



Pitfalls in diagnosis and treatment of infectious keratitis

Vincent Borderie

Centre Hospitalier National d'Ophtalmologie des 15-20, Paris,
France

No conflicts of interest

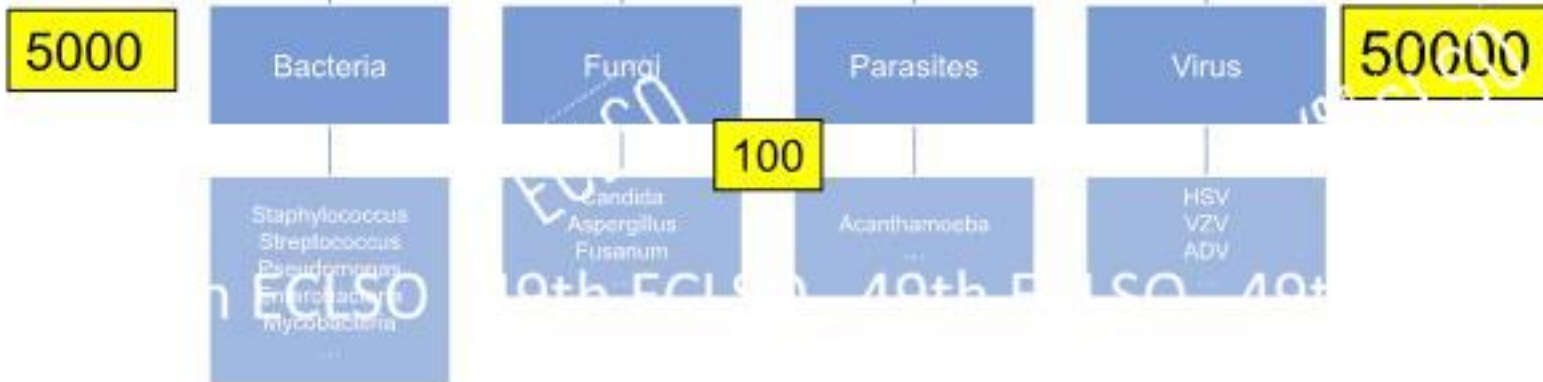
KERATITIS

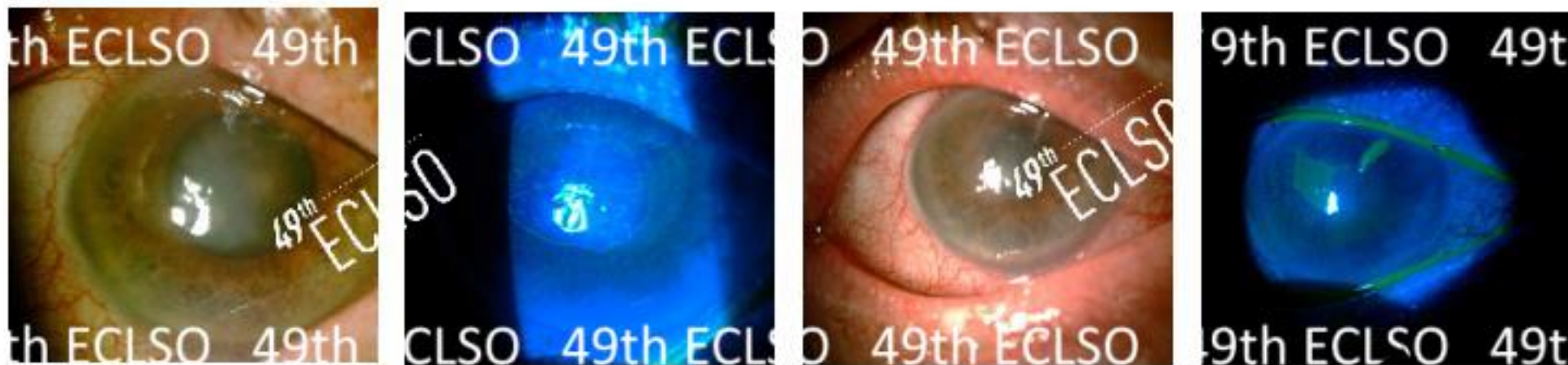
**TRAUMA
TOXIC**



**DRY EYE
ALLERGY
IMMUNE**

INFECTION

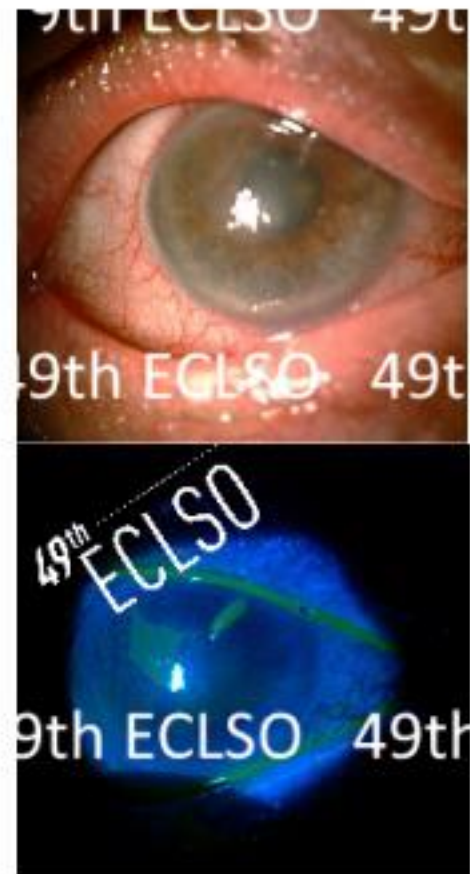




74-y old male patient, unilateral
recurrent disciform keratitis

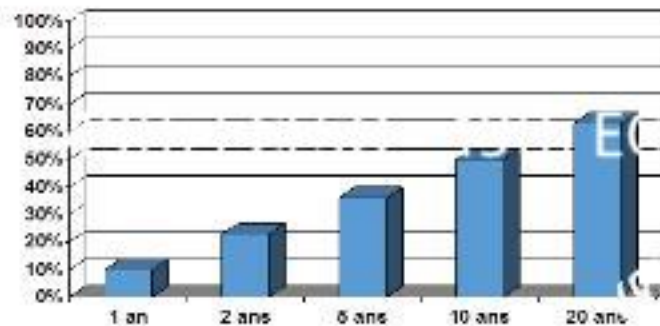
74-y old male patient, unilateral recurrent disciform keratitis

- History of ocular trauma
- 2017: disciform endotheliitis, geographic ulcer
- Visual acuity, 20/200; IOP, 20 mm Hg; CCT, 570 μm
- Treatment: intravenous acyclovir + topical steroids
- Pain relief, no visual improvement
- 2019: central corneal edema, cataract; VA, 20/2000
- Treatment: triple DMEK, valacyclovir
- 2020: no recurrences, clear cornea; VA, 20/20; CCT 457 μm



Epidemiology

HSV



VZV

- Prevalence of herpetic infections:
- Incidence
- Prevalence
- Incidence
- Recurrence rate: 10%

Prevalence of herpetic keratitis: 1.5%

Association of Ant. and Serum With C. Presumptive
 Pflieger M, Boudier M, Laurent-Bachelier M

FIGURE 1. HSV-1 seroprevalence according to patient age in 144 patients with suspected herpes simplex keratitis.

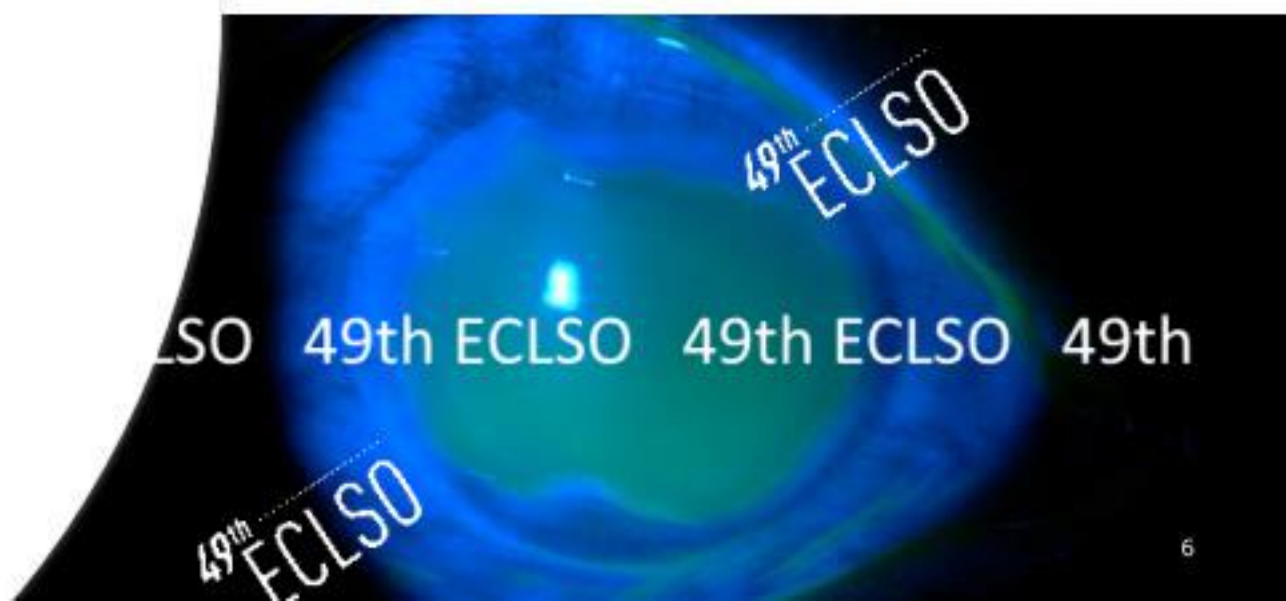
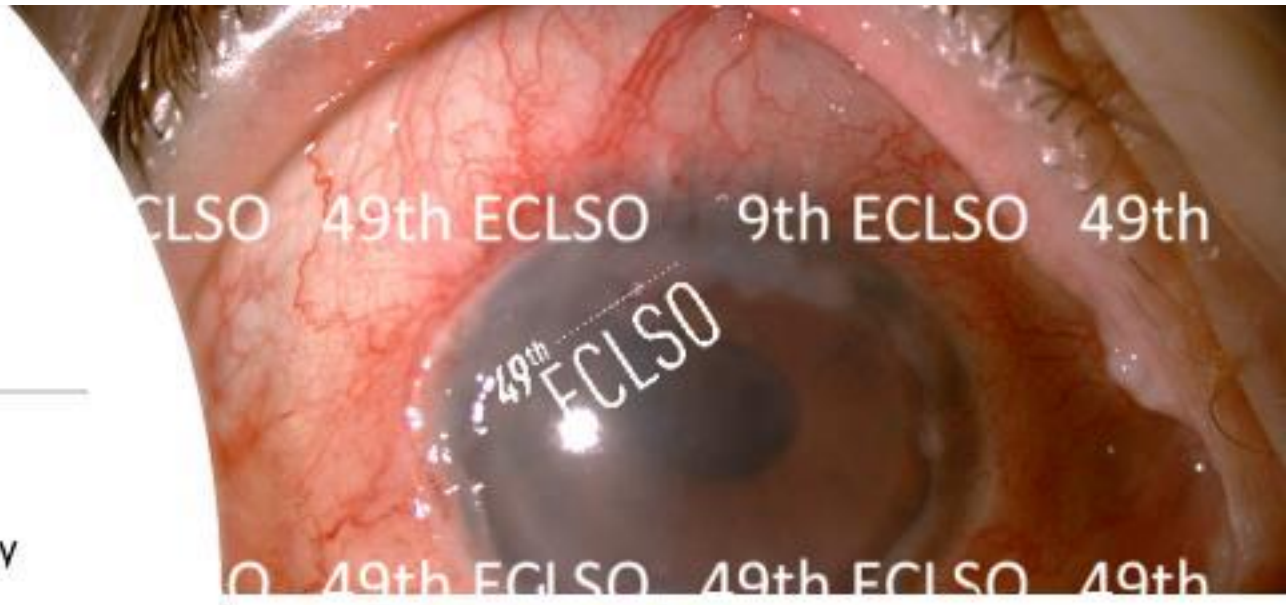
Age	Prevalence (%)
0-20	80%
21-30	85%
31-40	88%
41-50	90%
51-60	92%
61-70	95%
71-80	98%
81-90	100%

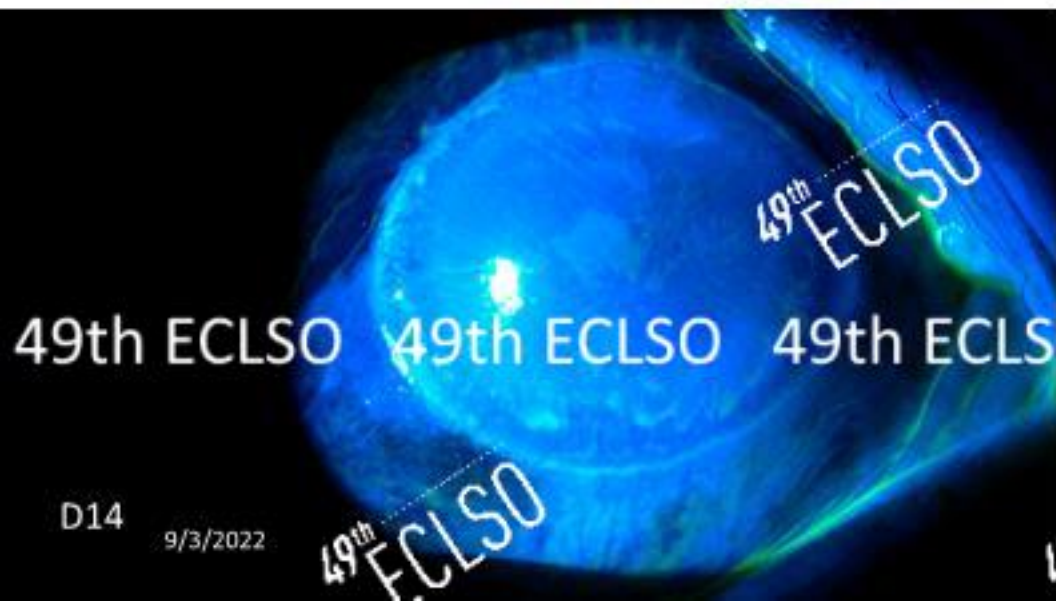
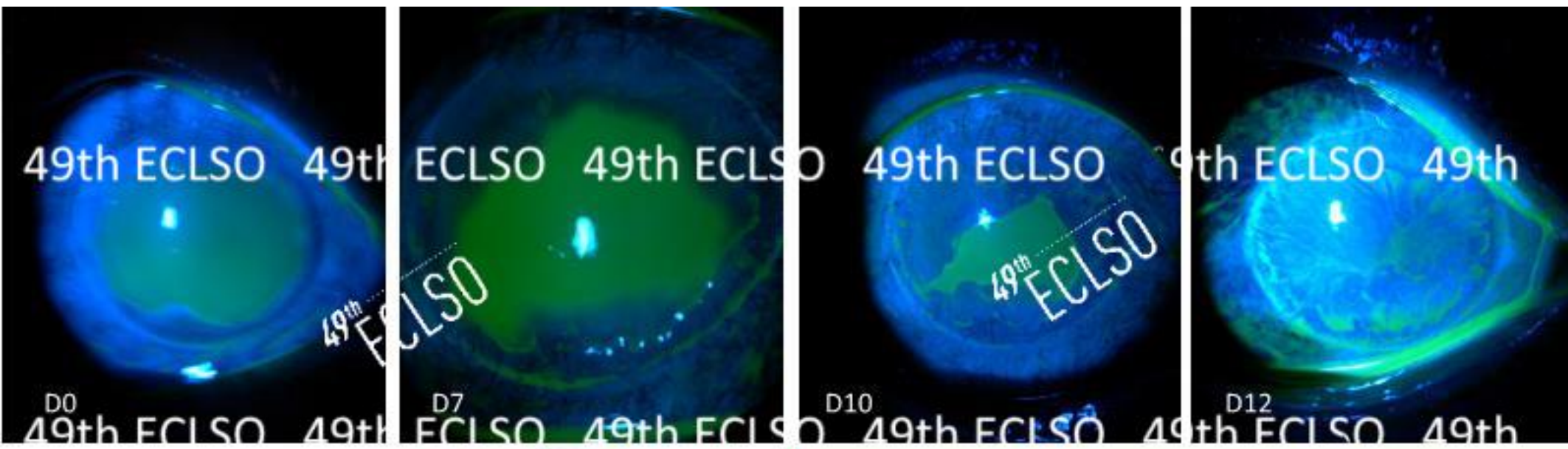
- Prevalence of herpes zoster: 20%
- Incidence increase
- Prevalence of ophthalmicus: 4%
- Rate of trigeminal nerve involvement: 9 to 16%
- Recurrence rate: 20%

Prevalence of herpes zoster ophthalmicus: 4%

83-y old female patient

- 2002: cataract surgery
- 2010: pseudophakic bullous keratopathy
- 2011: DSAEK
- Postoperative hypertonia: topical treatment
- 2012: corneal ulcer
- Diagnosis?





83-y old female patient

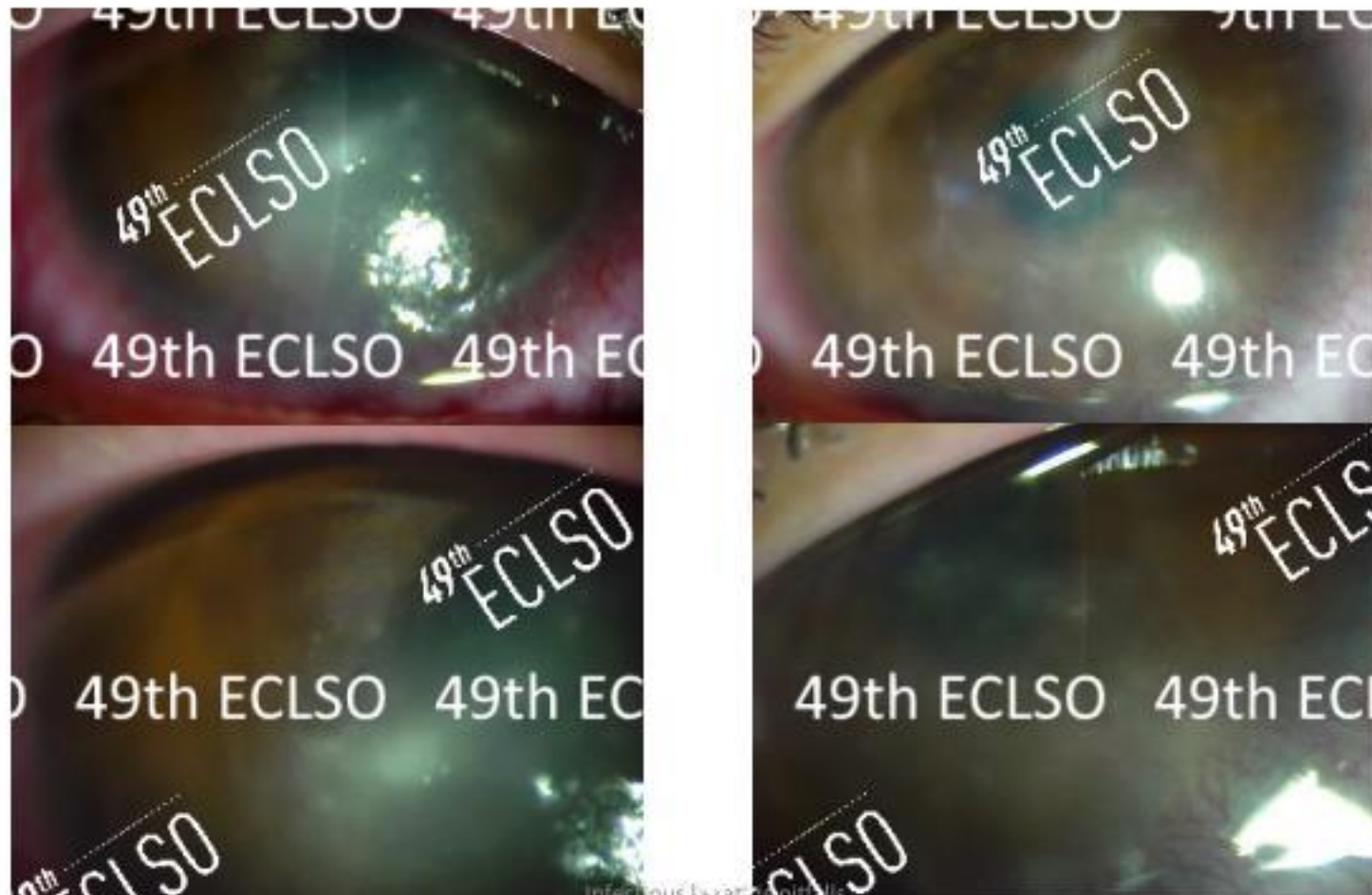
- Corneal scrapping: HSV PCR +
- Valacyclovir per os + topical steroids
- Deep sclerectomy x 2

Based on infectious keratitis pitfalls

Is corneal scraping useful in case of presumptive viral keratitis?

• YES:	• NO:
<ul style="list-style-type: none">- severe clinical presentation- uncertain diagnosis- long-term anti-viral treatment required	<ul style="list-style-type: none">- superficial keratitis- clinical evidence- short-term anti-viral treatment

Bilateral keratitis in a young contact lens wearer
(Renu with moisture lock) treated for HSV keratitis



49th ECLSO 49th ECLSO 49th ECLSO 49th ECLSO
On the scalp in contact with case
PCR + for *Acanthamoeba*

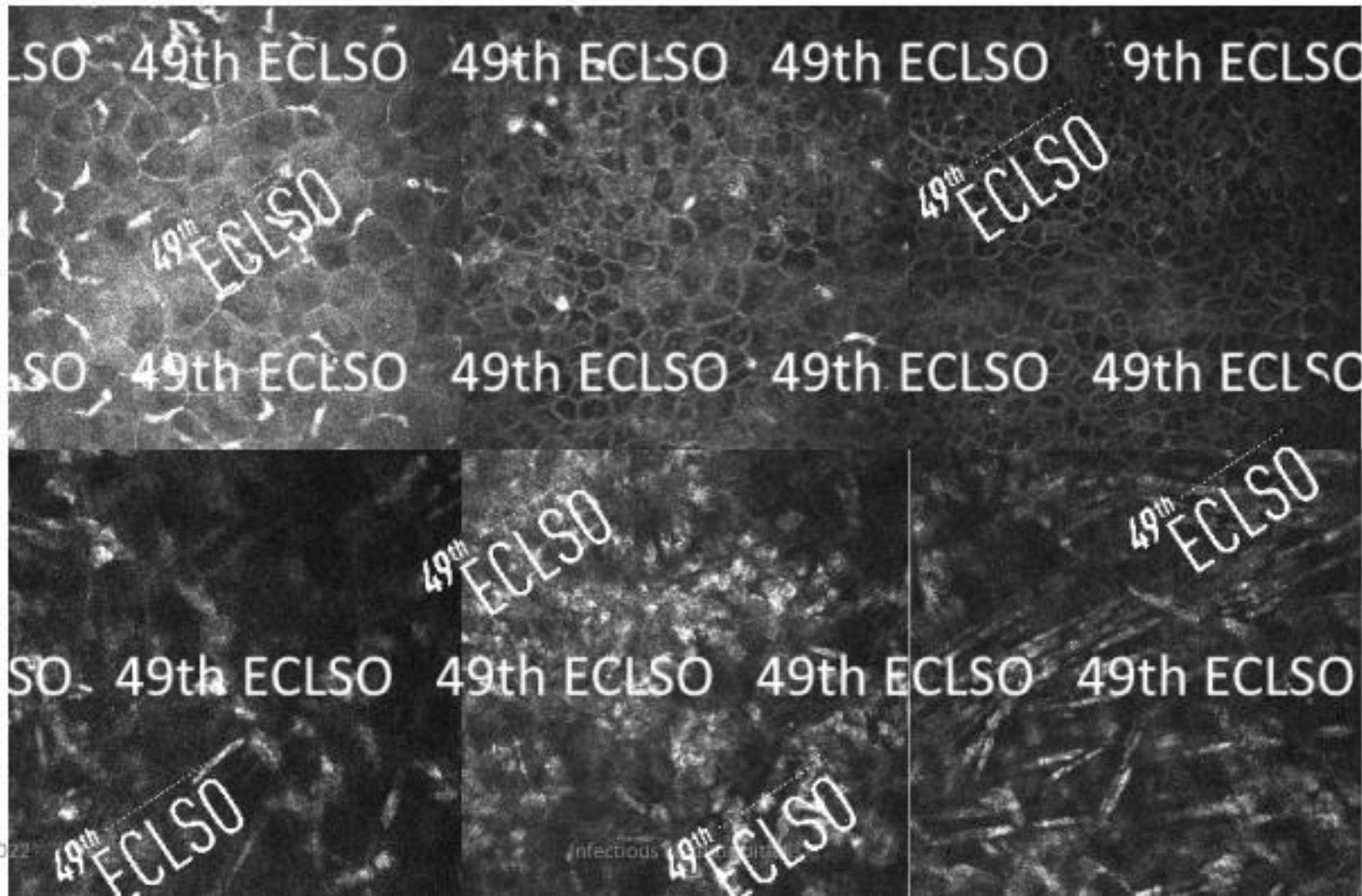
No improvement under topical PHMB + hexamidine

What to do next?

49th ECLSO 49th ECLSO 49th ECLSO 49th ECLSO

49th ECLSO 49th ECLSO 49th ECLSO 49th ECLSO

In Vivo Confocal Microscopy



Results: The most frequently identified fungi were *Fusarium* (61%), *Aspergillus* (6.5%) and *Candida* (5%). Thirty out of 44 cases examined with *in vivo* confocal microscopy (IVCM) presented filaments. Ten required conventional cross-linking, 9 therapeutic penetrating keratoplasty, and 2 enucleation. Risk factors significantly associated with the absence of response to medical treatment were patient age ($p = 0.02$), presence of a deep stromal infiltrate at presentation ($p = 0.04$) and high numbers of filaments in IVCM images ($p = 3.0$). The two populations were comparable in age, but not in sex ratio males/females (18/44 versus 37/26 in the previous study; $p = 0.001$). The frequency of contact lens-associated infection increased from 35.5% to 71% ($p = 0.0001$) between the two periods. Since then, filamentous FK increased from 69% (44/64) to 95% (59/62) ($p = 0.0001$). A history of keratoplasty was less frequently reported during the last period (3.2% (2/62) versus 17% (11/64) of cases ($p = 0.01$)). A clear decrease in the frequency of therapeutic keratoplasty was noted from 39% (25/64) to 14% (9/62) ($p = 0.02$).

Conclusion: The frequency of filamentous keratomycosis is currently increasing. Elderly patients and the presence of numerous filaments in IVCM are associated with poor clinical outcomes.

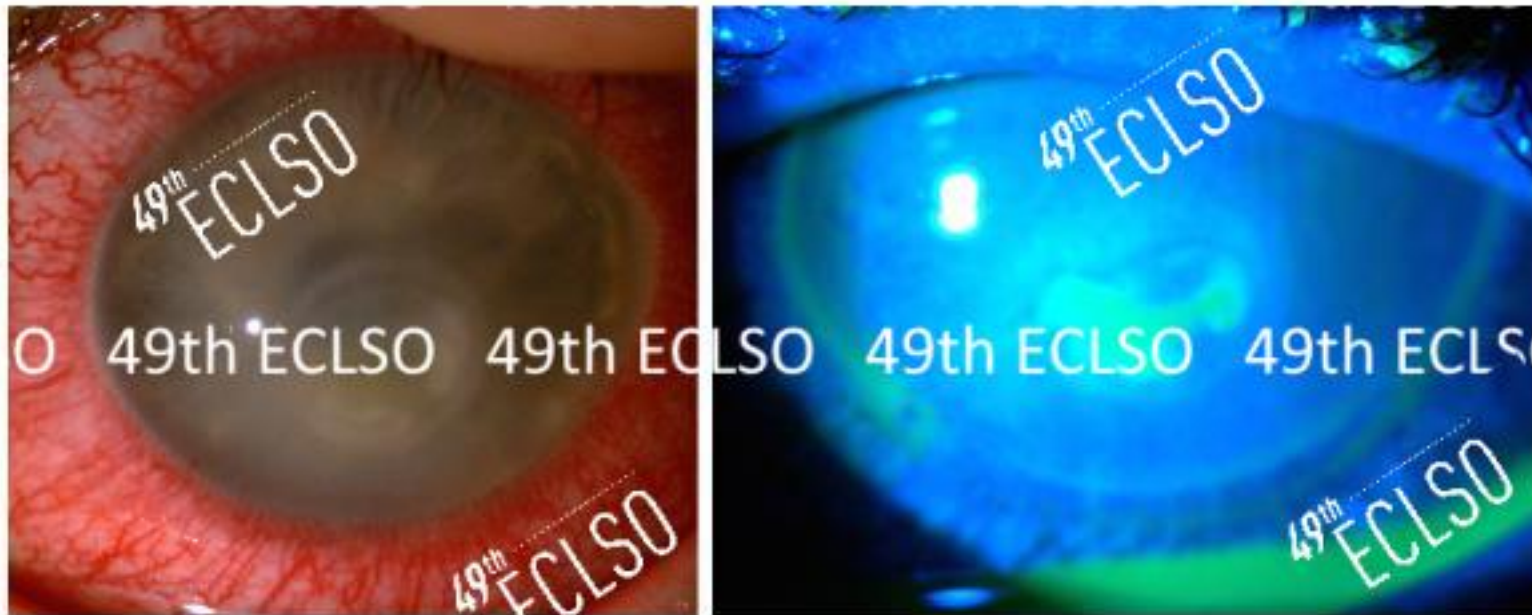
Table 1. Population characteristics at baseline and clinical outcomes ($n = 62$), shown as numbers and percentages.

Parameter	Present study period (2014-2018) $n = 62$	Previous study period (1993-2008) $n = 64$	p-Value
Population characteristics			
Age (mean \pm SD)	46 \pm 17	50 \pm 18	0.23
Sex ratio (male/female)	0.41	1.42	0.0012
Associated conditions			
Contact lenses	45 (73%)	24 (37%)	0.0008
Soft	41 (71%)	15 (35.9%)	
Rigid	4 (7%)	1 (1.5%)	
Ocular trauma	6 (10%)	12 (19%)	0.15
Topical corticosteroid	14 (23%)	15 (23%)	0.91
Pre-existing penetrating keratoplasty	2 (3%)	11 (17%)	0.01
Immunosuppression (HIV, diabetes, cancer, SLE)	5 (8%)	5 (8%)	0.96
Initial visual acuity			
>20/40	26 (42%)	14 (22%)	0.05
20/50-20/80	11 (18%)	18 (28%)	
<20/200	24 (39%)	32 (50%)	
Final visual acuity			
>20/40	32 (53%)	18 (44%)	0.23
20/50-20/80	11 (18%)	10 (16%)	
<20/200	16 (26%)	26 (40%)	
Infiltrate characteristics			
Depth			
Anterior stroma	54 (87%)	43 (67%)	0.008
Posterior stroma	8 (13%)	21 (33%)	
Area			
<5 mm	37 (59%)	12 (50%)	0.3
5-15 mm	14 (23%)	11 (27%)	
>15 mm	11 (18%)	14 (22%)	
Number			
Unique	48 (77%)	30 (78%)	0.92
Multiple	14 (23%)	14 (22%)	
Anterior chamber inflammation			
None	22 (37%)	18 (59%)	0.1
Trivial	28 (45%)	11 (17%)	
Typical	11 (17%)	15 (23%)	
Response to medical treatment			
Good response	47 (76%)	19 (61%)	0.07
No response	15 (24%)	25 (39%)	
Surgical treatment			
Penetrating keratoplasty	9 (15%)	19 (30%)	0.02
Crosslinking	10 (17%)		
Enucleation	2 (3%)	6 (9%)	0.16

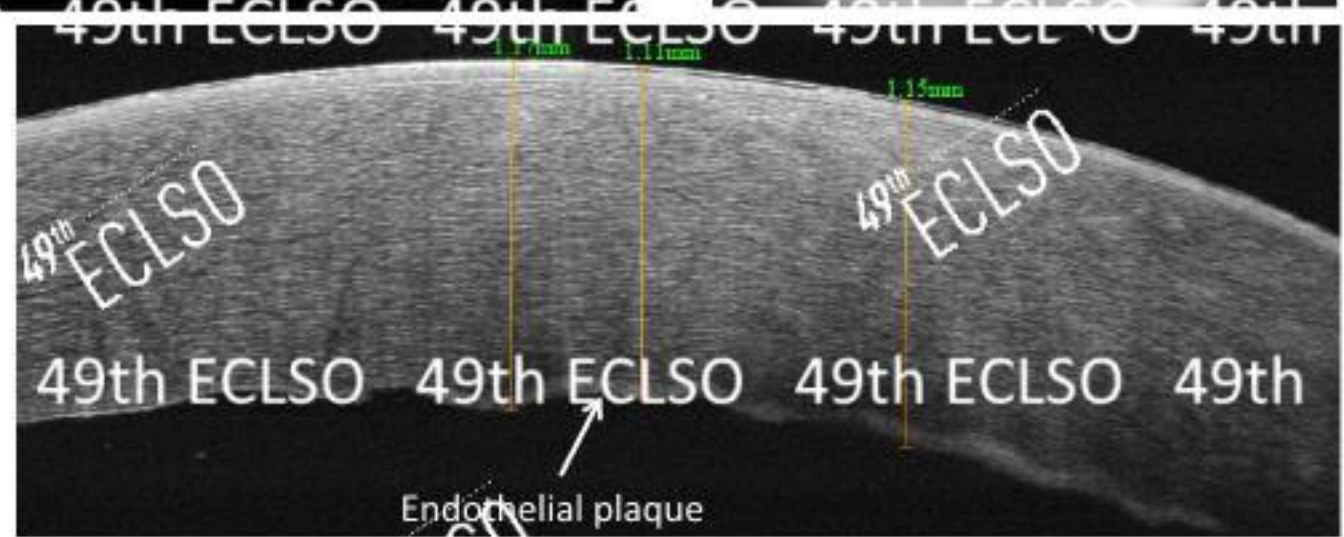
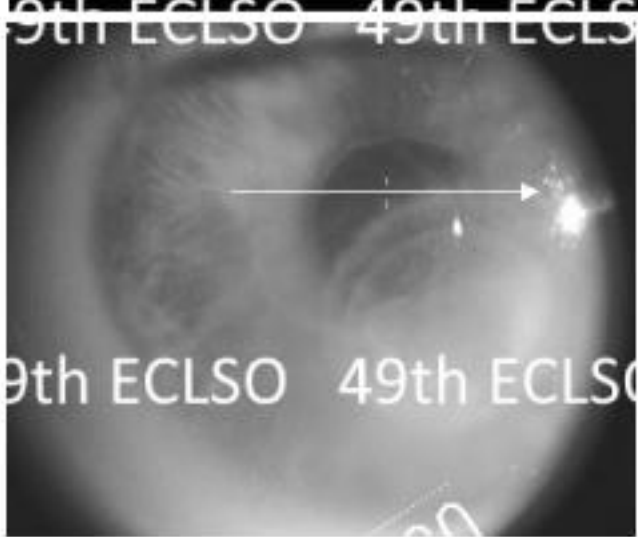
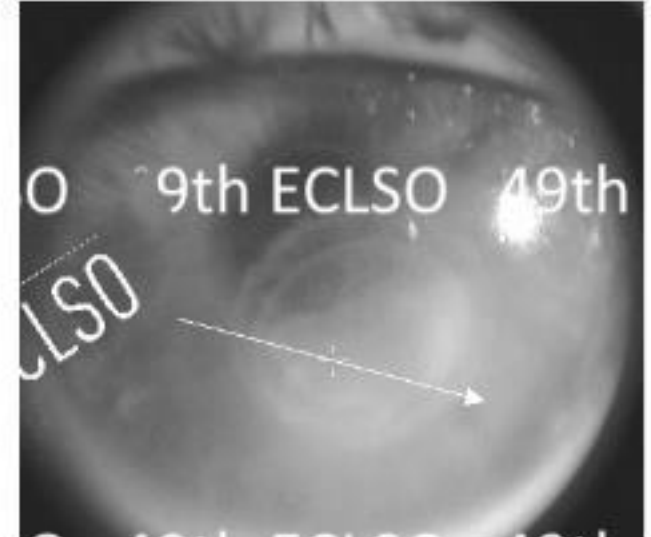
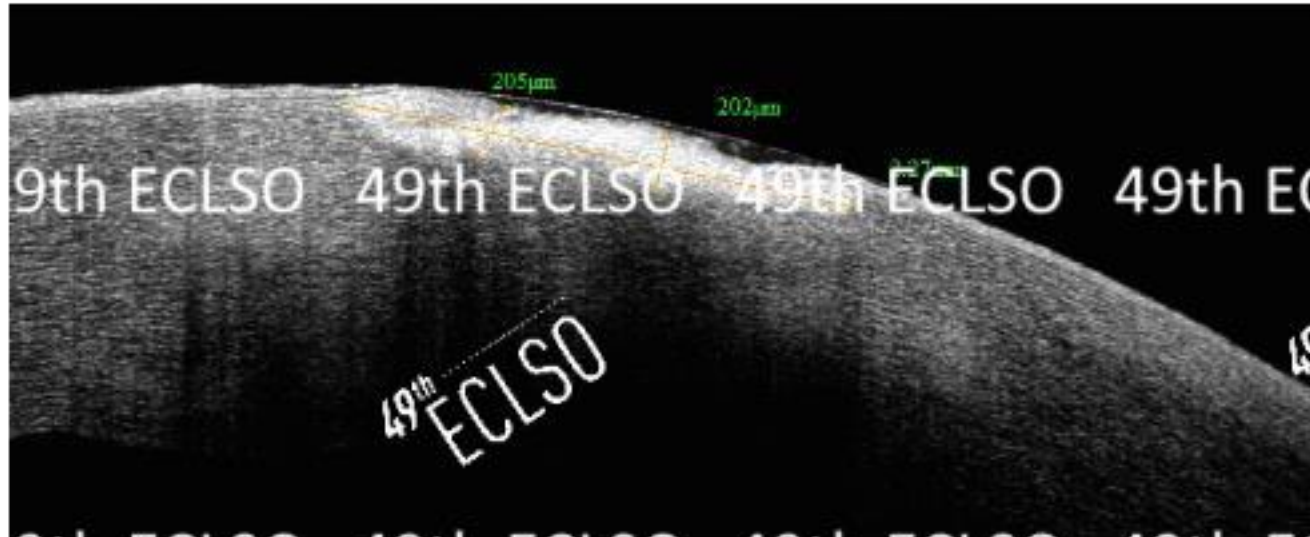
Update on fungal keratitis in France: a case-control study

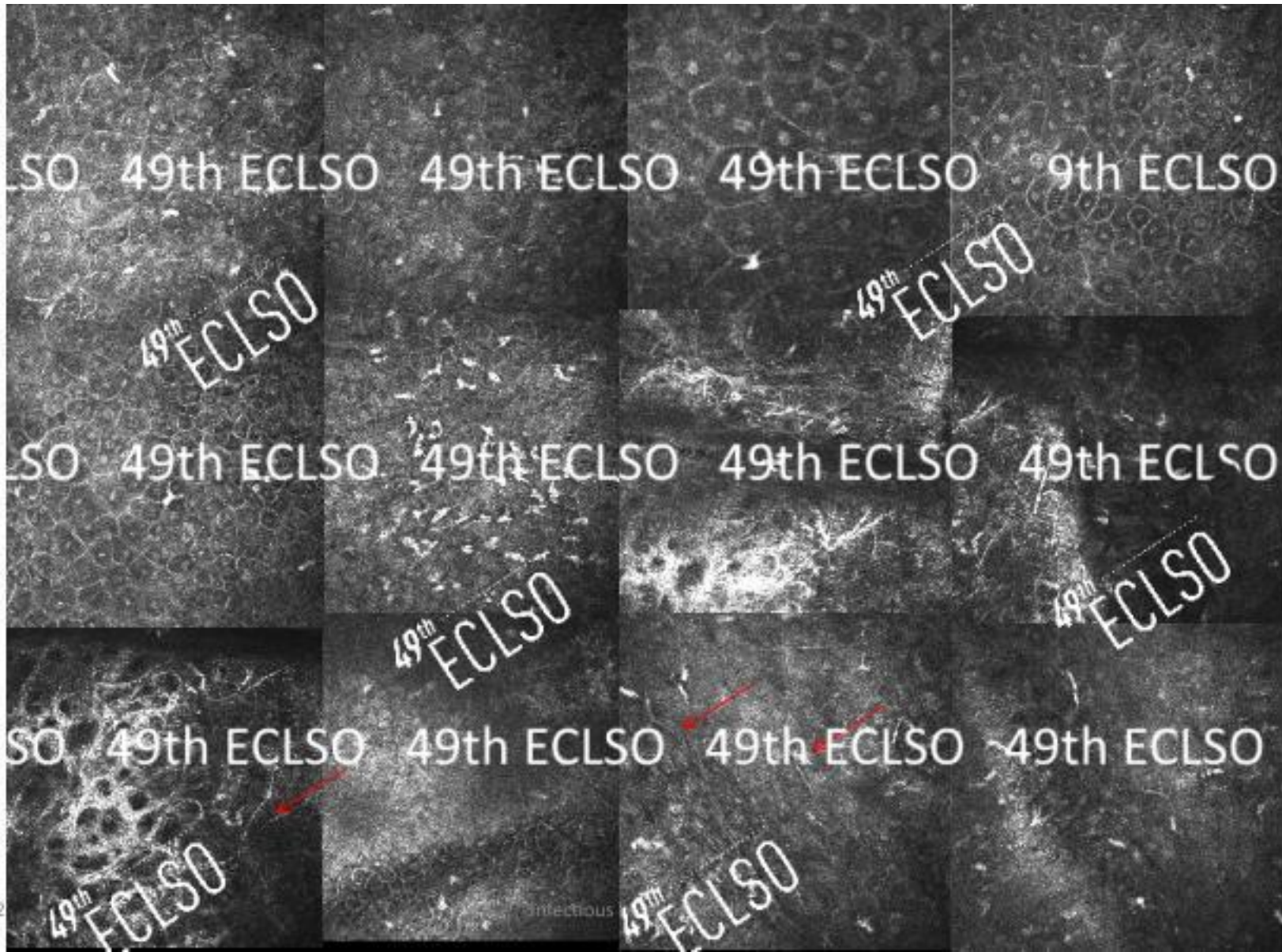
Roxane Olivier,^{1,2} Juliette Knoeri,¹ Loïc Leveziel,¹ Pierre Negrier,¹ Cristina Georgeon,¹ Alfred Kobal,¹ Nacim Bouheraoua,^{1,3} Christophe Baudouin,^{1,3} Jean-Philippe Nordmann,¹ Françoise Brignole-Baudouin,^{1,2} Lilia Merabet¹ and Vincent Borderie^{1,2}

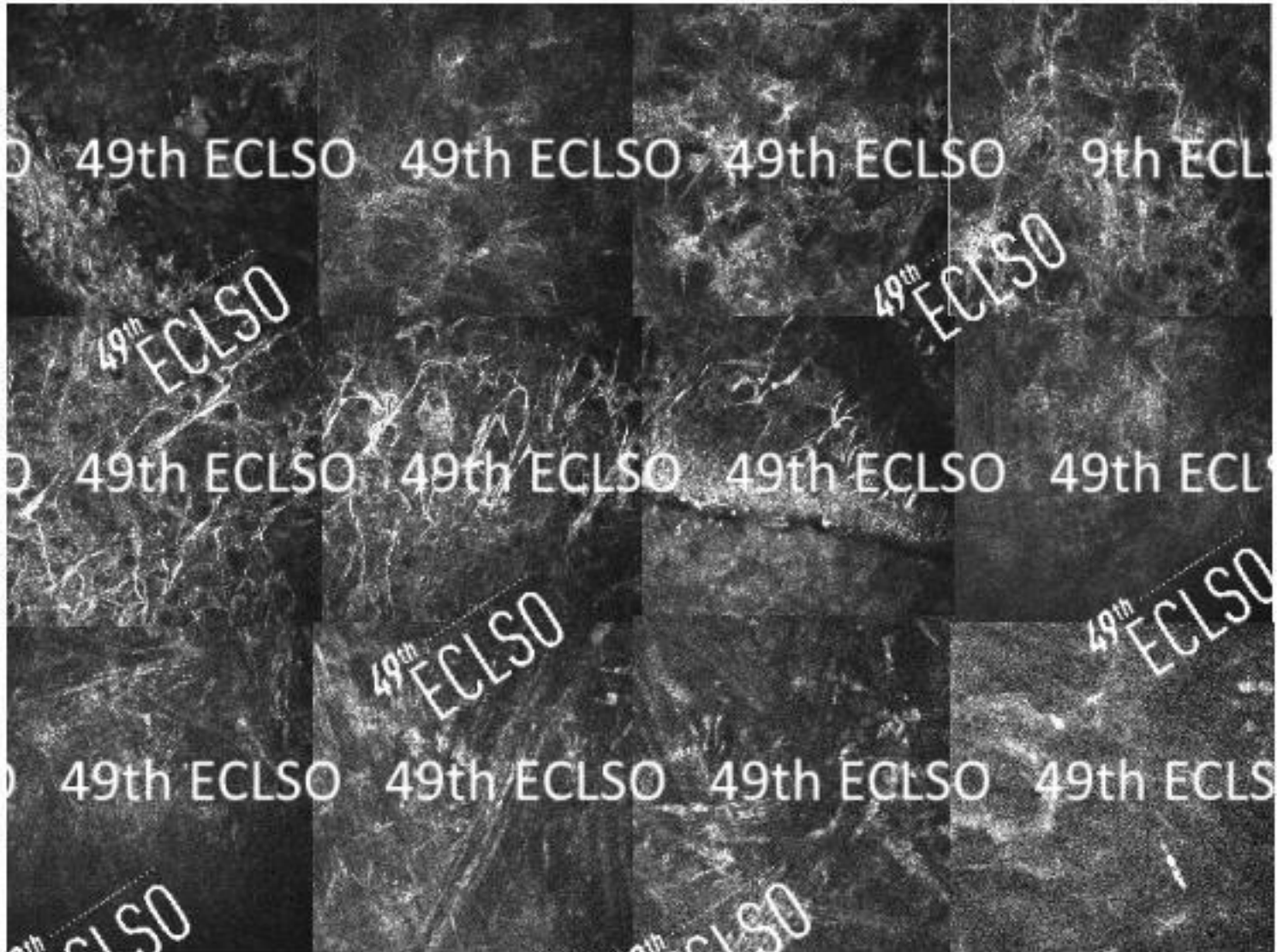
26-y old male patient

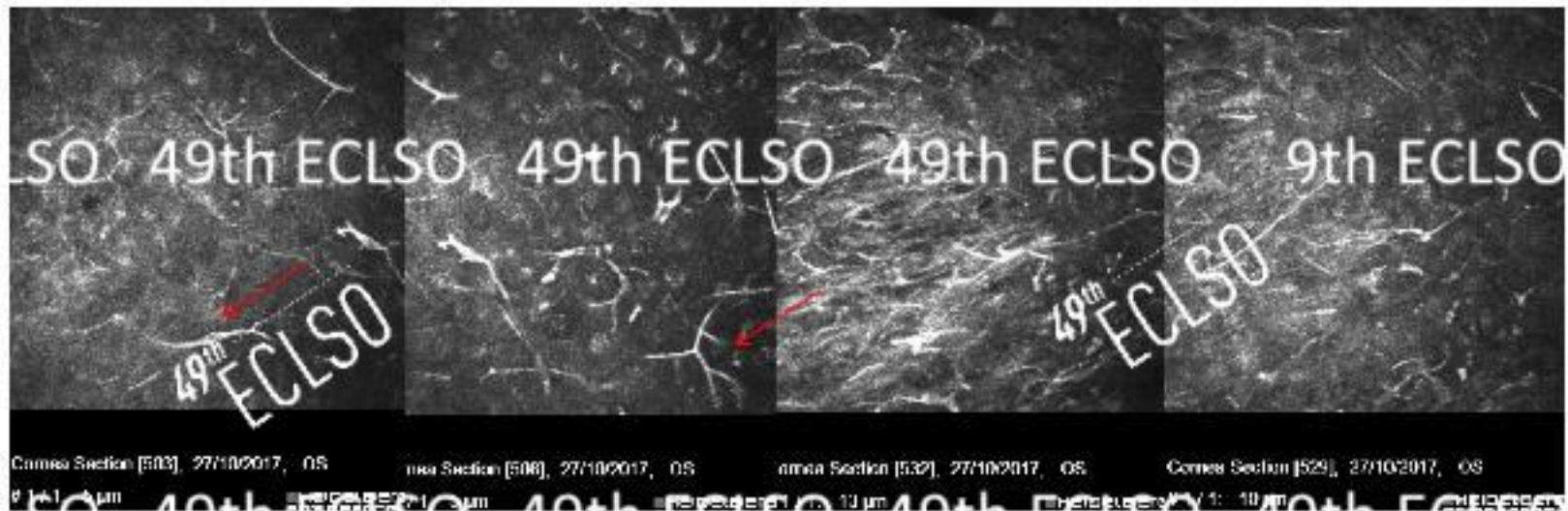


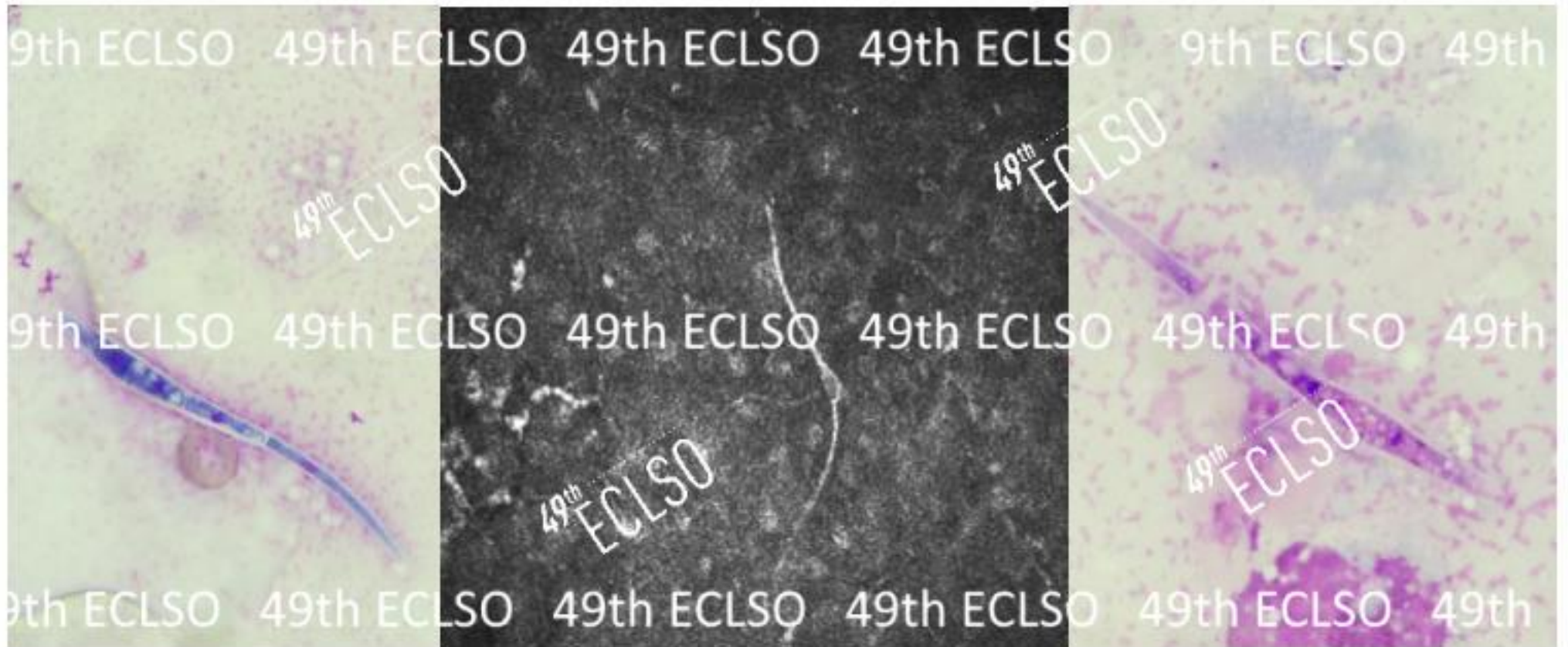
- Eye trauma with suitcase in Bali
- Corneal abscess with hypopyon, negative microbiological tests
- Treatment: topical levofloxacin 1,5% + ciprofloxacin per os, then natamycin + Cefazoline 50 mg/ml + gentamycin 14 mg/ml
- Hypertonia treated with Ganfort
- Pain relief, hypopyon resolution, repatriation in Europe











Corneal imaging workup for infectious keratitis

- **Slit lamp photos:**
 - Size of ulcer and infiltrate
 - Tyndall, hypopyon
 - Follow-up
- **SD-OCT:**
 - Size and location of lesions
 - Stromal necrosis assessment
 - Inflammation assessment
 - Follow-up
- **IVCM:**
 - Inflammatory infiltrate, type and location
 - Infectious agent visualization if size $> 1 \mu$
 - Corneal innervation condition
 - Diagnosis & follow-up

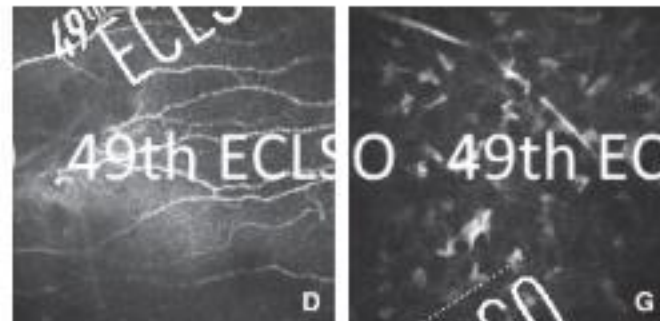
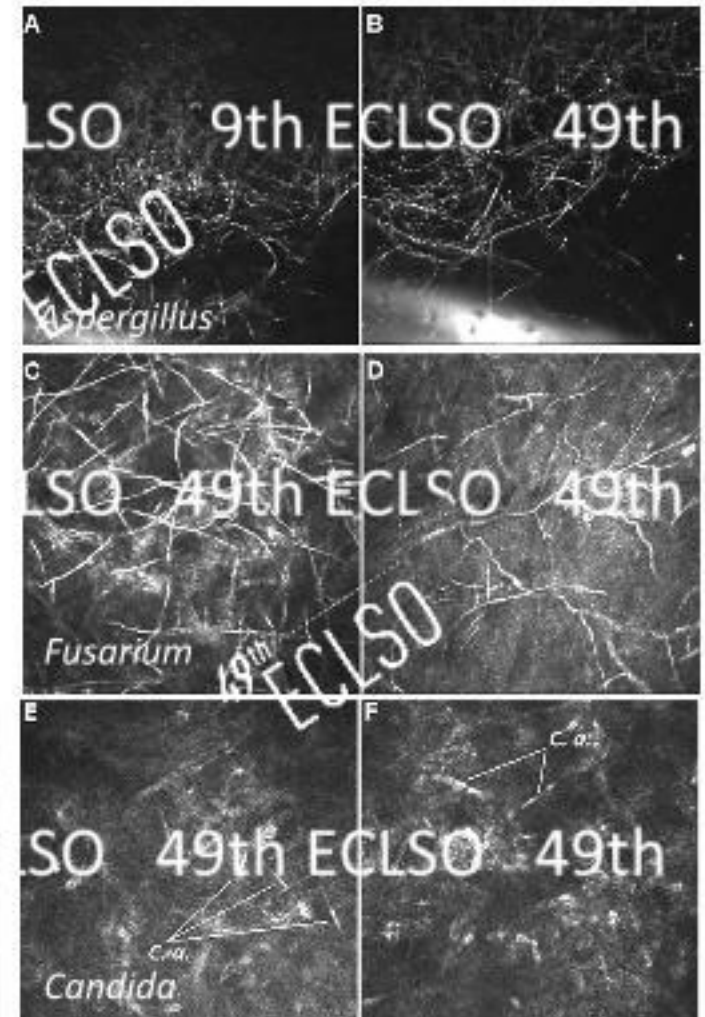


The use of *in vivo* confocal microscopy in fungal keratitis – Progress and challenges

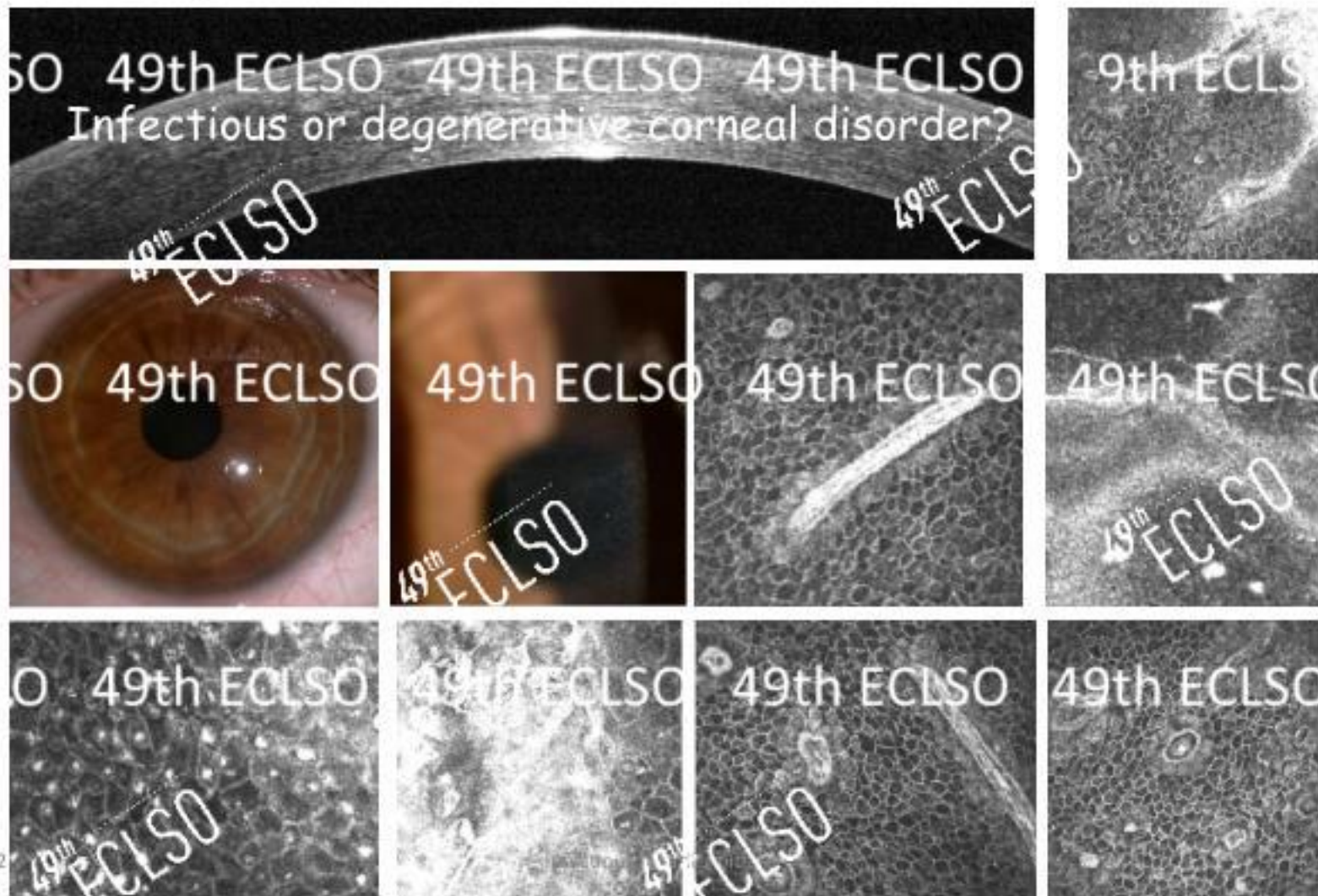
Ingrid M. Bakken¹, Catherine J. Jackson^{1,2}, Tor P. Uthrin^{1,2,3}, Eduardo Vilson^{1,2}, Pedram Hamrah⁴, Ahmad Kasribah⁵, Robin Nelson⁶, Scott Hua^{1,2}, Neil S. Lynn^{1,2,3,6}

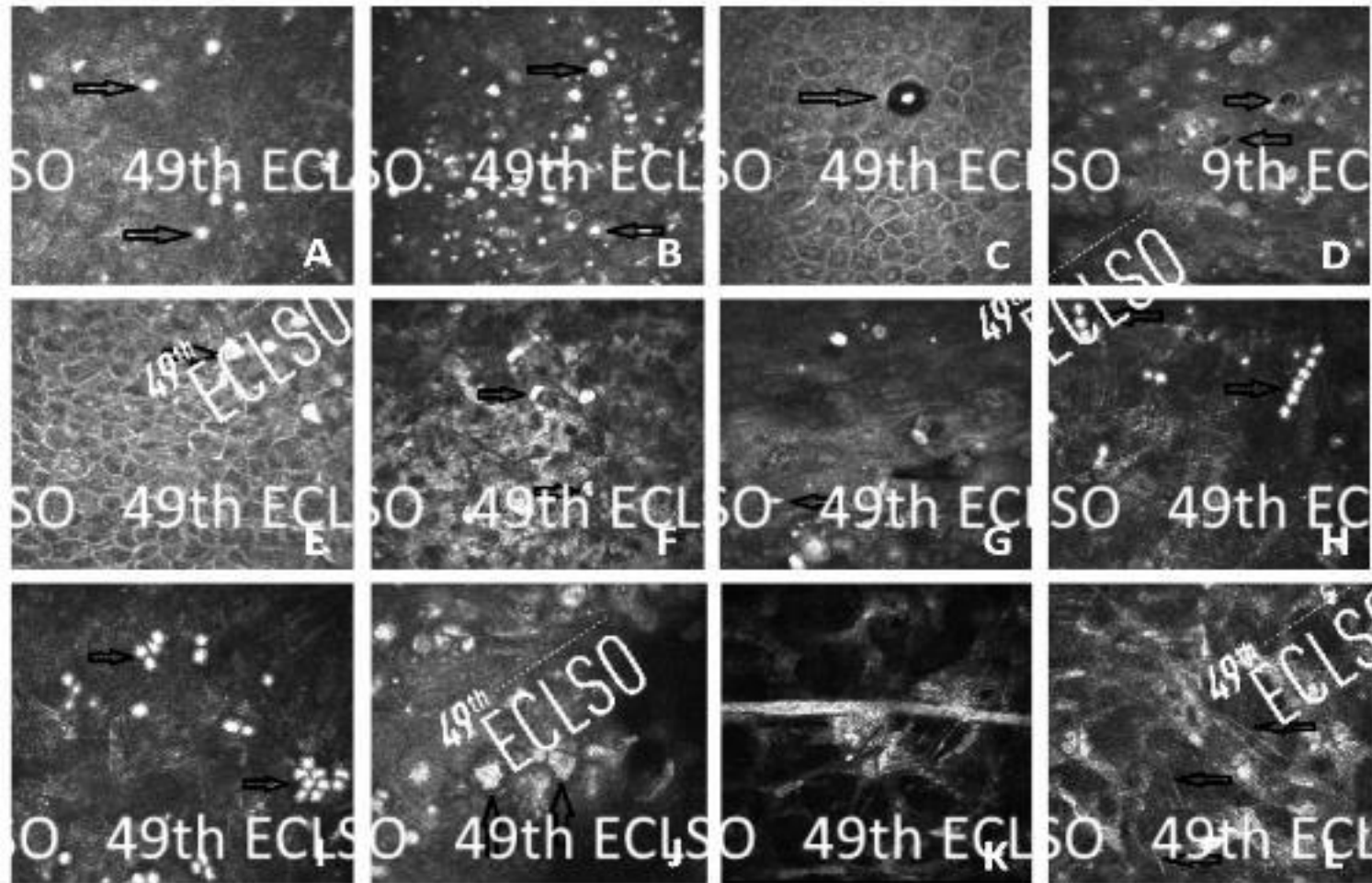
IVCM

- *Aspergillus* : filaments with septa, dichotomic branching (45°), 5-10 μ in diameter
- *Fusarium* : filaments 200–300 μ m long, 3–5 μ m in diameter, 90° branching
- *Candida* : pseudo-filaments 10–40 μ m long, 5–10 μ m in diameter
- Non-fungal branching linear structures:
sub-basal nerve plexus, dendritic cells, stromal nerves
- Sensitivity: 89-94%
- Specificity: 78-93%



Subacute superficial keratitis in a contact lens wearer

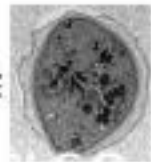




A. Round/ovoid hyperreflective images with no double wall (bright spots), B. Round/ ovoid hyperreflective images with double wall, C. Round/ovoid hyperreflective images with hyporeflective halo (target sign), D. Signet sign image , E. Polygonal/ stellate hyperreflective images, F. Coffee bean hyperreflective images, G. Rod hyperreflective images , H. Single file of hyperreflective images, I. Cluster of hyperreflective images, J. Trophozoite images, K. Keratoneuritis image, L. Spindle shaped images



IVCM
ECLSO



IVCM images associated with *Acanthamoeba* keratitis



Assessment of Confocal Microscopy for the Diagnosis of Polymerase Chain Reaction Positive *Acanthamoeba* Keratitis

A Case-Control Study

Sophia De Groot, MD, MSc, David J. Brown, MD, Christopher J. Rapraeger, MD, PhD, Philip K. Kaiser, MD, PhD, Vincent A. DiGirolamo, MD, PhD

- At least 1 of the 3 feature images (target, cluster/file of hyperreflective images, trophozoite-like images)
 - PPV = 87.5%
 - NPV = 58.5%

30 y-old lady

First symptoms in 2002

Contact lens wear

Wine trader, vegetal corneal trauma

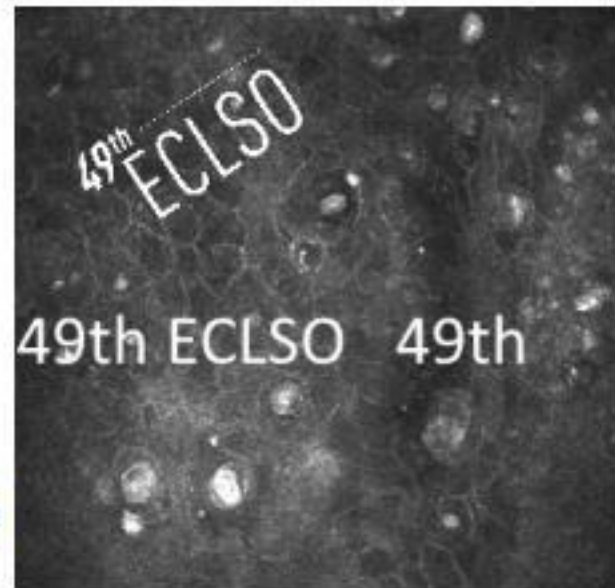
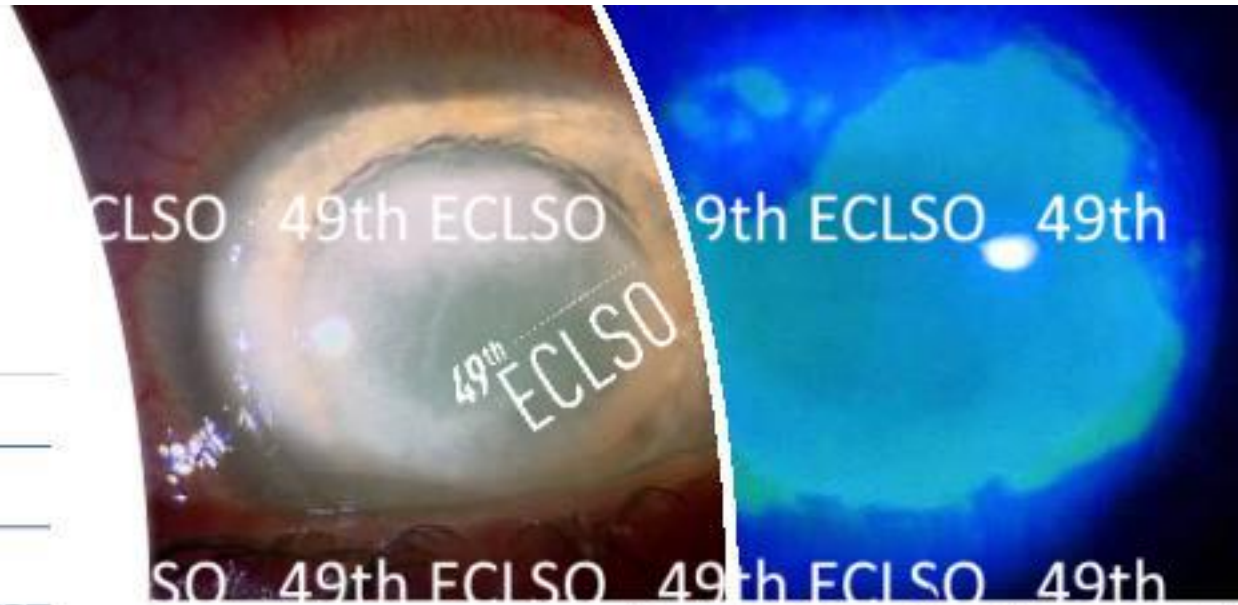
Corneal ulcer during the last 2 months

Severe pain

Corneal scrapping: Acanthamoeba PCR+

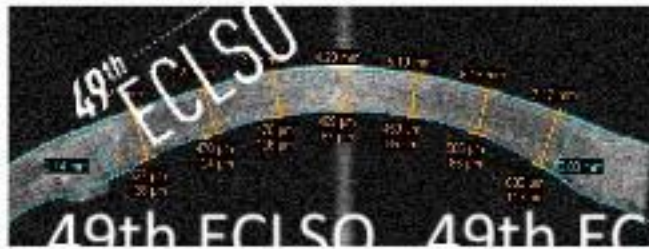
Chronic ulcer under topical treatment

No resolution after several AMT

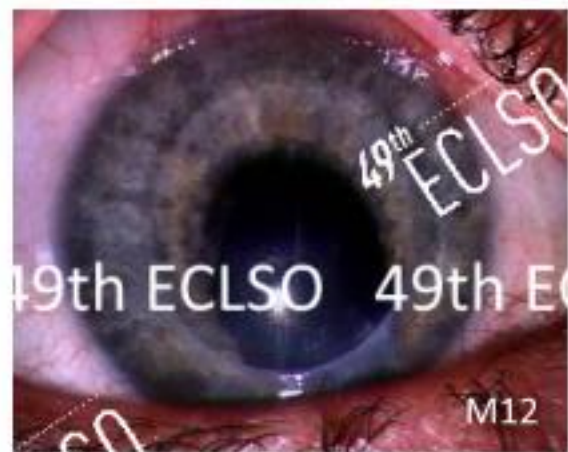
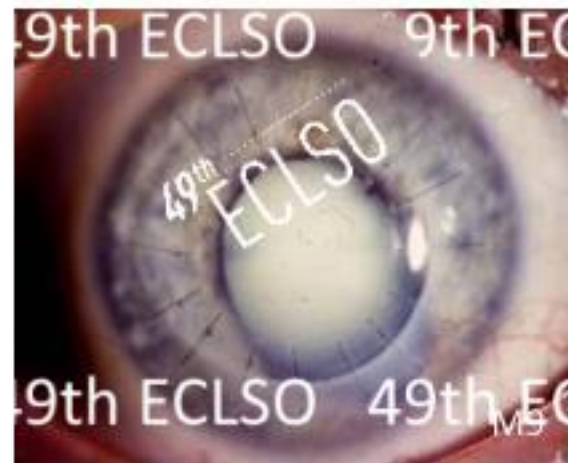
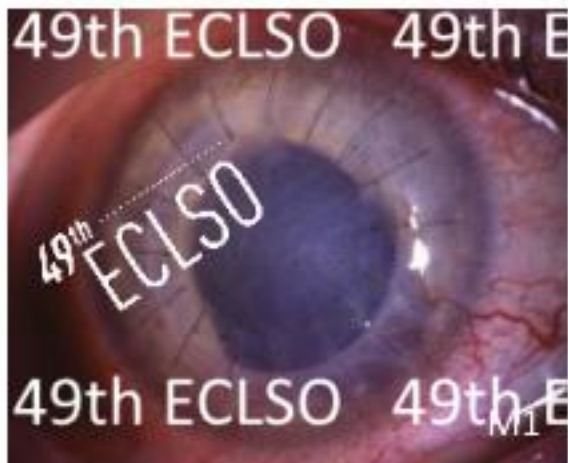


Further management?

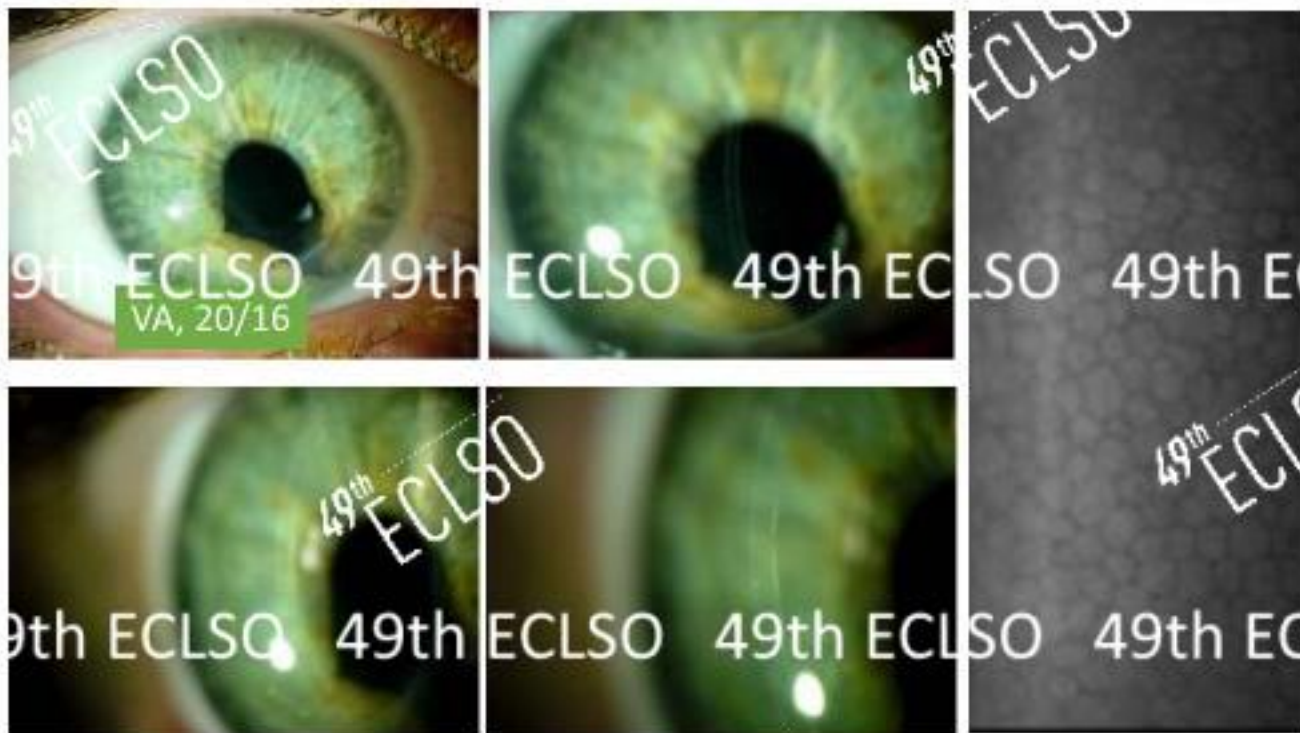
Deep Anterior Lamellar Keratoplasty



DALK outcomes



Nine years latter...



30-year-old woman



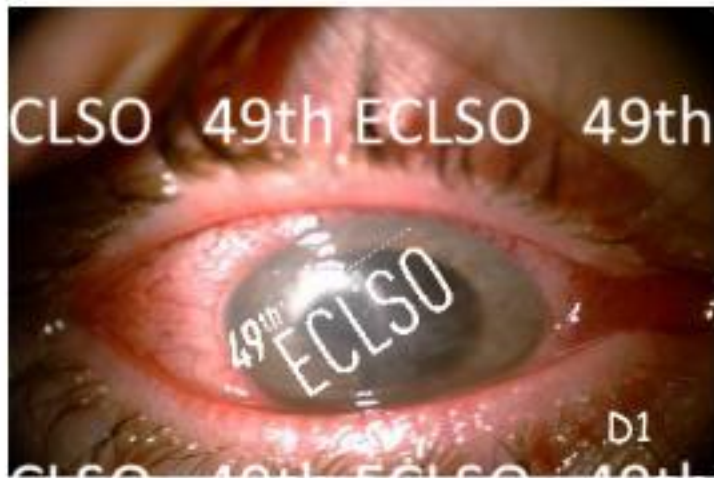
VA: 20/200

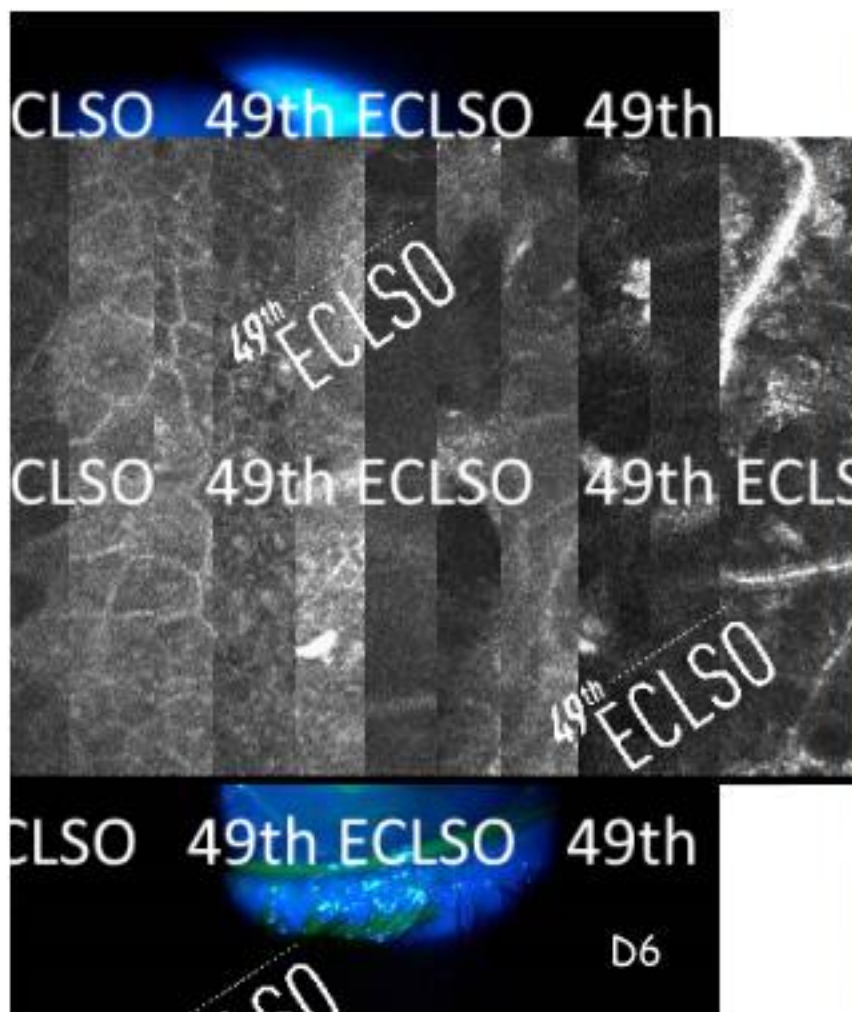


Contact lens wear
 Exposure to swimming pool water in Thailand
 Referred in 2010 for keratitis treated with
 topical dexamethasone and tobramycin for 10 days



Acanthamoeba, fungi:
 - Corneal scraping for wet microscopic
 examination after May Grünwald Giemsa staining, culture on
 chocolate PolyViteX agar, Schaedler broth with globular
 extract, and Sabouraud with antibiotics agar
 - VCM





First treatment:

- Topical amphotericin B
- Topical voriconazole
- Topical bacitracin
- Topical colmifyoin
- Topical atropine

Oral voriconazole D4

CLSO 49th ECLSO 49th ECLSO 49th ECLSO

Microbiology D6 (corneal scrapping):

Propionibacterium acnes

Acanthamoeba PCR

Corneal biopsy (Grocott) D6:

Large sparsely septate hyphae

CLSO 49th ECLSO 49th ECLSO

Topical caspofungin D11

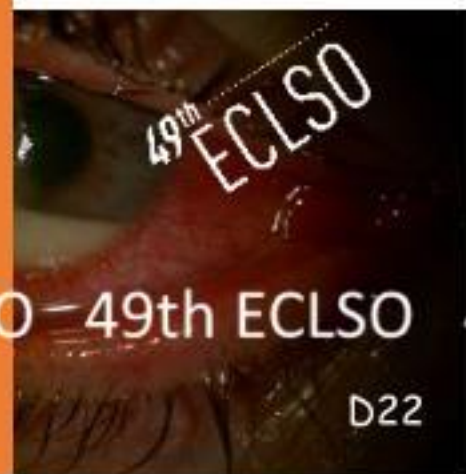
Intravenous caspofungin D14



Microbiology (corneal biopsy) D14:
Corynebacterium renale
Staphylococcus capitis

Treatment:

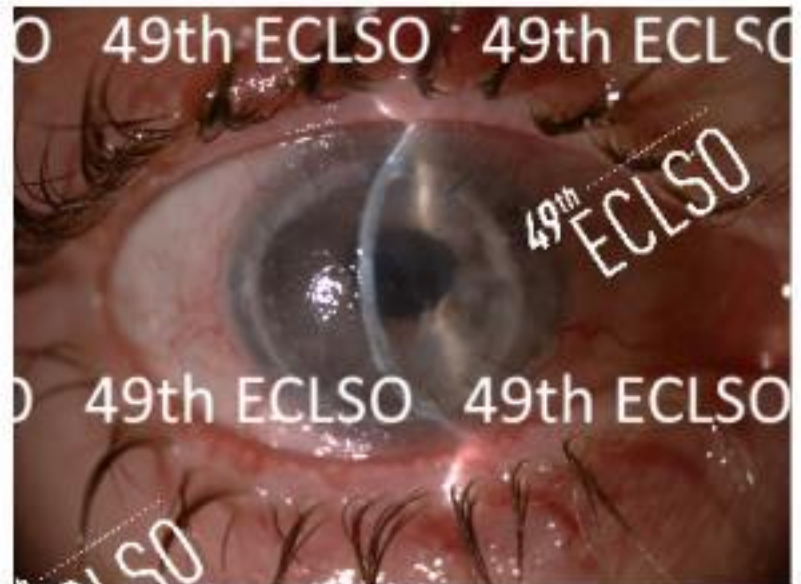
- Topical amphotericin B
- Topical voriconazole
- Intravenous caspofungin
- Oral voriconazole
- Topical moxifloxacin
- Topical gentamycin
- Topical vancomycin
- Intravenous imipenem



Voriconazole intrastromal injection
D30

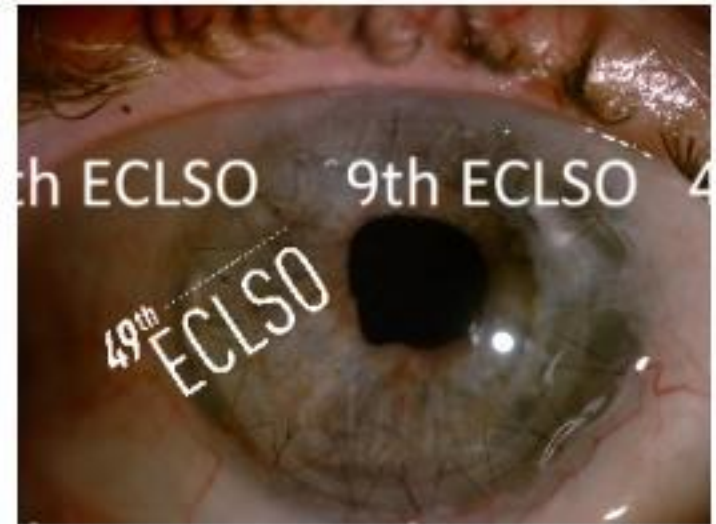
First PK outcome

- 1st rejection episode at 15 months
- 2nd rejection episode at 30 months
- 3rd rejection episode at 36 months
- Immune failure



Second PK (2015)

- Cataract surgery at 12 m
- Topical CsA + dexamethasone
- Last exam (2022):
VA: 20/20
clear graft



Pythium insidiosum

- **Protist:** eukaryotic organism (one with cells containing a nucleus) that is not an animal, plant or fungus.

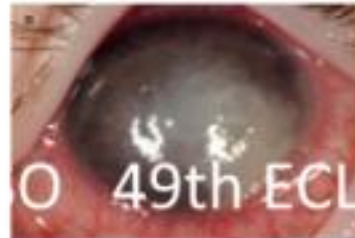
Phylum:	Oomycota
Class:	Oomycetes
Order:	Pythiales
Family:	Pythiaceae
Genus:	<i>Pythium</i>
Species:	<i>P. insidiosum</i>

- ❖ A protista is divided into several simple groups ;
 1. Protozoa (animal-like protist)
 2. Algae (plant-like protist)
 3. Myxomycetes and Oomycota (fungus-like protist)



- Tropical and subtropical areas (Asia, USA, South America, Australia, NZ)
- Endzootic area in Thailand (80% of reported cases)
- Pathogenic in mammals particularly in horses, dogs and humans
- Two forms:
 - hyaline hyphae with few septates
 - zoospores : infectious form, survival in wet environment
- Water contains motile zoospores or other propagules (zoospores or hyphae), also found on grass and soil
- Contact lens use, trauma and contact with water: common risk factors of ocular pythiosis
- The infection is not contagious

Human pythiosis

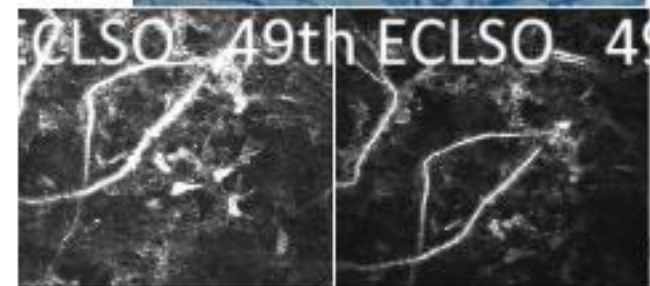
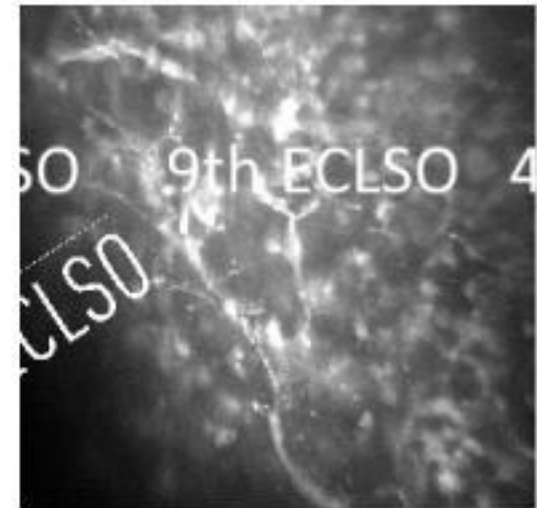


- Cutaneous or mucous membrane contact with water contaminated with zoospores + trauma
- Risk factors: agriculture (Thai farmers), activity in wet environment, thalassemia
- Ocular diseases (33%):
 - Keratitis (contact lens wear++)
 - History of ocular trauma
 - Severe forms may involve the orbit and lead to early endophthalmitis which usually requires radical surgery in the form of enucleation or evisceration
- Cutaneous diseases (5%):
 - Skin ulcers
 - Face & legs
- Vascular diseases (59%):
 - Arterial occlusions and aneurysms
- Disseminated diseases (3%)

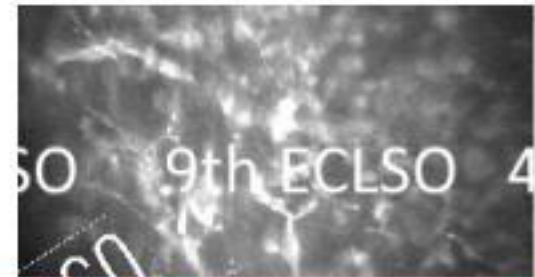


PYTHIOSIS DIAGNOSIS

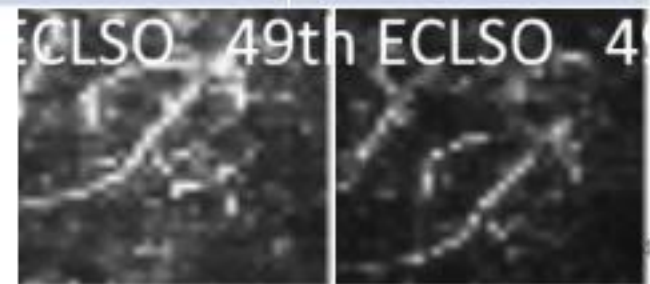
- **Polymerase Chain Reaction:**
 - DNA extraction with phenol-CHCl₃-isoamyl alcohol method
 - amplification of internal transcribed spacer regions (ITS1 and ITS2) target genes
 - gene sequencing
 - blast search : NCBI GenBank database
 - 91.7% sensitivity, 81.8% specificity
- Presence of zoospores is an important microbiological evidence of pythiosis
- The filaments stain positive for acridine orange hydrochloride and lactophenol blue on histopathological examination.
- **In vivo confocal microscopy:**
the filaments exhibit more flexible patterns like X or Y compared to fungal hyphae



PYTHIOSIS TREATMENT



Study	Keratoplasty	Evisceration/ enucleation/phtthisis	Other
Kunavisarut S. Pythium keratitis ulcer in Ramathibodi Hospital. J Med Assoc Thai 2003	10/10 (100%)	7/10	
Agarwal S. Clinical profile of pythium keratitis: perioperative measures to reduce risk of recurrence. Br J Ophthalmol 2018	7/10 (70%)	2/10	
Bagga B. Leap forward in the treatment of Pythium insidiosum keratitis. Br J Ophthalmol 2018	65/87 (75%)		
Agarwal S. Clinical profile, risk factors and outcome of medical, surgical and adjunct interventions in patients with Pythium insidiosum keratitis. Br J Ophthalmol 2019	45/46 (98%)	5/46	surgical adjunct (cryotherapy/ alcohol) with 1PK efficient in 13/14
Hasika R. Pythium keratitis in South India: Incidence, clinical profile, management, and treatment recommendation. Indian J Ophthalmol 2019	48/71 (68%)	16/71	





- Epidemiology necessary for first diagnosis orientation
- Clinical features provide first presumptive microbiological diagnosis
- Multimodal imaging mandatory for diagnosis, assessment and follow-up
- Acanthamoeba and fungal keratitis, although rare, remain major diagnosis and treatment concerns
- Not only virus, bacteria, fungi, and *Acanthamoeba* can lead to infectious keratitis
- ... but also aliens!
- Keratoplasty needed when medical treatment fails

Thank you

Groupe de Recherche Clinique 32,
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Vincent Borderie

Diida Ghoubay

Kristina Hirsch

Nacim Bouheraoua

Juliette Knoeri

Otman Samad

Marie Borderie

Christina George

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