to their patients.

The main target of this work are professionals in the vision science area, to make them better understand which clinical signs to look for, the risk factors to what patients are exposed in order to prevent these incidents and what message is important to their patients to minimize these risks.

2. Methodology

The methodology adopted for this review work involved an intensive research in several databases as PubMed, Google Scholar, B-On, ScienceDirect and Web of Science for scientific articles and books related with the topic, both used in this paper.

The minimum goal of this paper included the analyzes of 150 articles related with the theme. Overall, 170 articles were analyzed.

Initial search method in online databases involved specific keywords and their variations, from the most inclusive to the most restricted, in the order in which they are referred:

1. *Acanthamoeba*
2. (*Acanthamoeba* and contact lens) and (Contact lens and *Acanthamoeba*)
3. (*Acanthamoeba* and contact lenses) and (Contact lenses and *Acanthamoeba*)
4. (*Acanthamoeba* and contact lens and review) and (Contact lens and *Acanthamoeba* and review)
5. (*Acanthamoeba* and contact lenses and review) and (Contact lenses and *Acanthamoeba* and review)
6. *Acanthamoeba* keratitis
7. *(Acanthamoeba* keratitis and contact lens) and (Contact lens and *Acanthamoeba* keratitis)
8. *(Acanthamoeba* keratitis and contact lenses) and (Contact lenses and *Acanthamoeba* keratitis)
9. *(Acanthamoeba* keratitis and contact lens and review) and (Contact lens and *Acanthamoeba* keratitis and review)
10. *(Acanthamoeba* keratitis and contact lenses and review) and (Contact lenses and *Acanthamoeba* keratitis and review)
11. (*Acanthamoeba* keratitis and disinfecting solutions) and (disinfecting solutions and *Acanthamoeba* keratitis)

Then, in addition to the articles withdrawn first, were analyzed the available articles proposed by first ones.

3. Acanthamoeba

3.1 Definition

Acanthamoeba is an opportunistic unicellular[1] free-living protozoan[2–10] of genus *Acanthamoeba*[11,12] which is environmental widespread[13] and can affect the eye.[14] *Acanthamoeba* comes from term "Acanth" who means "spikes" or "peaks"[15] and "amoeba" that refers to the surface "spin-like" structures.[2] Has two different strains: pathogenic and nonpathogenic.[13]

Sometimes *Acanthamoeba* is called parasite but the term can't be applied because the amoeba can survive and reproduce on their own, it doesn't need a host.[16]

As it's the most common protozoa[17] and has an ubiquitous distribution in the environment, human exposure is inevitable[2] through inhalation or airborne cysts[1] and is frequent to find specific IgA[1] antibodies against *Acanthamoeba* in healthy patients.[18,19]

3.2 Distribution of *Acanthamoeba* in nature

Acanthamoeba lives in soil, dust, air and water.[2,13,16,20–22] Because of its ubiquitous presence in the environment it's common to have contact with the amoeba frequently.[2]

Acanthamoeba can be found in many water sources and supplies such as, tap and bottle water, distilled water bottles, jacuzzi tubs, spas, sea water, pond water, beaches, ocean sediments, fresh and salt water lakes, swimming pools,[2,18] rivers, springs, irrigation water for crops, aquariums dental treatment units,[13] shower heads and sewage.[2,23]

It has also been isolated from water-air interface, air, soil, mud,[24] vegetation and animals like mammals, amphibia, fish and reptiles.[17] Some hospitals, surgical instruments, sterile intravenous saline,[25] dialysis machines,[2,13] filters of ventilation systems[8] and air-conditioning units, eyewash stations, CL, lens cases[2,11,18,23,26–28] and CL solutions[2,11,18,23,27,28] has been identified with varied *Acanthamoeba* strains.[2]

This amoeba was recovered from human tissues such as corneal biopsies, human nasal cavities, skin lesions, brain necropsies and cerebrospinal fluid, lung tissues, throat, human faeces,[2] kidneys[18] and intestines of healthy people.[29]

Human contamination is more likely to occur through CL, cases and solutions infected with this free-living amoeba.[30,31] When *Acanthamoeba* is under certain conditions can be a considered a pathogenic microorganism.[32]

Acanthamoeba is sometimes retrieved from CL surfaces and storage cases not only in symptomatic but also in asymptomatic patients.[33]

3.2.1 Effect of *Acanthamoeba* in nature

Acanthamoeba is an organism present in soil that promotes the presence and regulation of bacterial populations and maintenance of ecosystems. This combination improve the mineralization of nitrogen, phosphorus and carbon.[2]

3.3 Live cycle of *Acanthamoeba*

Acanthamoeba it's a resistant dimorphic organism that presents two distinct forms: an active form – trophozoite – and a dormant stage – cyst.[2,17,27,34,35]

Trophozoites are the active form of *Acanthamoeba* and presents vegetative growth. Is characterized by having an irregular, oval, round[29] or pear-shaped,[7] acanthopodia (protoplasmic projections) that emerge from the clear hyaline ectoplasm laterally and anteriorly,[36] the size varies depending on the author and the genotypes but is comprehend between 15-50 micrometers (μm),[4,15,29] a large numbers of mithocondria,[2] and different kind of vacuoles: small digestive vacuoles and a large and refractive contractile vacuole responsible of osmotic regulation (Figure 3.1).[18] In this stage, trophozoites are able to replicate, move, feed, adhere to surfaces and prey through acanthopodia on the surface.[12,18]

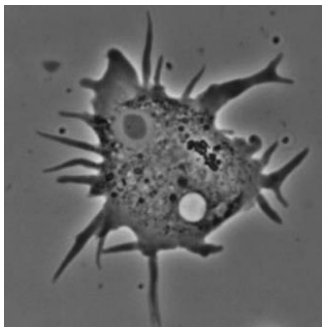


Figure 3.1 - Trophozoite form of *Acanthamoeba* (Magnification x1000) (Reproduced from Walochnik *et al.*, 2015).[19]

The movement of trophozoites is due to the formation of hyaline pseudopodium.[4,17] They're able to connect to lesions on corneal surface created by trauma with CL or other injuries through specific mannosylated proteins.[13]

In periods of environmental adversity, trophozoite is put under unfavorable conditions of pH, temperature of -20 to 56°C,[25] dryness, dessication,[30,33] ultraviolet radiation (UV), presence of chemicals, accumulation of metabolic products and toxins, changes in osmolarity,[37] nutrient limitation and has the ability of differentiate into a double-cyst wall developing two walls, exocyst (exterior wall) and endocyst (interior wall).[38] In the cystic form, *Acanthamoeba* is more resistant to disinfection[11,16] and can survive under this conditions during extended periods of time.[1] Cellular dehydration among other causes led to a great loss of volume from trophozoite to cysts.[18] It's possible to revert into the trophozoite stage when the situation is favorable, called excystment (Figure 3.2).[17,38]

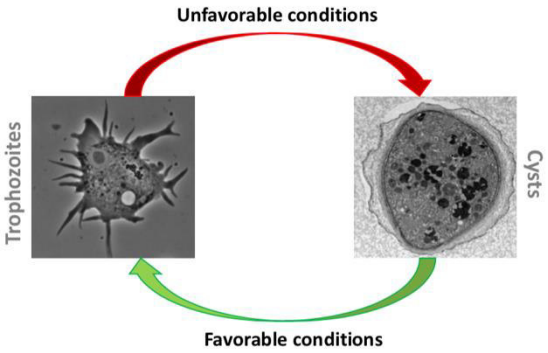


Figure 3.2 - Schematic representation of the *Acanthamoeba* life cycle. The trophozoites, which are able to reproduce and produce infection, when put under harsh conditions transforms into cyst (encystation). The cyst, more resistant under unfavorable conditions, it's able to transform back into the trophozoite form when the conditions become favorable (excystment) (Adapted from Siddiqui and Khan, 2012).[2]

Cysts are the inactive form of *Acanthamoeba* that presents minimal metabolic activity[2] and is the cause of persistent infections (Figure 3.3).[15] They can be found in two different forms: mature and immature.[13] The size varies depending on the author but is comprehended between 5-30 μm,[15,18,29] stellate form with 6-8 rays, round, ovoid, triangular, hexagonal or polygonal bodies,[27,28] that varies morphologically between isolates. The structure of the cyst contains a cellulose double wall with a fibrous laminar exocyst and a granular smooth endocyst. The composition of cyst wall has 1,4 b-glucan, also known as cellulose[39], and other polysaccharides that makes the wall more robust for protection.[40] The endocyst is mainly constituted by proteins and cellulose and the wrinkled exocyst is composed of polysaccharides[41] and proteins.[2] Between this two walls exists a blank space that is thinner around a series of pores, known as ostioles. Possesses an operculum where the trophozoite exits the cyst stage when extern conditions allow.[18] The cyst cytoplasm is composed by dense granules of food particles, mitochondria, lipid droplets and glycogen.[29]

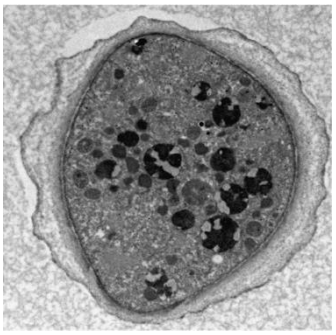


Figure 3.3 - Cyst form of *Acanthamoeba* (Reproduced from Siddiqui and Khan, 2012).[2]

After decades of storage, cysts can remain pathogenic and viable.[42] According to Niszl *et al.* "cyst walls become thicker as the cyst ages"[43] and mature cysts are more resistant than immature cysts.[25] As they are very resistant to CL chemical and disinfection solutions containing antimicrobial agents, are hard to treat and are able to contaminate lens storage cases.[44,45]

3.4 Biology of *Acanthamoeba*

Acanthamoeba species "belong to the phylum Amoebozoa and the family Acanthamoebidae".[46]

Acanthamoeba is constituted by the following main structures: nucleus, cytoplasm, vacuoles, membrane, endoplasm, ectoplasm and pseudopods (Figure 3.4 and 3.5).[2,15]

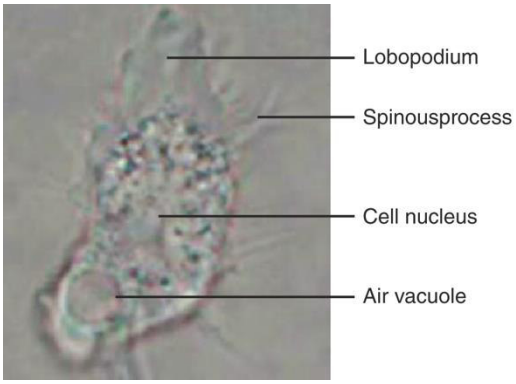


Figure 3.4 - Trophozoite of *Acanthamoeba* with identification of the cell nucleus, spinous process and air vacuole (Magnification x1000) (Reproduced from Sun and Wang, 2018).[29]

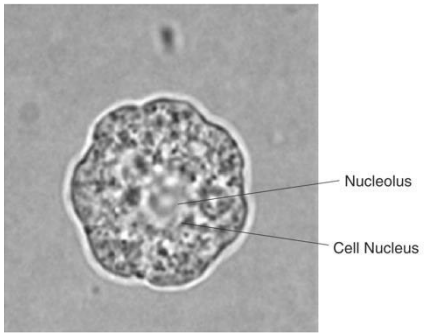


Figure 3.5 - Cyst of *Acanthamoeba* with identification of the cell nucleus (Magnification x1000) (Reproduced from Sun and Wang, 2018).[29]

It's usually uninucleate, local where possesses the genetic information, that control the activities of the cell and the expression of different genes.[2,15]

The membrane define the limit of *Acanthamoeba*[15] and presents contractile vacuoles that maintain osmotic regulation by the control of water quantity inside the cell.[2,15]

Under Transmission Electron Microscope (TEM) observation can be perceived the division of the trophozoite membrane in three coats and the cyst wall is marked by two layers. The internal and external layers are separated by a transparent space that split the tenuous fibrils inner coat of wrinkled and fibrinous outer coat. These layers may merge and form spinous foramens established in the equatorial zone.[29]

Plasma membrane is a trilaminar membrane that enclose the cytoplasm and it's composed by proteins (33%), lipophosphonoglycan (29%), phospholipids (25%) and sterols (13%).[2] The main phospholipids found in *Acanthamoeba* are phosphatidycholine (45%), phosphatidylethanolamine (33%), phosphatidylserine (10%), phosphoinositide (6%) and diphosphatidyglycerol (4%).[2] There are also chains of fatty acids like "oleic acids (40-50%) and longer polyunsaturated fatty acids (20-30%)".[2]

Cytoplasm has glycogen, lipid and food vacuoles. The last ones regulate digestion[15] and are also present in trophozoites lysosomes,[2] abundant mitochondria, Golgi complex, free ribosomes, microtubules and smooth and rough endoplasmic reticulum.[17,29]

Endoplasm includes the central portion of the cell while the ectoplasm represents the outer superficial film.[15]

The pathogenicity and virulence of the ameba can be define by the conditions in which the specie of *Acanthamoeba* develop and grow.[47] Pathogenicity was also determined by the observation in Scanning Electron Microscope (SEM) of an elevated quantity of acanthopodia on the surface.[48]

3.5 Reproduction of *Acanthamoeba*

Acanthamoeba reproduction occurs on the trophozoite stage by binary fission[17,28] and mitotically division[41] when the conditions are favorable.[2] The binary fission is divided into different stages: prophase, metaphase, interphase, anaphase and telophase.[29]

The prophase consists on the split and disappearance of the nucleolus. Next, in the metaphase, the division in two parts starts in the equatorial zone with a banding shape of chromatin. After metaphase, interphase which englobes the adherence of the dense long column of centrosome with nucleus cells to tubular structures. The next phase, anaphase, refers to the arrival of the fine fiber's chromatin to the two poles of the cell. In the final phase – telophase - exists the reformation of the nuclear membrane and circle the new formed cells, completing the formation of the two new trophozoites.[29]

3.6 Classification of *Acanthamoeba*

According to Marciano-Cabral and Cabral, Volkonsky proposed a classification system for *Acanthamoeba* in 1931 based on the presence of acanthopodia.[17]

Both Bouheraoua *et al*. and Sun refer the classification system of Pussard and Pons presented in 1977 that establish a division of 18 species in three groups based on the cyst morphology (size and shape of cysts).[15,29] Assigning one of three groups depend on the average diameter and characteristics of the outer and inner layer:[29]

- Group I: englobe the cysts with average sizes of eighteen micrometers or more. The inner layer presents a star shape, the outer layer can be wrinkled or smooth, ostioles present at tips of the rays,[36] and the space between both layers is large.[29]

- Group II: englobe the cysts with average sizes of less than eighteen micrometers. The inner layer presents different forms like round, oval, triangle, polygon or star shape; the outer layer is wrinkled[17] and/or wavy, ostioles present at angles of the rays,[36] and the space between both layers differ. Most of pathogenic species was attributed to this group.[29] Human pathologies are mostly implicated by *A. polyphaga* and *A. castelanii*, species of this group.[15]
- Group III: englobe the cysts with average sizes of less than eighteen micrometers. The inner layer soft presenting three to five angles, the outer layer may or may not present wrinkles and ostioles are single or obscure. Although most of pathogenic species belong to group II, *A. culbertsoni* belongs to group III (Table 3.1 and Figure 3.6).[29]

Table 3.1 - Classification of *Acanthamoeba* based on morphology (Adapted from Sun and Wang, 2018).[29]

Group I	Group II	Group III
A. astronyxis	*A. castellanii*	*A. palestinensis*
A. comandoni	*A. mauritaniensis*	*A. culbertsoni*
A. echinulata	*A. polyphaga*	*A. lenticulata*
A. tubiashi	*A. lugdunensis*	*A. pustulosa*
	A. quina	*A. royreba*
	A. rhysodes	
	A. divionensis	
	A. paradivionensis	
	A. graffini	
	A. tringularis	
	A. hatchetti	

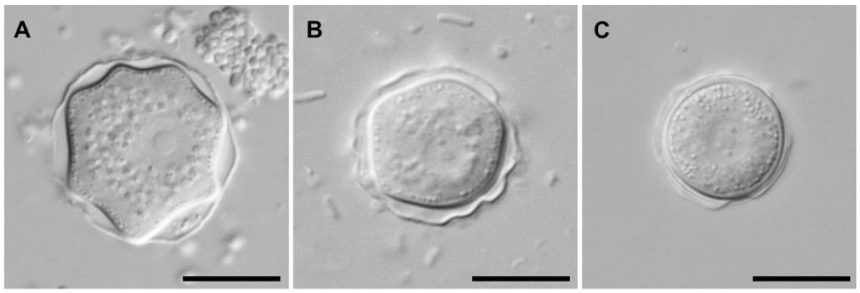


Figure 3.6 - Acanthamoeba cysts in interference contrast microscopy (A) morphological group I, (B) morphological group II, (C) morphological group III. Scale bar: 10 μm. (Reproduced from Lorenzo-Morales *et al.*, 2015).[47]

It was identified that group II and III, particularly, have more virulent characteristics when related with human keratitis.[45]

In the year of 1991, some species of *Acanthamoeba* like *A. tubiashi* and *A. hatchetti* was added to group I and II, respectively, based on their characteristics.[29]

In 1999 was created a new system of classification by Stothard *et al.* based on the analysis of "18S nuclear subunit ribosomal RNA gene" that allow the discover of 53 isolates[17] and the division in 12 groups (T1-T12).[15,49] It's possible to relate both classification system of Stothard and Pussard and Pons establishing the next groups:

- Group I: T7, T8 and T9
- Group II: T3, T4 and T11
- Group III: T1, T2, T5, T6, T10 and T12.[17,19]

3.7 Genotyping of *Acanthamoeba*

The benefits of genotyping are allowing us to understand what the epidemiological links are so that it's possible to established drug susceptibility, virulence factors, infectious isolates, and the most relevant clinically, the clinical outcome.[50]

The division by genotype of the specie *Acanthamoeba* is based on the rRNA gene sequences and has to have a difference of 5%, at least, so that they are considered different

sequences[2] Exists both nonpathogenic and pathogenic strains in *Acanthamoeba* genotypes but the pathogenic ones happens equally in nature.[13]

To date are recognized 25 species[29] and 20 different genotypes of *Acanthamoeba* (T1-T20).[30,48,51–54] The genotype T4 is identified as the most common clinical isolate[28–30,38,52,55] of the majority of human infections and in 90% of cases is linked to *Acanthamoeba* keratitis (AK)[2,19,32,50,53,56,57] and 79,3% of cases in brain, skin and lung infections.[13] Unlike the genotype T4 that is abundant in nature,[54] the genotypes T3 and T11 are rare in the environment.[15]

Genotypes related to corneal infections as AK are: "T2, T3, T4; T5, T6, T10, T11, T13, T15 and T16".[2,29,48] The genotype T4 is related with both CL and non-CL related keratitis[58] particularly the species *A. culbertsonii*, *A. castelanii* and *A. polyphaga*[1,4] that have higher pathogenic potential. This pathogenic potential is related with the capacity of binding to corneal tissues, extracellular protease production, specific enzymatic activity and reproduction speed.[29,50]

According to Ledee *et al*. the genotype T5 is believe to be not pathogenic to the human species but with the increase of CL use is possible to start finding this isolate more often.[50]

Recently, in a 2018 report of Orosz, E. *et al* was identified for the first time the presence of genotype T8 as a cause for AK related with the use of CL.[51]

The species of *Acanthamoeba* that are recognized for causing *Acanthamoeba* keratitis are *A. culbertsoni, A. castelanii, A. polyphaga,*[1] *A. hatchetti, A. griffin, A. rhysodes, A. lugdunensis, A. quina*.[17,27,36,38,41]

3.8 Alimentation

Acanthamoeba trophozoites feeds on algae, bacteria,[20] fungus,[9,16] cells, yeast,[17] other protozoans, organic matter,[2,41] deskerocytes[15] and keratocytes in the cornea.[4] Between the bacterial selection, they show a preference of coliforms and non-fermentative Gram negatives bacteria.[8,59]

The feeding procedure occurs by two distinct methods: phagocytosis and formation of food cup sucking.[17,29] The ameba forms temporary structures on its surface like food cups to help ingesting food and nutrients.[17]

According to Qureshi *et al.* species as *Pseudomonas aeruginosa* can be lethal to *Acanthamoeba* trophozoites or cysts[60] and Neff *et al.* refers that the growth of the ameba species is favored by death cells of *Saccharomyces cerevisiae* and live or death *Escherichia coli* and *Aerobacter aerogenes*.[61] In Qureshi *et al*. was refered that beyond the species refered anteriorly, also species of bacteria like *Pseudomonas paucimobilis, Xanthomonas maltophilia* and *Flavobacterium breve* enhance growth of *Acanthamoeba.*[60]

3.8.1 Relationship between *Acanthamoeba* and *P. aeruginosa*

Acanthamoeba and *P. aeruginosa* have lethal relation when exist contact between *P. aeruginosa* and the cells on the surface of *Acanthamoeba*. Despite this relationship, they are both similar eye pathogens. Both are capable of penetrate in damaged corneal tissue through "tissue destructive" enzymes, contaminate CL cases and care systems.[60]

The production of different exoenzymes as elastase (destroy epithelial cells in humans), blue-green pigment pyocyanin (able to affect human nasal epithelial cells by inhibiting the cells function) and phospholipase C are capable of injure *Acanthamoeba's* membrane.[47,60]

3.9 What *Acanthamoeba* can cause

The ameba can cause rare human infections[36] like AK[62], potentially blinding infection of the cornea;[1,63] granulomatous amoebic encephalitis (GAE), fatal disease that affects the central nervous system;[17] sinus infections, cutaneous lesions[2,16] and, depending on the strain, serve as a host for other microorganisms.[1] This infections are related with immunocompromised individuals and all users of CL.[2,11]

Acanthamoeba is contracted by contact with contaminated water, soil, dust, air or the inhalation of cysts[20] that invades the human body through several ways.[18]

4. *Acanthamoeba* Keratitis

4.1 Definition

AK is a rare but severe corneal infection[15,64] caused by *Acanthamoeba* species,[5,25,65–70] painful and difficult to diagnose and treat[1,7] that can lead to blindness or even enucleation.[18,23,30,33,35,40,58,71–74] The devastating risk of vision loss is connected with scarring and corneal ulceration[75] causing massive ocular injury.[52] Sight-threatening AK is mostly found in soft CL wearers, young ages and agricultural workers even though it can be present in non-CL wearers (Figure 4.1).[76]

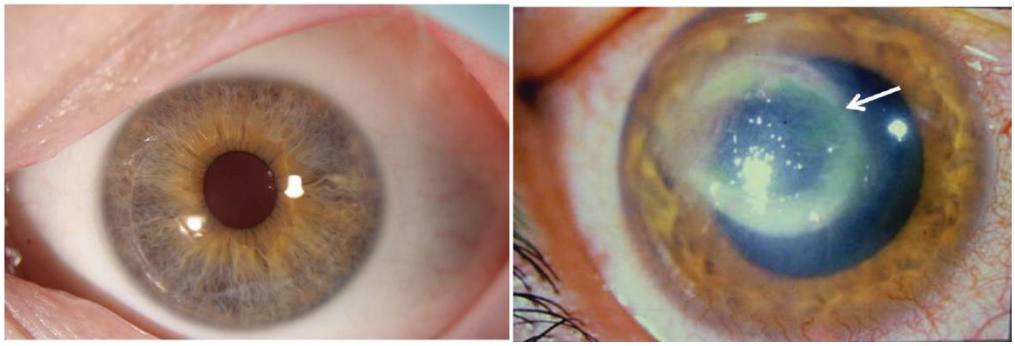


Figure 4.1 - A) Normal human eye; B) Eye with AK. The ring-like stromal infiltrate (pointed with the arrow) is a characteristic feature of AK. (Reproduced from Neelam and Niederkorn, 2017).[41]

When AK isn't associated with the use of CL usually presents in an advance stage with worse diagnostic and poor visual outcome with acute vision damage.[1,77]

Cases of AK are normally connected with corneal trauma, lack of personal hygiene, biofilm formation, poor cleaning of CL and contact with contaminated water, materials or solutions.[20] There are also reported cases of AK after LASIK and radial keratoplasty.[18]

Infection by *Acanthamoeba* starts on the epithelium and progress slowly into the stroma. When treatment isn't adequate, AK can lead to perforation or corneal melting.[78] This process of infection is complex and requires both host and pathogens factors.[47]

In most of the cases AK is unilateral but can also affect both eyes[18,35,55,79,80] like 7.5% in UK.[15] AK can also be found in animals like monkeys, fish, reptiles, bulls, dogs, kangaroos, buffalo and echinoderms.[18]

Patients with AK may lose stereopsis due to central corneal inflammation and presents difficulties on the daily tasks like reading.[16]

4.2 Incidence of AK

As state by several authors in scientific literature[18,22,55,81–84] the first report of AK was described in 1974 by Nagington.[85] In the following years, AK was mainly related with ocular trauma.[34] Although the ubiquitous presence of *Acanthamoeba* species in nature and the appearance from all over the world,[76] the infection caused by this ameba still is rare.[13]

After the first recognition of the disease was verified an increase in the incidence of AK in parallel with growing of CL users.[76] The incidence of AK differs between develop and developing countries, geographical areas and within the countries.[25]

As reported by Jain *et al.* in 2015 in a developing country the incidence of AK is lower when compared with a develop country. In this last one, the majority of the cases are liked with the use of contact lenses[86], more specifically soft CL[76,87] in 85-88% of cases[15,40,52] even though it can be present with other kind of CL like rigid lens[34] and orthokeratology.[78] In developing countries, as India, where the use of CL is not considerable, most cases are reported in non-CL wearers[76] and associated with trauma.[16] In cases of non-CL wearers the diagnostic of AK is, most of the time, late due to the low incidence around 3-15% in UK and USA[15] and are associated with trauma in corneal tissue with contaminated water, soil, vegetal material[18] and surgical trauma. Often found in farmers or artisans.[15]

According to Seal *et al.* in 1995 the incidence of AK in USA was low about 1 in 250.000.[11] A study realized in the US around the decade of 1990 pointed numbers of 1.65-2.01 cases of AK per million of CL wearers in the US and numbers of 17.54-21.14 per million of CL in the UK.[6,31] This contrast is thought to be due to the use of water storage tanks in house lofts in UK.[37] In 1988, was announced the first incidence of this disease in Japan[81] and in the southeast and southern

regions of Brazil.[53] In countries like Japan where the prevalence of myopia is high and orthokeratology lenses are popular, leads to the increase of infection[78] by fifteen more times than United States numbers.[7,83] In China the first case of AK occurred in 1991,[70] and it's similar to Japan since presents a high adherence to orthokeratology for myopic control and high rates of AK when compared with other countries like Nepal and India.[88] Other than orthokeratology, AK in China is also very evident in cases of soil-contaminated matter and corneal trauma.[66]

As reported by Lorenzo-Morales *et al.* in 2013, in the years of 1980 to 1990 the incidence of AK was relatively low of 1-2 cases per million of CL wearers.[18] The relationship between the use of CL and AK was first established in 1984.[34] With the increase of wearers was observed a direct increase of reported cases. The recognition of risk factors like the incorrect use of non-sterile homemade saline prepared from salt tablets and tap water for rinsing and storing CL[34] lead to a decrease in AK numbers until the year 2000.[18] Even though the introduction of silicone-hydrogel lenses in the late 1990s, which was expected to reduce the incidence of AK, the numbers never back down.[58]

The inefficacy of certain CL care systems like Complete MoisturePlus (Abbott Medical Optics, Santa Ana, CA, USA) and ReNu MoistureLoc (Bausch&Lomb, Rochester, NY, USA) were associated with outbreaks of AK and Fusarium, respectively. After the recall of this products from the market, the numbers in the US remained higher than the anterior baseline levels pointing to other risk factors.[42]

The difference of incidence of AK between countries varies depending on diverse factors as the prevalence of CL use, virulence of *Acanthamoeba* strains, diagnostic techniques used to detect AK, risk factors, contact with contaminated water in domestic or public environments, climate, amoebicidal efficacy of CL care system[15] and the lack of care with the maintenance of CL and cases.[65] This lack of maintenance of CL cases can lead to the formation of biofilm that acts like a food source to *Acanthamoeba* and makes disinfection difficult due to the resistance of polysaccharide conglomerates of microorganism.[1]

Factors as younger age and gender also appears to have relevance in the incidence of AK. Even though several reports refer that the female gender present a greater risk for contracting AK because of lack of care and disregard for clinical evaluation[30,74,88,89], there is one report that defend

that the male gender seems to be at higher risk of contracting AK due to less compliance to hygiene behaviors.[68]

As it was referred by Bouheraoua *et al.* the increase incidence of AK in warmer periods like summer and early autumn is associated with the increase in the number of amoeba in nature and the number of water activities.[15]

The increasing number of CL wearers all over the world due to refractive errors or cosmetic reasons is leading to the increase of AK cases every year.[32] This increase in the develop countries is often caused by contamination with *Acanthamoeba* during cleaning procedures.[53]

4.3 Pathogenesis of AK

In most cases pathogenesis of AK involves the contaminated CL as a transmitting vector of *Acanthamoeba.*[11,90,91] The principal ways by which the protozoa comes in contact with CL is through water and air (Figure 4.2).[12,19]

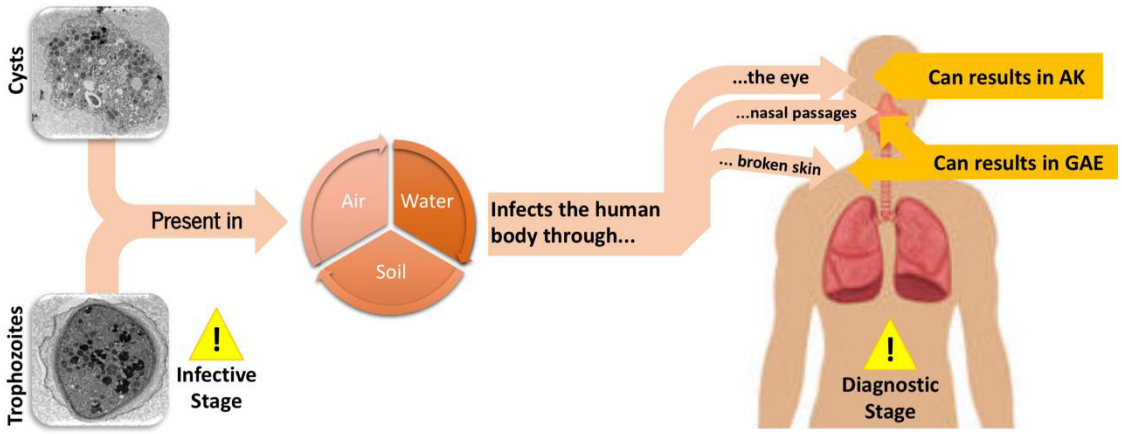


Figure 4.2 – Schematic representation of *Acanthamoeba* life cycle in humans. Trophozoites and cysts are present in contaminated air, water and soil and can infect the human body through the eye, nasal passages and broken skin which can result in AK and GAE, respectively (Adapted from Maycock and Jayaswal, 2016).[4]

CL wear can induce hypoxia in corneal epithelial cells that decreases metabolic rates and debilitate the capacity of resistance of the cornea, particularly in overnight wear. Other than

hypoxia, soft CL can also cause decrease of in tear exchange and minor mechanical trauma in eyelids causing alterations in corneal cells.[72,92]

Some theories point CL storage cases as one of the principal source of infection and where *Acanthamoeba* can adhere to all kinds of CL material and then transferred to the cornea.[11,93]

Most of the time, AK is caused by T4 genotype because it seems to attach to host cells more easily and therefore it's the genotype more commonly found AK cases.[1]

First, *Acanthamoeba* is enclosed in the epithelium. The proliferation to the stroma occurs with the progression of the disease that causes inflammation and severe damage.[17] A number of steps occur before keratitis happen like adhesion of the amoebae to CL, transference to the cornea, adhesion to the corneal surface,[94,95] disintegration of the epithelial barrier, stromal invasion and final stromal necrosis with blinding consequences.[2,15] This invasion mainly occurs when corneal surface is damage, otherwise the amoebae is rapidly removed through tears, blinking[1] and host immune system.[41] During the use of CL the posterior surface of the CL is relatively immobile so the microorganisms can adhere and proliferate more easily to the corneal surface.[1]

Adhesion to the corneal surface, more specifically to the epithelium surface, depends mainly on the number of acanthopodia on the trophozoite surface and mannose-binding protein (MBP).[18] Both this characteristics are influenced by the degree of pathogenicity once non-pathogenic trophozoites present low capacity of binding to the host cell.[18] Pathogenic trophozoites presents over than a hundred acanthopodia per cell and higher levels of the adhesion MBP protein, non-pathogenic trophozoites has a maximum of twenty acanthopodia per cell and low levels of MBP.[18]

Breakdown of the epithelial barrier is facilitated by the abrasion of corneal surface through the use of soft CL and/or trauma and contact with the inoculum of *Acanthamoeba.* Once in the surface of the epithelium trophozoites destroys corneal integrity through the fabrication of an enzyme denominate cytotoxic serine protease. A 136-kDa MBP adheres to the exposed mannosylated glycoproteins in the surface of the corneal epithelium.[17,18,45,53,58] This event is only possible because of the high quantity of both this proteins in injured corneal surfaces that are essential for the adherence of *Acanthamoeba* to corneal epithelium.[18]

Trophozoites releases MIP133, protease able to dissolute cells, when exposed to mannose.[1] *Acanthamoeba* is also able to produce three different types of proteases, like cysteine proteases,

serine proteases and metalloproteases, that are enzymes capable of degrade protein substrates. Serine proteases ease are capable to corrupt collagen type 1, present in corneal stroma that regulates corneal integrity,[17,18] secretory immunoglobulin A (sIgA), immunoglobulin G (IgG), laminin, fibrinogen, fibronectin, fibrin, lasminogen, hemoglobin and bovine serum albumin (BSA).[18] Enzymes as elastases act on connective tissues degrading them and phospholipase A causes demyelination of brain nerves.[18]

Stromal invasion derives from the destruction of epithelial cells and perforation of Bowman's membrane caused by the formation of an enzyme of serine protease and the secretion of collagenolytic factors from trophozoites lead to the dissolution of stromal matrix.[13,53] This stromal degradation provides a deeper invasion of the cornea.[4]

During the process of invasion by *Acanthamoeba* the amoeba induces cytolytic mechanisms that cause changes in the osmotic pressure inside the cell as well as ionic disruption causing apoptosis of the host cell.[18]

The last stage involves death of corneal cells and corneal nerves[94] due to the activation of the caspases-3 and -10 dependent apoptosis cascade.[13,96] Absence of trophozoites in the anterior chamber is connected with the robust neutrophil response.[66] In some cases, the protozoan destroys all layers of corneal tissue and can lead to perforation.[67]

In the cornea *Acanthamoeba* trophozoites are able to convert into cysts which may prolong the presence of the protozoa in the cornea during infection.

4.4 Immune response to AK

Although the contact with *Acanthamoeba* is common the infection is not. When healthy people get in touch with the amoeba triggers a humoral immune response and production of specific antibodies against the protozoa.[29]

Antibodies have an important role in the prevention or regulation of *Acanthamoeba* adhesion to the host[18] as they restrain trophozoite movement and/or neutralize cytotoxic factors of the amoeba.[17] Individuals affected with AK possesses low levels of IgA[1] and serum IgG[41] in tear fluid

when compared with non-affected individuals.[1] As referred in the anterior chapter serine proteases are capable of destroying IgA and IgG antibodies present in human tears.[9,17]

Tears present natural defenses like anti-pathogenic substances capable of dissolve and kill pathogenic microorganism. IgA is the principal immunoglobulin in tears and function as the main obstacle for adhesion.[18] IgA antibodies acts on *Acanthamoeba* MBP inhibiting the adhesion to corneal epithelium and IgA-independent factors restrain the activity of cytotoxic proteases protecting from cytopathic effect on the cornea.[1]

Other than antibodies exist a cellular response to the site of infection. In the first moment there is a flow of neutrophils[97] followed by macrophages. These types of inflammatory cells are found around the tissue containing the amoeba. Langerhans cells (LC) are known for postpone AK progress by migrating from the peripheral to the central cornea and retarding hypersensitivity. This kind of cells might act by destroying the trophozoites or inhibiting the adhesion to the epithelium.[29] T-cells appears around the final stage of infection. The affluence of neutrophils cause corneal infiltration and a significant humoral response result in corneal opacity or perforation.[17]

Corneal infection caused by *Acanthamoeba* doesn't produce immunity so recurrence of this infection is frequent.[2] This persistence is due to the capacity of *Acanthamoeba* to avoid human response to the infection and it's able to persist in host tissues during extended periods of time.[29]

4.5 Symptoms of AK

Symptoms of AK are most of the time incapacitating driving from periods of improvement and setbacks[16] where severe ocular pain out of proportion is a distinct characteristic.[20] In the majority of times is unilateral,[29] even though it can appear in both eyes.[98]

The symptoms may varied between discomfort,[20] blepharospasm, conjunctival hyperemia, foreign body sensation, eyelid ptosis, edema, excessive tearing,[36,99] blurred vision, decrease of visual acuity,[34] photophobia and severe ocular pain disproportionate to clinical signs [18,30,41,47,68,81,82,100,101] even though it could not present some of these symptoms.[1,4,15,16,31,102]

Pain is related with the inflammatory process[67] and the intensity of pain derives from nerve affectation.[103,104] When AK is presented with no pain associated could be connected with the

retention of infection in the epithelium, treatment with steroids that decreases inflammation and cornea could be already neurotrophic.[104]

At an early stage visual loss is not significant and gets worse with the delay of diagnosis, as well as all other symptoms.[29] The symptoms may persist during a long period of time and according to Jain *et al*. that may vary from 3-240 days.[76]

The symptoms reported above are common to other bacterial, viral and fungal keratitis what makes the diagnose difficult and facilitates misdiagnoses.[18,54]

4.6 Signs of AK

Some clinical signs aren't specific and may be confused with other ocular disease[15] and so it gets worse over time.[15] The clinical sign characteristics of AK is the ring infiltration that results from the stromal invasion of *Acanthamoeba.*[29]

In the early stage of AK most of the signs are confined to the epithelium and presents like epithelial lesions as punctate epithelial erosions, pseudodendrites (Figure 4.3),[1] subepithelial and perineural opacities (Figure 4.4),[16,27] conjunctival hyperemia, eyelid ptosis[17] and watering.[40] These initial signs are often confused with herpetic keratitis.[5,67,105]

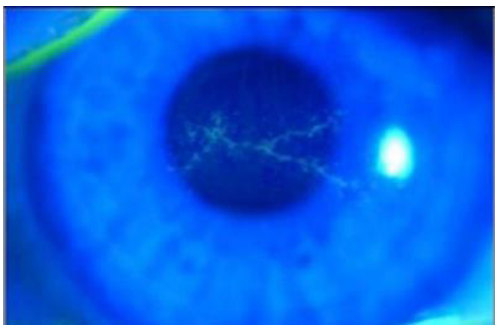


Figure 4.3 - Pseudodendrite from an AK case (Reproduced from Maycock and Jayaswal, 2016).[4]

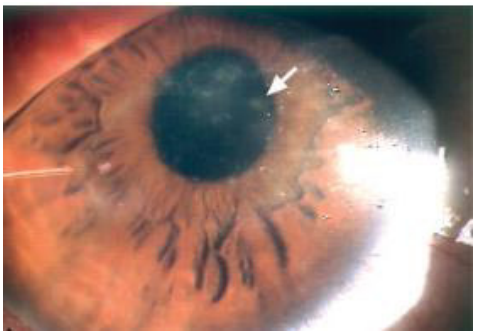


Figure 4.4 - Cornea with subepithelial infiltrates, pointed by the white arrow (Reproduced from Wong *et al*.,2007).[124]

The progression of the disease through the stroma can produce neovascularization,[83] anterior stromal infiltration,[76] scarring,[103] ring infiltrate (Figure 4.5),[18,80] central inflammation which causes increase vision loss, corneal opacification, stromal thinning, ulcers with increase diameter and unclear boundary,[29] hypopion[15] and scleritis.[1,16,34,106,107] In a late stage the patient can present severe headache and orbital pain.[29]



Figure 4.5 - Ring infiltrate from a late advanced stage of AK (Reproduced from Garg *et al*., 2017).[29]

During the process of inflammation corneal sensitivity decreases.[15] When trophozoites invade the corneal nerves they are able to cause neuritis and, consequently, necrosis.[17]

In some rare cases, AK can produce cataracts, glaucoma[30,34,105] and dacryoadenitis.[101]

According to Tu *et al.* there are five classifications depending on the severity of disease: epithelitis, epithelitis with keratopathy, previous stromal involvement, deep stromal involvement and immune ring and scleritis, respectively, from stage one to five.[108,109] Sometimes in more acute stages surgery may be required and also enucleation as a last resort.[110]

4.7 Risk factors of AK

As referred in chapter 3.2, in industrialized countries about 85% of AK cases are related with the use of CL.[41,51,68,81,89,99,111] Trauma is the main cause of AK in developing countries, farmers[70] and non-wearers of CL.[16,22,70,100,112,113] Other factors are also pointed for the increased number of infections related with *Acanthamoeba* as CL multipurpose disinfecting solutions,[77] CL materials,[18] contact with contaminated water,[77,114,115] use of contaminated storage cases,[116] trauma with soil or vegetation,[1,17] ocular surface disease, ocular surgery,[84] systemic diseases[117] and climate.[29] The majority of the patients with AK are immunocompetent CL wearers.[6,18,23]

The following describes some risk factors associated with this ocular complication:

Age: AK is more likely to appear in younger patients,[77] with ages under 26 years, but can also be present in the oldest, with ages above 55 years.[1,16] As orthokeratology is associated with younger ages and less hygiene, the risk of contracting AK is higher.[118]

Gender: female patients are at higher risk of contracting AK. Normally, women wear CL for cosmetic reasons that sometimes leads to cheaper choices without eye examinations. Furthermore, the application of makeup as mascara can induce the adhesion of pigments to the surface of CL facilitating the adhesion of microorganism.[30]

Systemic Risks: decreased immunity, genetic mutations, diabetes, thyroid disease and general health problems.[117]

CL wear: is considered the major risk factor of AK.[4,17,18,30,33,42,59,72,74,77,94,119,120] The main risk behaviors are related with lack of hygiene habits with CL,[17,36,52] absence of rubbing the lens during the process of disinfection, hand hygiene, overwear of CL,[121] extended wear,[1,16] non-compliance with replacement schedules,[111,119,122] overnight wear[75,105,123,124] and biofilm formation in both CL and their storage cases.[110] The overwear issues are related with many characteristics as lens type, oxygen transmission and permeability.[72]

CL material: soft CL are mostly related with AK,[74,125,126] although AK can appear in all types of lens materials[1] as rigid gas permeable (RGP), hard CL,[27] cosmetic lenses,[127] hybrid lenses and orthokeratology lenses.[75,77,78,128]

CL disinfection methods: like Multipurpose solution (MPS) are easy to manipulate but they are not completely effective against AK.[4,18,95,129,130] Solutions as "ReNu MoistureLoc", "Advance Medical Optics Complete Moisture Plus"[64] and "Moisture Plus Complete No-Rub" were withdrawn from the market because they were not effective against some species that cause keratitis, within which *Acanthamoeba* was included.[18] CL maintenance solution and hygiene procedures of CL have an impact in the onset of the disease.[1] Risk behaviors like topping off[1,89,131] or reuse of the solution,[119] expired CL solutions,[40] failure to follow manufacturer's protocols,[9,37,132] the use of home-made saline solution from distilled water and salt tablets and chlorine based disinfection show increased risk to the disease.[3,5,14,25,27,123,133] This subject will be explored in chapter 4.

CL lens storage cases are the source for many pathogenic agents.[42,58,134] Some of the main risk behaviors are infrequent replacement of storage cases,[12,121] wash and rinse the CL storage case with tap water[21] and then keep the case wet when it should be air-dried.[120] A contaminated storage case with bacteria, protozoa and fungus offer a useful environment to the developing of *Acanthamoeba* once the bacteria work as food supply to trophozoites.[7]

Contaminated water: This contact may occur in daily activities such as shower, hot tub, lakes,[34] tap water,[120,135] storage water tanks, swimming,[14,22,57] fishing, snow and water sports.[1,25,100,106] The exposure of CL to water during the process of disinfection such as rising lens,[14] placement and cleaning the lens with tap water before placed them in the storage case[1] is very prejudicial. Tap water despite being treated to be drinkable isn't sterile and it's not free of microorganisms that can cause keratitis.[89] Hard water supplies are favorable environments for the development of *Acanthamoeba* species and can originate a decrease efficacy of some disinfection systems.[79]

Other risk behaviors: Sleeping with CL[118,121] presume close lids and absence of lid movements, which leads to corneal hypoxia, edema, subepithelial infiltrations, central corneal thinning[72] and can result in superficial punctate keratitis.[30] More, rubbing the eyes with CL on causes corneal trauma facilitating the invasion by *Acanthamoeba* species.[30] Sometimes, the purchase of CL online is seen as a risk factor once the seller doesn't require a prescription and the user doesn't see the purpose of a thorough visual examination and important procedures of disinfection.[136]

Climate: According to Sun *et al.* in the summer there is an increase of cases when compared with other seasons,[29] which is associated with the increase in the number of amoeba in nature and the number of water activities.[15]

4.8 Diagnostic of AK

Diagnostic of AK is complicated due to unspecific signs and symptoms.[31] AK is very often confused with herpes simplex keratitis[34,53,115,137] and other types like viral, fungal or bacterial keratitis.[2]

Early diagnosis and treatment is essential to maintain visual capacity since the delay of diagnosis leads to worse prognosis.[76,138] Suspicion is vital to obtain an early diagnosis and avoid delays.[79] As state by Chynn *et al*. and Sharma *et al*. the time for the diagnosis is about half in CL wearers when compared with non-users of CL.[100,126]

In cases of ocular trauma and all types of CL wear,[5] contact with contaminated water or soil associated with severe pain should alert to the suspicion of AK.[2,15]

Improvement of diagnostic tools and evaluation technics should allow a correct interpretation of signs and symptoms. The delay on the diagnosis or the wrong one can lead to permanent blindness or the need for corneal transplant.[68]

Since diagnosis based on clinical signs is difficult, diagnostic methods such as biomicroscopy, corneal scraping, histopathology, Polymerase Chain Reaction (PCR)[16] and confocal microscopy[76] allow faster and more accurate diagnosis[66] with higher sensitivity.[6]

Initially, biomicroscopy remains an important diagnostic tool to detect modifications on corneal integrity and evaluation of clinical signs even though it doesn't allow direct visualization of pathogens.[29] Despite advances in other diagnostic techniques, PCR method doesn't replace the biomicroscopic evaluation with fluorescein which allow the visualization of damage area.[139]

Corneal scraping is the gold standard method for AK diagnosis with a specificity of 60%. This procedure starts with the extraction of a small part of the already fragile epithelium and separated in two parts. After this division, half is cultivated with non-nutritive agar cover with E. Coli, that function as food for *Acanthamoeba,*[16] or inoculates into liquid medium.[17] Trophozoites appear first with slow and contractile moves and cysts just develop after a few days when the food is over.[1,16] The other part of the tissue sample is submitted to microscopy with tension immunohistochemisthry.[16]

When corneal cultures results are negative should be elaborated cultures in CL, lens cases[18] and lens-cleaning solutions[17,27] of patients.[105]

Histopathology includes the staining of tissue sample and visualization of microorganisms and antigens under fluorescent microscopy or light. During the biopsy procedure the extraction of trophozoites can provoke the damage in the amoeba cells.[1] Stains normally used by this technic are Gram-stain,[80] Calcofluor White (Figure 4.6) and Periodic Schiff.[1] The different stains allow the visualization of structures in detail:

- Calcofluor White Stain: gives a pale blue tone to the cyst wall because of the chitin presence in the cysts wall.
- Acridine Orange Stain gives an orange-red tone to *Acanthamoeba*
- Lactophenol Cotton Blue Stain: gives a blue tone to cyst wall and thorn-like projections of trophozoites.
- Iodine Tincture Stain: gives a yellow tone to *Acanthamoeba* cysts and trophozoites because of the levels of glycoproteins in *Acanthamoeba* surface.
- Fuchsin Stain: gives a pale brown tone to *Acanthamoeba*'s nucleolus.[29]

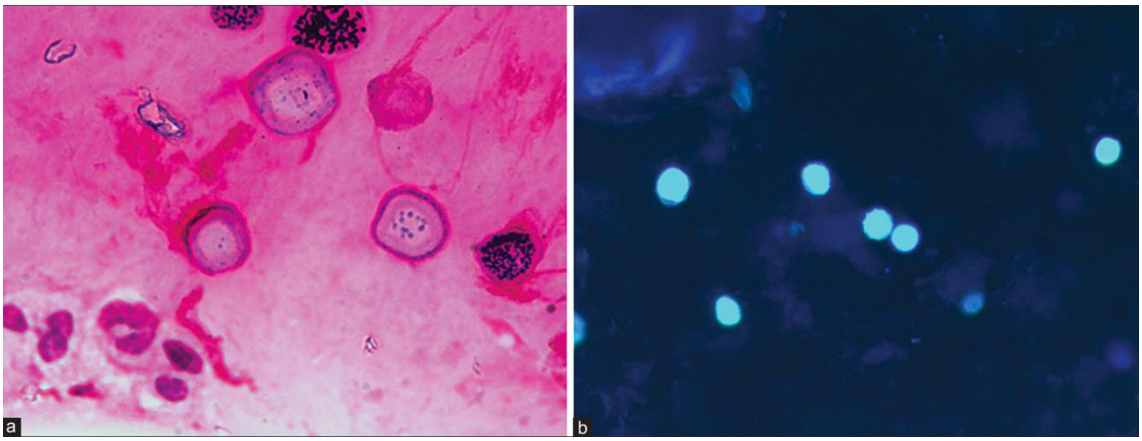


Figure 4.6 - Microscopic features of *Acanthamoeba* cyst in corneal scraping – Gram stain (a) and calcofluor-white (b). Note hexagonal inner wall of the cysts (Reproduced from Garg *et al.*, 2017).[80]

PCR has a sensibility of 85%,[16] specificity of 94-100% and sensitivity of 80-84%.[15,76] This molecular method consists in the identification of microorganisms through the gene sequence of 18rRNA gene collected from corneal scrapping.[1,16,106] This technique uses "oligonucleotide primers to an *Acanthamoeba*-specific target sequence"[27] but doesn't allow to distinguish living beings from the dead ones making it difficult to realize if the cornea is healthy or the infection is still active.[1] It allows the identification of *Acanthamoeba* strains[28] and others genotypic information.[50]

Garg *et al*. referred a new method of loop-mediated isothermal amplification where the DNA sequence is amplified under isothermal conditions in a quickly and efficient way. When compared with PCR technique this method takes two or three hours less.[80]

Confocal microscopy has a specificity and sensitivity of 90%.[15,76] It's rapid, non-invasive[18] and in-vivo method[36] using the scanning confocal microscope through the visualization of all layers of the cornea for identification of trophozoites and double wall cysts of *Acanthamoeba.*[17] Cysts presents as hyperreflective, round or oval form and diameter of 15 to 30 μm.[15] Sometimes cysts are confused with macrophages, neutrophils and immune cells[1,15] and trophozoites with keratocyte, leukocytes[47] and inflammatory cells.[80] For this technique is required a cooperative patient.[27] For some authors like Zhong *et al*. this method is considered as the best diagnostic technique due to its characteristics such as the high levels of sensitivity and specificity.[66] With this procedure it's possible to repeat it during the evaluation of infection and the treatment,[29] verifying if the cornea has any sign of infection with living cysts or trophozoites before it's submitted to a corneal transplant, if necessary.[22]

According to Rodríguez-Martin *et al*. the Schirmer test showed a sensitivity of 100% and it can be used to acquire samples from the ocular surface in an aseptic and fast form which allows the study of the pathogens.[140]

To confirm the diagnostic of AK is necessary to be fulfilled with one or more of the following criteria:

- Positive result from a culture of corneal scraping, corneal biopsy or keratoplasty specimen;
- Histologic evidence from corneal biopsy or keratoplasty specimen and detection of cysts and/or trophozoites;
- Clinical manifestation of at least one of the symptoms presented in the chapter 2.6.[14]

4.9 Treatment of AK

Good visual prognosis derives from an early diagnosis and correct treatment,[2] but the risk of recurrence is high.[40] Treatment should act on trophozoites, cysts and inflammation.[141] Its aim is to removal of all pathogens from corneal tissue with therapy with anti-amoeba agents and elimination of inflammatory response by the use of anti-inflammatory drugs.[1,4,80,106]

In the course of diagnosis with epithelial debridement it's thought that in early stage of disease this procedure allow the removal of some of the pathogens from the corneal tissue and helps in the penetration of medication.[1,4,17,34,67,137] Before diagnostic confirmation of AK, the treatment with corticosteroids, antiviral, antibacterial or antifungal may cause some improvement in the condition but is followed by periods of deteoration.[17]

Therapeutic principles depend on the gravity of the disease and stage of infection, the combination of drug therapies for first line of treatment, duration of treatment longer than six months and a surgical option must be placed when the drug therapy isn't effective.[29]

Treatment outcome is mostly related with the initial stage of the disease in the time of diagnosis.[70] When early diagnosis is performed with organisms still limited to the most superficial layers of the cornea there is a probability of minimal scarring.[103] On the contrary, late diagnosis usually leads to risk of corneal transplants and/or permanent blinding.[142] Thus, with the monitorization of DNA copy number the treatment can be conducted or modified accordingly the stage of disease.[143]

The first response to a successful treatment includes a combination therapy which is more effective than a monotherapy[69] because one agent is not enough to act against all genotypes of *Acanthamoeba.*[2,27,47,56,58,82,140] While trophozoites are sensitive to different therapies as antiprotozoals, antiseptics, antibiotics and antifungals, cysts are highly resistant to treatment[1,16,20,80] and responsible for the persistent infection since they can remain in corneal surface for a long period of time.[4,20] There is no single agent that proved to be effective for both cysts and trophozoites.[17]

There is no specific treatment directed to AK but there is evidence that exist effective substances to treat this disease. In cases where treatment is delayed, the progression of the infection through the corneal layers and treatment can be continued for periods of 1 year or more.[17] The effectiveness of treatment also depends on the *Acanthamoeba* strain,[16,48] since they present

different drug susceptibilities during the different growing phases,[29] and virulence of the strain,[20,144] health of corneal tissue, degree of the disease and the experience of eye health professional.[58] Due to the factors mentioned before is difficult to create a link between in vivo and in vitro efficacy.[18,47]

Recommended effective topical treatments for AK comprehend the use of aromatic diamidines, biguanides, aminoglycosides and imidazoles. Successful therapy englobe the use of biguanides together with diamidines,[56,69,74,84,106,140] although the biguanides can be used without the use of diamidines.[15] This comprehends multiple agents as polyhexamethylene biguanide (PHMB), chlorhexidine, propamidine isethionate, dibromopropamidine and hexamidine.[129]

- Biguanides
  - First choice in clinical treatment of AK.[29]
  - These cationic surfactants interact with phospholipids on the cytoplasmic membrane, damaging both respiratory enzymes and cell components with modification of configuration and membrane fluidity.[4,106,145] After, the interaction with the biguanides, there is a contraction of cyst's contents and swollen of cyst wall.
  - They are often combined with aromatic diamidines to enhance treatment of AK.[140]
  - The most used biguanides are:
    - 0.02%-0.06% PHMB[1–4,15,16,27,34,36,47,54,56,67,99,118,140,146] – effective in low concentrations against both trophozoites and cysts[105] of different strains of *Acanthamoeba* however causes toxic reactions in corneal cells[18,47,107,129] when combined with other substances.[17]
    - 0.02%-0.2% chlorhexidine[1,2,4,15,16,27,36,54,56,67,140] - effective against both trophozoites and cysts and in low concentrations doesn't cause toxic reactions in corneal cells.[18,47]
  - Use of biguanides with concentrations above 0.02% are used in cases of resistance to treatment.[15] In this cases, PHMB and chlorhexidine concentrations may be increased to 0.06% and 0.2%, respectively.[4,29]
- Diamidines
  - The cationic surface effect produced by the bipolar molecule in diamidines can modify cell membrane structure and increased permeability,[106] leading to the escape of ions and biomolecules from the interior of the cell and, consequently,

the cell death.[29] After penetrate into the cytoplasm they cause denaturation and coagulation of cytoplasmatic enzymes and structural proteins, acting effectively against cysts and trophozoites,[4] by interfering with the synthesis of nucleic acids.[29]

- Present an additional anti-amoebic effect[140] and effective cysticidal effect often used in combination with chlorhexidine.[4,15]
- This kind of treatment is tolerated by ocular tissue but the extension of treatment can lead to toxic corneal reactions.[4,15]
- The most used diamidines are:
    - 0.1% propamidine isethionate[1,2,56,118,4,15,17,18,27,34,36,47] – active substance against trophozoites of *Acanthamoeba* but not suitable for prolonged used due to the possibility of causing signs of corneal toxicity.
    - 0.15% dibromopropamidine[17,18,29,47]
    - 0.1% hexamidine[1,2,4,15,47,56,67] – has been proven to present a faster effect against both forms of *Acanthamoeba*[4]
    - Pentamidine isethionate[29]
    - Diminazene aceturate[29]
    - Hydroxystilbamidine isethionate[29]
- Combination of 0.1% propamidine isethionate and 0.1% hexamidine proved clinically effective against both forms of *Acanthamoeba* even though hexamidine present a faster amoebicidal response when compared with propamidine isethionate.[15]
- According to Marciano-Cabral *et al*. combination of chlorhexidine and propamidine isethionate is rapid and efficient in AK infection.[17,36]
- Diamidines can't work as a monotherapy, always have to be combined with other kind of treatment.[15]

- Triazoles – has successful results when combined with biguanides and diamidines, in cases with resistance to therapy and limiting the contamination of scleral tissue from corneal tissue. Voriconazole appears effective against clinical strains of *Acanthamoeba*[140] and also proved effective in both topical and systemic application.[18] It's sometimes used in cases where the patient doesn't respond to the conventional treatment plan.[80]

- Imidazoles
  - Broad-spectrum antifungals.[29]
  - Should never be used alone, usually combined with other therapies[147] like biguanides.[29]
  - This compound influenced the permeability of the cell membrane and leads to death of the microorganisms, but the concentration used in medical treatment isn't enough to kill them, they only inhibit their activity.[29]
  - The most used imidazoles are:
    - Miconazole[18,20,29]
    - Ketoconazole[29]
    - Clotrimazole[29] – only effective against cysts[34]
    - Fluconazole[29] – used during keratoplasty to irrigate the anterior chamber impeding the contamination with ameba in the chamber.[66]
- Aminoglucosides
  - Main capacity of modifying the bacterial membrane permeability and inhibiting of the synthesis of bacterial protein.[29]
  - Clinically used in combination with biguanides.[29]
  - The most used imidazole in AK is neomycin, with a concentration of 0.5% in clinical use.[29]

Treatment is usually applied every hour during the first 48 to 72 hours followed by an hourly application only during daytime[80] with a minimum recommended of nine applications per day, from 3 weeks to many months.[54] This decrease in treatment dose is due epithelial toxicity derived from the excessive application of drugs.[1,4,45]

Guidelines suggested by Holland *et al.* (1987) in an example of treatment guide involves: 1 drop of propamidine isethionate every hour, miconazole and solution of neomycin-polymyxin-gramicidin every two hours when the patient is awake and propamidine isethionate before bed.[137]

Most ocular antibiotics aren't effective in this pathogens.[20] As refer by some authors, it was successfully used neomycin-polymyxin,[16,18] miconazole and propamidine isethionate.[18,20] Siddiqui *et al.* also recommended the use of chloramphenicol.[2] When AK is associated with bacterial infection is also necessary the use of antibiotics as neomycin or chloramphenicol.[2] Agents as clotrimazole and neomycin are only effective against trophozoites not cysts.[34]

The use of corticosteroids is controversy because of the possible aggravation of the infection[17], its associated with the increase of intraocular pressure,[1,16] the suppression of the immune activity present in the initial stage of infection,[16,18] leading to worse prognosis.[66,71] They are normally applied in situations of persistent inflammation[47] or stromal inflammation.[16] It has been discussed the benefits of corticosteroids to reduce vascularization, scarring, vision maintenance,[1] scleritis and corneal inflammation.[45,66,67,74] According to laboratory tests, dexamethasone amplify the pathogenicity of trophozoites and cysts, and also contribute to the excystment of cysts into trophozoites.[1,41,80]

As reported in the work of Sun *et al*. referring other works the use of glucocorticoids combined with amebicide therapy is safe for clinical use, only after the condition has improved with amebicide drugs for 1 to 2 weeks. Glucocorticoids can prevent the conversion process from trophozoites to cysts, increasing the effectiveness of treatment with the amebicide drugs and enhancing the probability of damage in corneal tissues.[29]

In the event of scleritis could be necessary oral corticosteroids and immunosuppressive therapy,[16] where sometimes cyclosporine is used.[1]

Severe infections can lead to permanent epithelial defect that can be resolved by amniotic membranes together with keratectomy.[16] The amniotic membranes transplantation after the removal of corneal lesions are capable of lower the quantity of pathogens and decrease inflammation on corneal tissues.[29]

In advance stages cases of corneal perforation, scarring or when both oral and topical treatment fails, corneal transplant is required (Figure 4.7).[16,18,29,47,67,80] This procedure allows visual rehabilitation and sometimes it's used to clean the cornea from pathogens although in this case it isn't highly successful.[16] When *Acanthamoeba* cysts or trophozoites aren't completely removed from corneal tissue before transplant it's possible that there occur a colonization of the new cornea.[18] Most of graft failure after penetrating keratoplasty (PK) appears to be caused by repeated AK infection.[14] In cases where the patient presents neovascularization PK isn't recommended[97] because of the high risk of rejection.[1] After surgical procedures medical treatment must be continued for months to ensure that there is no residual organisms in corneal tissue.[47]

Keratoplasty must be performed with 1mm larger than the lesions and it's also important to use a solution of 0.2% fluconazole to irrigate the anterior chamber. When the lesion caused by AK is too extensive to be treated in a conventional keratoplasty, a corneoscleral transplant is necessary.[66]

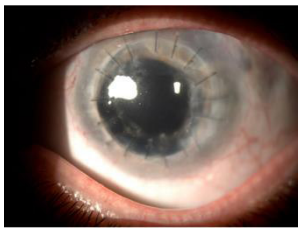


Figure 4.7 - Final outcome after penetrating keratoplasty. Diffuse observation at 12× magnification (Reproduced from Arance-Gil *et al.*, 2014).[22]

Deep Anterior Lamellar Keratoplasty (DALK) is technically challenging. Should prevent amoebas from entering the anterior chamber during surgery and maintain the endothelial cell functions[47] in patients with affectation of visual axis. The successful of this treatment prevent the risk of rejection like in PK procedure. Risk of DALK include the permanence of amoebae and the perforation of Descemet membrane with possible invasion of the anterior chamber.[14]

Alternative surgical treatments as cross-linking, cryotherapy and photorefractive surgery showed some efficacy in vitro.[1] Crosslinking start being used for a possible adjuvant treatment of AK by reinforcing the resistance to *Acanthamoeba* enzymes with the use of collagen, although still controversial.[15] The combination of riboflavin (R) with ultraviolet-A radiation (UVA) should inactivate isolates of virus and bacteria but the standard dose is not enough to eradicate cysts or trophozoites of *Acanthamoeba*.[148] The success of this treatment is conditioned by the depth to which the microorganisms are, since when they are located at depths greater that 300μm the treatment is unsuccessful.[22] Photorefractive surgery was used to remove a large corneal stromal abscess with laser phototherapeutic keratectomy.[4] Both this procedures can present downsides as corneal melting in crosslinking and endothelial failure in cryotherapy.[1]

As a last option, in cases of scleritis associated with pain is sometimes necessary to resort to enucleation. This kind of treatment was more used in the past and nowadays isn't so common due to immunosuppressive therapies.[16] Enucleation can also be considered in cases that medical and surgical treatments aren't effective.[17]

AK presents a higher duration when compared with bacterial keratitis with a resolution time between four and six months.[16] In some severe cases treatment can last up to three years.[1] The extend duration and relapses are mainly linked to the difficulty on removing cysts from the corneal surface[1,4,16] so monitoring is essential to avoid recurrence.[17,27] Prolonging treatment time may lead to toxic reactions in corneal cells that can result in keratophaty[31] and follow-up is indispensable.

Lack of success of some treatments is frequent and mainly due to resistance to drugs, difficult in agent penetration and insufficient treatment duration.[17] The resistance to some agents gets worse in an advance late of infection when trophozoites invade deeper tissues.[17] The capacity of encystment of *Acanthamoeba* trophozoites usually occurs with the start of treatment with different medicine and prolongs disease duration[35] by reemergence of trophozoites after the end of treatment.[50]

Treatments sometimes result in complications as secondary glaucoma which is difficult to control without surgery.[141]

After full recovery of this disease, when the patient wishes to wear CL, daily disposable CL are recommended even though there are also cases of infection in these types of lenses. In cases of corneal irregularity, it's more suitable to fit rigid gas permeable CL. When this solution alone can improve visual performance it isn't necessary resort to keratoplasty.[16]

4.10Visual Prognosis

As previously reported AK is a severe disease that reflects in serious visual complications when it's not diagnostic early and treated effectively.[4,15,47,66,140] The possibility of vision recovering is lost a little every week when the diagnosis is delayed.[128] Early diagnosis and increased aware of vision care professionals are a good combination for a better results.[32,55,126,149]

Visual prognosis is affected by the corneal positioning of the inflammation, age, time of diagnosis,[16] severity of disease in time of diagnosis and the time between start of symptoms and the effective therapies.[4,15] When corneal inflammation is located in a central position the patient may suffer from stereopsis loss[16] with difficult on depth perception.[1] Advance age is also associated with poor results.[1] After three or more weeks of infection without a correct diagnostic

and onset of treatment, the prognosis gets worse.[4,15] The severity of disease can be evaluated by duration of the disease, quantity of visual loss and cost .[117]

In 25% of the cases patients suffers with vision loss and need queratoplasty.[16] The option of queratoplasty shouldn't be considered when visual capacity is improved with the correct adaption of RGP CL, which decreases the effect of scarring, corneal irregularity and irregular astigmatism.[1]

Diagnosis time is crucial for the visual acuity outcome. Therefore, when the diagnosis was performed within one month after the onset of symptoms the patients were able to reach visual acuities of 20/40 or more. When this time limit is exceeded, the best visual acuity was not more than 20/40.[100]

A reduction in visual capacity affects directly the patient life, with a decrease in the quality of life, productivity and the possibility of progression in career.[97]

In extreme cases AK can cause total blindness of the infected eye[17,40,122] and enucleation becomes an option.[22,27]

5. *Acanthamoeba* and Contact Lenses

5.1 Effect of Contact Lenses in Eye Structures

The first polymer used in hydrophilic CL was used in 1960 in Czechoslovakia. Before this period, polymethymethacrylate (PMMA) lenses were the available CL. The new CL brought a way of comfortable vision correction with a superior diameter and flexibility than the anterior lenses.[27]

This approach for visual correction is the method of election for millions of users all over the world.[27] CL are capable to contribute to safe and effective visual correction[89] if care properly[68] but in case of wrong use can interfere in the tear film spread,[23] the natural defenses of the eye and allows microbial survival and contamination.[58]

Use of CL can produce minor corneal abrasions, damaging the integrity of corneal epithelium and affecting the strong barrier that the tight junctions create against invasion of microorganisms.[72] Other than the direct contact, CL can produce indirect damage through the hypoxia and hypercapnia that they cause in corneal cells. Factors like this are exacerbated with low oxygen transmissibility CL and risk behaviors as sleeping, which are in part associated with absence of blinking. The decrease of oxygen in corneal cells leads to an accumulation of carbon dioxide and significant changes in normal metabolic pathways.[72]

This break in the protective barrier is essential to the adherence of protozoan to the epithelium and continued invasion of the remaining corneal layers. Without this breach the infection may not developed to an AK.[72]

Other than the traumatic or metabolic effect, CL can serve as a vehicle for the transmission of *Acanthamoeba* to the eye.[41,72,150] Some risk behaviors like poor cleaning habits, showering, swimming and wrong storage procedures increase the risk of contamination of CL leading to an increase of *Acanthamoeba* infection.[21] This risk behaviors like water raising CL also increase the contamination in lenses and lens cases with species like Pseudomonas spp., Gram-negative bacteria, between others.[21]

CL also influence eye dryness by destabilizing the lipid layer which leads to a worse spread of the tear film which leads to a reduction on the lipid layer in the pre-lens tear film and, consequently, the intensification of evaporation of the tear film.[23] This disturbance caused by CL

leads to reduced CL wearing time and visual acuity and increased risk of infection, bacterial adhesion and desiccation of ocular surface.[23]

5.2 Contact Lens Types

CL and respective disinfection systems are both considered as medical devices and, in the United States, are regulated by Food and Drug Administration (FDA).[151]

Soft lenses wearers are more likely to be contaminated with *Acanthamoeba*[8,12,15,93] and lead to an increase of AK.[1,64] When compared with rigid lenses, the risk of contracting AK is higher for soft lenses wearers.[5,15]

Silicone-hydrogel (Si-Hi) contact lenses were introduced to the market in the late 1990s.[58] It's possible to divide silicone-hydrogel lenses in different generations, based on lens characteristics. In the first-generation of silicone hydrogel CL, there was an increase of transmissibility through the introduction of silicone[58] in the materials of conventional hydrogel CL.[150] CL of second-generation presents a decrease in hydrophobicity with the incorporation of an internal wetting agent. Third-generation CL present an increase in wettability with the introduction of a surface treatment with gas plasma.[73,150] This enhance of transmissibility was thought to extinguish signs of hypoxia and consequently lead to a decrease in structural, physiologic and pathophysiologic corneal changes.[152]

Daily disposable CL don't require a disinfection process therefore they present a lower risk of infection by *Acanthamoeba* and less severe infection.[16,117] In this lenses there is no exposure to some risk factors like poor disinfection habits and contact with contaminated storage cases[79] and solutions.[3]

Rigid CL are less linked to AK appearance although there are references to some clinical cases connecting rigid lenses to AK.[72] RGP lenses are usually used in cases of high or irregular astigmatism or for orthokeratology treatments.[75]

Scleral CL are usually used in the correction of high or irregular astigmatism, in cases of refractive surgery complications, keratoconus, keratoglobus, pellucid marginal degeneration,

between others. This kind of lenses with large diameter is firm on the sclera, without corneal touch, and a gap between the lens and the cornea that is filled with saline solution.[118]

Orthokeratology lenses acts as a non-surgical treatment for the reduction of myopia progression and cases of AK have been reported.[153] Theses lenses are used overnight[22] and cause the modification of the central cornea, with reverse geometry causing corneal flattening,[22,78] with possible hypoxia during using hours.[75] The nightly use of CL it's one of the risk factors.[15]

Cosmetic soft lenses are mostly used at young ages, mainly in women, for modification of eye color or appearance.[110,122] They are associated with lack of maintenance and hygiene habits. As these types of lenses are easily bought without prescription and proper education, they present an increased risk for AK.[58,154]

5.3 Adhesion of *Acanthamoeba* to Contact Lenses

Acanthamoeba adhesion to CL is one of the essential first steps in pathogenesis of AK.[73] The adhesion of protozoa can occur in all types of CL[57,64,122,136] and depend on many factors like CL material,[94] form and concentration of *Acanthamoeba* species, surface tension,[27] water content, ionicity and material of CL[8] and exposure time to the protozoa.[72]

CL material is directly related with the capacity of *Acanthamoeba* adhesion[94,116] and posterior transference to the corneal surface.[57,72] *Acanthamoeba* adhere to both soft and rigid lenses.[34,58] Comparing the rate of adhesion between trophozoites and cysts it was noted that trophozoites can adhere more to CL materials[36,63,72]. According to Ibrahim *et al*. and Chynn *et al.* there is a higher adherence of cysts in soft lenses when compared with the rigid ones,[72,74,100] this reduced adherence is due to the smoother surface and easier removal in RGP lenses.[146] The adherence of trophozoites was noted with high affinity with the silicone acrylate of rigid lenses and PMMA lenses.[72,146] Adherence of *Acanthamoeba* trophozoites in RGP lenses depend on their surface.[146] Within soft lenses, trophozoites adhere more to new silicone hydrogel rather than the conventional hydrogel lenses.[8,64,72,73,150] This difference in attachment preference it's related with the silicon type.[72] Between generations of silicone-hydrogel CL, first-generation lenses had a high affinity with *Acanthamoeba* species[150] when compared with second and third-generations.[73] In cosmetic lenses was verified a higher adherence in the colored area when compared with the non-colored

area, due to higher roughness induced by the colored areas with pigments.[155] The difference in roughness between both areas can be observed in this SEM image of a cosmetic lens (Figure 5.1).[154]

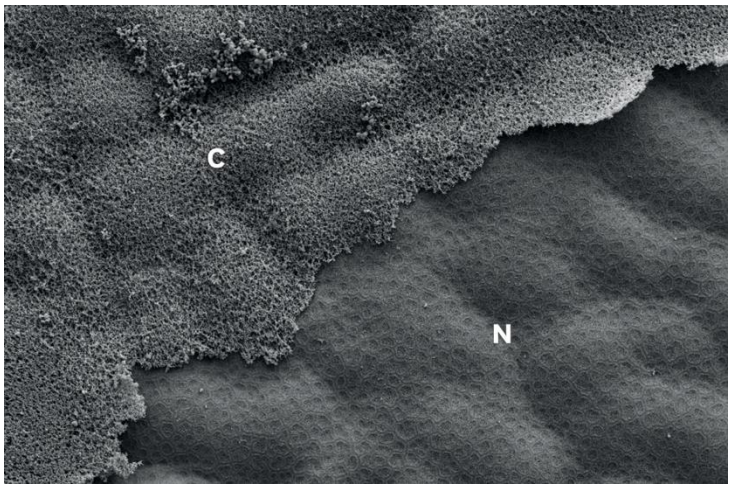


Figure 5.1 - SEM image from a cosmetic lens surface. C represents colored areas and N represents non-colored areas of contact lenses (Original magnification x 1000) (Reproduced from Lee *et al.*, 2018).[154]

CL water content and ionicity interferes with the adhesion of cysts and trophozoites depending on FDA hydrogel lens groups.[36,58,72] In disposable lenses there is a larger variety of non-ionic and high-water content lenses when compared with ionic and low-water content lenses.[34,36,58,72,73,94,133,150] Lenses with higher water content provide a comfortable environment for patients leading to an increased wear time and consequently an increase in exposure time to *Acanthamoeba*. This reason can be one of the explanations for the appearance of AK in daily disposable soft CL.[72] The relationship between the extent of adhesion and water content/ionicity it's not proportional.[94]

Bacterial adhesion is highly influenced by the surface roughness so rougher surfaces present more adhesion rate than smoother surfaces.[58] This fact may explain the preference in adhesion to the first generation of silicone-hydrogel CL.[58]

Biofilm formation in CL and storage cases enhance resistance for disinfectants, higher CL adhesion,[47] feeding, development, survive and adhesion of *Acanthamoeba*.[72,93]

Surface treatment with plasma in silicone hydrogel lenses to increase wettability causes irregularities in CL surface that may increase the capacity of *Acanthamoeba* to bind to the surface.[73,146] According to López-Alemany *et al*. this kind of treatment creates an irregular surface that leads to high rates of adhesion.[156]l

Replacement schedule has a direct implication on the adhesion of microorganisms to the lens, largely because of biofilm formation and deposits accumulation.[64] Protein deposits enhance the likelihood for *Acanthamoeba* adherence[33] as other organisms like *Pseudomonas aeruginosa* provide food for *Acanthamoeba*. Worn CL with repeated use presented a higher quantity of Acanthamoeba organisms than unworn lenses,[72,73,94] because of the rougher surface of used lenses that enhance the presence of biofilm.[24]

Exposure of CL to contaminated environments, even minimal, increases the risk of contracting AK.[146] In only seconds of exposure in contaminated solutions or environments, trophozoites show immediately adherence to CL[92,111] but the numbers tend to increase with increase trophozoite concentration and exposure time.[21,58,72] Even when unworn hydrogel CL are exposed to contaminated environments for a short period of time, also allows adhesion of *Acanthamoeba*.[58,111]

Adhesion mechanisms to CL may occur through ventral surface of the ameba. In trophozoites, this contact is established by filopodia, formed by thin, long and steady pseudopods located on the base of the ameba, that creates focal places of adhesion.[58,92] This stage is denominated “associated contact”. This *Acanthamoeba* adhesion mechanism differs from bacterial mechanism of adhesion which occurs by biofilm, piluslike structures, bacterial polysaccharide layer or bacterial pili.[92] In cysts doesn’t exist the formation of filopodia and the adhesion can occur through adhesive substances in the exocyst.[33]

5.4 Disinfection methods of CL

AK can have an association with all types of disinfection systems because most of them are ineffective against *Acanthamoeba.*[13,72] This fact is due to the difficulty in establishing a standard methodologies effective in both trophozoites and cysts.[13] Currently, no efficacy against *Acanthamoeba* is required by the International Organization for Standardization (ISO)[33] and FDA,[10,34,157,158] for the majority of disinfecting solutions during the process of fabrication because it’s

considered as a rare cause of infection.[18,38] Brocious *et al*. refer that in the standardized methodology tests must be considered the encystment techniques, strain type, grown method and life-cycle changes. Other than that, this standard method would be helpful in determine specific criteria for disinfection evaluation.[38]

CL care solutions must also be efficient against bacterial population since they can enhance the development of amoebic growth.[18] It's important that the solution maintain its stability after opened during a limited certain period of time.[159] The expected effect is affected by temperature of the solution,[149,159] time of exposure, *Acanthamoeba* strain and concentration.[36,94] MPS system present more disinfection efficacy in trophozoites when compared with cysts.[37] This higher resistance of cysts its related with the difficulty of disinfectants for penetrating thick and old walls,[43] and varies if the cyst are mature or immature,[10,24] once mature cysts are less sensitive to disinfection than immature cyst.[24]

Most common solutions used to disinfect CL are hydrogen peroxide systems and MPS,[95] due to the convenience.[28,107,129]

MPS is recommended in about 90% of all disinfection systems prescribed in soft CL.[160] This single solution is composed by a complex system[42] used in cleaning, disinfecting and rewetting the CL.[28,160] Soft CL MPS consists of several components as an aqueous liquid medium, antimicrobial and preservative, surfactant, chelating, wetting, buffering,[42] tonicity and viscosity-inducing[73,107,154] conferring stability and efficacy in the disinfection process.[42] These solutions are used to clean CL and respective cases through wiping, rinsing and rubbing procedures which causes cyst and trophozoites displace and decrease the bacterial amount associated.[72] MPS efficacy varied between strains and solutions,[42] with limited efficacy in *Acanthamoeba* trophozoites, and specially, cysts.[57] MPS with a combination of chlorhexidine and PHMB presented reduced trophozoite adherence in the surface.[146]

Peroxide based solutions (six hours) can be used as an effective disinfection system for AK which allows the destruction of trophozoites and cysts[34,59] through oxidation.[28,150] One-step peroxide system use a catalyst as a neutralizing,[34,59] and the amoebic action could be enhance if the neutralization phase was delayed.[24,95] The author also affirmed that one-step peroxide showed less efficacy in some strains as *Acanthamoeba castellanii*, *Acanthamoeba polyphaga*, *Acanthamoeba lenticulate* and *Acanthamoeba hatchetti*.[95] Two-steps peroxide system is preferred by Grey *et al*.

once this system allows a chemical neutralizing for a suitable time forcing the exposure to the peroxide.[59] Hiti *et al.* also argue that two-steps cleaning solutions are more effective against cysts than one-step solutions.[161] The exposure time of *Acanthamoeba* organisms to the active solution components is directly proportional to the disinfection capacity of hydrogen peroxide.[95]

Components of disinfecting solutions with active role in *Acanthamoeba*:

- Chlorhexidine – effective in the destruction of trophozoites and cysts of *Acanthamoeba castellanii* and in the development delay of both trophozoites and cysts of *Acanthamoeba polyphaga*.[162]
- Chloroquine – According to SEM evaluation, chloroquine causes more modifications in cell morphology than 3-methyladenine.[107]
- Ethylenediaminetetraacetic acid (EDTA) – chelating agent without effect in destroying *Acanthamoeba* trophozoites.[125]
- Hydrogen Peroxide – shows different efficacy between strains, that is some cysts are destroyed after four hours of disinfection and other strains are still active after eight hours. When combined with other stabilizing substances the result may be different.[43]
- Myristamidopropyl dimethylamine (MAPD) – present in MPS causes a decrease in adhesion of organisms in cosmetic and conventional lenses.[154]
- Polyquaternium-1 – disinfection base that doesn't act on *Acanthamoeba* and fungi but is effective in bacteria as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Serratia marcescens*.[37] Polyquad present viable cysts in all strains even after seven days of exposure.[43]
- PHMB – when used in high concentrations are effective with *Acanthamoeba*.[43] If the concentration of PHMB is low, the amoebicidal efficacy is directly affected.[163] As it's an antiseptic cationic agent it's able to affect both cysts and trophozoites of Acanthamoeba through the inhibition of the cell function.[164]
- Polyaminopropyl Biguanide (PAPB) – is completely ineffective in *Acanthamoeba* cysts even after seven days of exposure.[43]
- Propamidine isethionate – present efficacy on the destruction of trophozoites and, to some extent, the cyst.[9]

- Propylene glycol – hydrophilic organic compound that acts on patients comfort with wetting, demulcent and osmoregulatory components, which might induce encystment[37,125] through the combination of nutrient deprival and higher osmolarity.[37]
- Chlorine increases the risk of AK[44] once it's ineffective on cysts so it's not safe to use chlorine release systems.[1,123] Cysts can survive up to ten times the amount of chlorine used in chlorine based disinfection systems.[44,105]
- Combination of thimerosal and chlorhexidine is effective in both cysts and trophozoites but its associated with reactions of hypersensitivity and toxicity because of the preservatives.[34]
- The majority of surfactants present in MPS like propylene glycol, poloxamer and poloxamine, are non-ionic substances that should not interfere in the PHMB activity but can also induce encystment and the formation of biofilm.[10]
- Elevated concentrations of chloroquine, autophagy inhibitors and 3-methyladenine reduce adhesion of *Acanthamoeba* in silicone-hydrogel lenses.[107]

Some compounds of solutions can encourage encystment or induce adhesion and viability.[94] Substances like dimethyl sulfoxide (DMSO)[99] and propylene glycol present in the solution Complete MoisturePlus (AMO, Santa Ana, CA) allow *Acanthamoeba* encystment leading to an increase resistance to disinfection.[1,14,37,119] The multipurpose solution Opti-Free presents a capacity for decreasing the adherence in soft CL, only when the procedures of cleaning, rising and soaking are followed according manufactures guides.[72] RGP lenses disinfected with Boston Simplus showed decreased adherence of trophozoites to the lens surface.[146]

Unlike soft lens disinfection solutions, Por *et al.* refer that cleaning solutions for RGP lenses can kill about 10^4 cysts in eight hours of disinfection.[14] Daily cleaner for RGP lenses works in the deposits removal using a combination of abrasive components and a high concentration of surfactants.[120] Most popular solutions for RGP lenses are based on PHMB or hydrogen peroxide, combined or not with chlorhexidine.[159]

Saline solution is sometimes used by patients as a method of disinfection but its use may enhance the risk of AK as it has no disinfectant activity.[58,68]

As state by Lindsay *et al.* and Ibrahim *et al.* the solution may serve as indirect source of protection if the disinfection solution is effective against the bacterial population, that it will directly affect the main food source of *Acanthamoeba*.[25,34]

It's important that patients follow the cleaning procedures as recommended by the manufacturers in detriment of causing corneal cell damage through the adhesion of the ameba.[18] As state in several articles, a good wash with rubbing moves promote the detachment of deposits,[160] cysts and trophozoites from CL surface[72] and improve the disinfectant efficacy.[160] Although the majority of articles state that CL should not be placed in contact with water, manufacturers of RPG cleaning systems still recommend water in the rising phase.[75] Even when all the manufactures rules are followed, there is no guarantee to work if the solution isn't effective in killing *Acanthamoeba* cysts and/or trophozoites.[43,72]

Unfortunately, there are still some solutions where the instructions suggest using running water to clean RPG lenses contrary to what is recommended by FDA guidelines, which may lead to patient confusion.[165]

Procedures using microwave irradiation is thought to be easy, convenient, effective, rapid, cheap, reliable in removing trophozoites and cysts of *A. castellanii, A. comandoni* and *A. hatcheti*[25] from storage cases in only three minutes of treatment,[17] with a capacity of 600W,[83] without damaging the product.[25] This method uses radio waves, with a frequency of 2450 MHz, which when in contact with material with water produces thermal and nonthermal effects. The thermal effect results from molecules vibration which extend kinetic motion and nonthermal effects are the result of relation between molecules and the electromagnetic field created by microwaves.[83]

UV radiation is also mentioned as a possible efficient method for CL and cases disinfection from bacteria, but the necessary level to disinfect can affect lenses properties as mechanical properties, parameters and biocompatibility. UV-induced changes has been resolved by Lonnen *et al.* with a creation of a new device that protects the CL from radiation exposing only the solution, but did not show expected results in *Acanthamoeba* cysts.[42]

Heat disinfection proposed by Lindsay *et al.* was directed for some soft CL but it's not recommended for more recent soft lens materials, rigid lenses[33] and high-water content lenses.[143]

The procedure involves a temperature of 80°C for at least ten minutes and effectively acts on both cysts and trophozoites,[25,34,162] especially in low-water soft CL.[25]

Disinfection is crucial not only for CL but also for the storage case.[30] The storage can be contaminated through contaminated fingers or CL. Topping off is a risk behavior that involves the addition of new solution on top of the old solution which leads to the dilution of active disinfectant concentration facilitating biofilm development and serving as food source for *Acanthamoeba*.[75] To a correct case disinfection must be used a correct cleaning systems, followed by air drying and storage.[30] The drying phase reduces the risk of contamination through partial elimination of organisms.[57]

The ideal goal is to improve the effectiveness of solutions against *Acanthamoeba* trophozoites and cysts,[18] particularly in cysts as they are the most resistant to disinfection.[42,159] When the solution can prevent encystation it will enhance the effect of MPS on *Acanthamoeba*.[129] The solution should also present capacity to impede the adherence of the organisms to the CL surface.[99]

6. Conclusion

AK is a rare but a potential devastating disease that can directly affect the eye. As the majority of events appear on CL wearers it's important to have a proactive behavior regarding care associated with cleaning and handling CL.[4] To avoid the infection and its potential complications it's important to recur to suitable CL care routines, effective sterilization[166] and, when needed, an early diagnosis[18,128] to achieve better outcomes in AK.[1,128]

One important initial step by the eye specialist to reduce the risk of infection is to cautiously choose the CL material and its main characteristics, preferably nonionic and low-water content material.[11,94] When fitting CL the eye care professional must make sure that the lens is correctly fitted and has no defects which may cause corneal lesions.[120] The use of daily disposable CL decreases the possibility of infection once there is no need for cleaning habits and the danger of contamination through the storage case does not exist.[1,72,119] In this cases it's important to emphasize the idea that daily lenses should be worn only once.[3,5]

Patient education to promote correct hygiene behaviors regarding CL is essential to decrease the risk of infection.[13,27,30,31,38,72,128,133,141,167] This includes changes of some behaviors like don't shower or swim with CL on[13,21,119] and avoid overnight wear when possible.[21,54] In orthokeratology patients, where overnight wear is required, following all handling and cleaning recommendations is essential.[75,152] Other than informing patients about the correct behavior they should also be informed about the visual consequences in case of wrong doing,[72] and let them be aware of signs and symptoms of infection.[21]

As referred by some authors certain daily activities where the use of glasses difficult the patient life, like going to the beach and pool, can be improved with the use of swimming goggles while wearing CL, which seems to decrease the risk of contamination.[16,136]

Some risk minimization techniques regarding the disinfection and handling of CL involve:

- Always wash hands before handling CL.[58,59]
- Avoid all contact of CL with tap water[122,136] or handling with wet hands.[57]
- Comply with the wear schedule and replacement as state by the eye specialist.[18,34,168]

- Use of the sterile solutions recommended by the eye specialist to clean and storage CL.[3,13,33]
- Don't leave the disinfecting solution open.[16]
- If the specialist recommends the use of peroxide, it is preferable to use the two-step system to improve efficacy.[59]
- CL should be rinsed and rubbed, for at least fifteen seconds[118], with the disinfectant solution before and after each use.[10,16,36,58,94,118,119,160]
- Cleaning procedures as recommended in manufacturer's guidelines.[18,94]
- Don't rinse CL with tap water.[89,120,134]
- Never use homemade saline.[4,25,59]
- Replace solution daily.[21]
- Don't reuse solutions.[117,119]
- Don't "topping off" solutions.[4,75,117]
- Careful CL remove before water contact, especially in showering and swimming situations.[18]
- Use of eye protection systems to prevent trauma in the cornea.[18]
- Always bring the actual prescription glasses and CL storage cases if the lenses need to be removed.[89]

In addition to CL care it's also important to consider some risk minimization techniques regarding disinfection and handling of CL cases:

- Creation of standardized manufacturer's recommendations for CL storage cases hygiene.[1]
- Avoid all contact of CL cases with tap water[16] or handling with wet hands.[16]
- Rinsed CL cases with sterile saline and never with tap water.[13]
- Must be rubbed,[16] air-dried[13,57] or dried with paper[16] after every use.[13,16]
- When possible, the storage case must be submitted to heat disinfection every month.[59]
- Replace storage cases regularly[33,57,59,95,139], maximum every three months.[13,89,119,136] If this time is reduced is even better, to reduce the possibility of case contamination.[134]

In addition to all previous recommendations, a vital procedure for monitoring CL wear should include a close involvement from the eye care provider with the planning of the recommended visits and advise, if necessary, to recur immediately to him in case of symptoms like blurred vision, eye pain, redness and discomfort.[89]

In cases where the patients don't follow the specialist recommendations, Anger and John advertise for the use of 3% hydrogen peroxide, if possible, with delayed neutralization[13] to decrease the probability of infection.[1] Should also be considered the discontinuation of CL wear if the patient doesn't demonstrate proper hygiene habits.[169]

There is an urgent need to enhance the efficacy of CL disinfection systems against all strains and both stages of *Acanthamoeba* through a creation of standardized methods,[18,24,27,28,56] which have been in continued evaluation by FDA to increase safety in the solutions.[170] One way of improving the efficacy of the disinfection systems can go through inhibiting the encystation of *Acanthamoeba*.[129]

In the near future it is important that some new methods will be developed and implemented related with the efficacy of CL disinfecting solutions, newer and effective diagnostic methods and treatments who can provide better outcomes for the patient.[16]

Some important steps concerning water sources and supplies must include a series of changes in the antimicrobial treatment of municipal water supplies. This changes interfere indirectly in the contamination of the municipal water distribution system with Acanthamoeba specimens affecting those non-compliant patients that don't follow manufacturers guidelines.[13]

The purchase facility of these medical devices without any kind of supervision must also be considered as a concern, once patients must be correctly informed about the use and care procedures with CL, have a first assessment to test what's the CL that is the most suitable for each patient, and the follow-up to decrease the possibility of some complications regarding CL use.[18]

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