Keratomalacia and corneal perforation in vitamin A deficiency: Anterior-segment optical-coherence-tomography and histological findings

Julia M. Weller *, Theofilos Tourtas, Friedrich E. Kruse

Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany

A R T I C L E I N F O

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A B S T R A C T

Purpose: To describe the histological findings and results of anterior-segment optical-coherence-tomography (AS-OCT) in patients with severe keratomalacia and corneal perforation due to vitamin-A deficiency (VAD).
Observations: Four patients (3 female, 1 male) with moderate to severe VAD were included in this single-center case series. Keratomalacia/corneal perforation was diagnosed by slit-lamp examination. The findings were documented using photographs and AS-OCT imaging (CASIA-2, *Tomey corporation, Nagoya, Japan)*. Ocular and general medical findings including causes of VAD are reported. VAD was severe (<20 ng/ml) in two patients with chronic alcoholism, and moderate (205/231 ng/ml) in two patients with cachexia. Corneal perforation occurred in 3 out of 4 patients. One patient had severe keratomalacia with impending perforation and massive conjunctival folds with Bitot's spots, which vanished soon after initiation of vitamin A supplementation. In three cases, corneal specimens obtained during anterior lamellar keratoplasty were assessed histologically by light-microcopy. The histological specimens showed massive epithelial thickening and a pronounced granular layer of the corneal epithelium.
Conclusions and importance: Keratomalacia leading to corneal perforation is a rare, but possible complication also in countries with sufficient general food supply. The

with night blindness/nyctalopia. In contrast to retinal dystrophies, night blindness/nyctalopia in VAD is reversible after supplementation of vitamin A.

The purpose of this study is to show the histological and anterior-segment optical coherence tomography (AS-OCT) findings in four patients with corneal affection and perforation in the setting of VAD.

2.1. Patients

In this retrospective, single-center study, the medical records at a tertiary referral center for Ophthalmology (Department of

– Summary of patients' characteristics, general medical findings, ocular findings, laboratory results, and treatment.

	Case 1	Case 2	Case 3	Case 4
Age at presentation	70 y	35 y	65 y	72 y
Sex	male	female	female	female
BCVA at presentation	OD: 20/630 OS: light perception	OD: 20/25 OS 20/200	OD: no light perception OS: 40/200	OD: 20/500 (advanced glaucoma) OS: 20/80
Cause for vitamin A deficiency	Suspected alcoholism, reduced general health state	Alcoholism	Cachexia (body mass index: 15.8 kg/m^2)	Liver cirrhosis, cachexia (body mass index 19.5 kg/m^2)
Laterality of eye affection	bilateral	unilateral	bilateral	unilateral
General history	Liver fibrosis with beginning cirrhosis, suspected alcoholism, nicotine addiction, psoriasis	Liver cirrhosis, ascites, alcoholism, asthma	Cachexia of unknown reason	Liver cirrhosis, anemia, chronic obstructive pulmonary disease, rheumatoid arthritis, Graves' disease, gastric ulcer, reflux esophagitis, chronic pancreatitis, osteoporosis, cardial decompensation, tricuspid valve insufficiency
Conjunctival alterations (Bitot's spots)	Bitot's spots, keratinized conjunctiva	No Bitot's spots	Mild conjunctival injection, no Bitot's spots, keratinization of the conjunctivalized cornea	Conjunctival hyperemia, no Bitot's spots
Corneal findings	OD: massive staining, focal infiltrates, erosion, corneal edema, Descemet folds OS: bulging of the inferior corneal hemisphere, keratomalacia, vascularizations, flattened anterior chamber	OD: normal OS: peripheral perforated corneal ulcer with iris prolaps	OD: scarred cornea, athalamia OS: superficial corneal ulcer, infectious nfiltrate	OD: normal OS: paracentral focal white stromal infiltrate with overlying erosion; stromal thinning
Corneal sensitivity (esthesiometry)	n/a	6/6	4/6	3/6
Microbiology	Negative	Negative	Negative	Positive for *Candida albicans* (first sample), positive for *Stenotrophomonas maltophilia* (second sample after 1 month)
Ocular diagnosis	OD: keratitis due to massive corneal xerosis OS: keratomalacia with large ulceration and retrocorneal fibrin	OD: without pathological findings OS: perforated corneal ulcer	OD: blindness due to assumed previous corneal perforation OS: superficial infectious corneal ulcer	OD: without pathological findings OS: mycotic corneal ulcer due to exposition keratopathy and vitamin A deficiency, followed by bacterial superinfection and perforation
Treatment	OD: topical antibiotics (cefuroxim, tobramycin) and lubricants OS: anterior lamellar tectonic keratoplasty with amniotic membrane patch	OU: lubricants OS: anterior lamellar tectonic keratoplasty	OD: lubricants, no surgery due to poor prognosis OS: antibiotic eye drops, lubricants, topical steroids	OD: lubricants OS: topcial antimycotics hourly, followed by antibiotics due to bacterial superinfection; finally anterior lamellar keratoplasty with lateral tarsorraphy due to corneal perforation
Vitamin A level (normal range: 300–800 ng/ml)	<20 ng/ml	<20 ng/ml	205 ng/ml	231 ng/ml
Laboratory	hypoproteinemia (48.8 g/l), hypalbuminemia (30.5 g/l) low vitamin B1 (25 µg/l) slightly reduced vitamin B12 (203 pg/ml) low 25-OH-vitamin-D3 (4.3 ng/ml) low transferrin (1.24 g/l)	high MCH (37 pg) high MCV (112 fl) high CRP (40.7 mg/l) high LDH (397 U/l) high gamma-GT (184 U/l) high white blood cells (12.300/µl)	high MCH high MCV gamma GT normal ALT/AST normal	hypoproteinemia (59 g/l) high gamma GT (77 U/l) high AST (60 U/l) high ALT (49 U/l) low platelets (94.000/µl) low hemoglobin (10.8 g/dl) high α-amylase (141 U/l) high lipase (219 U/l)
Follow-up time	–	15 months	2 months	2 months
Ocular co-factors	–	–	Blepharitis posterior	Exposition keratopathy

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alpha-amylase (normal <110 U/l).
ALT = alanine transaminase (normal <35 U/l).
AST = aspartate transaminase (normal <35 U/l).
CRP = C-reactive protein (normal <5 mg/l).
gamma-GT = gamma-glutamyltransferase (normal <40 U/l).
hemoglobin (normal 12–15.5 g/dl).
LDH = lactat dehydrogenase (normal <250 U/l).
lipase (normal <60 U/l).
MCH = mean corpuscular hemoglobin (normal 27–32 pg).
MCV = mean corpuscular volume (normal 83–98 fl).
serum protein (normal 60–68 g/l).
serum albumin (normal 35–55 g/l.
transferrin (normal 2.0–3.6 g/l.
25-OH-vitamin-D3 (normal: 30–70).

Ophthalmology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), Erlangen, Germany) were screened for cases with proven VAD and ocular morbidity. Four patients fulfilled these criteria. Written informed consent was obtained from all patients included in this study. The institutional review board waived the need for approval of this study.

Main study parameters were reason for consultation, age of patient at presentation, sex, best corrected visual acuity, cause of VAD, ocular findings at the anterior segment, clinical course, treatment modalities, and follow-up time.

2.2. Methods

Best corrected visual acuity (BCVA) was measured using standard number optotypes in a clinical setting. Slit lamp examination was performed in all patients with fluorescein staining of the surface. Dependent on the slit lamp findings, further measurements (ultrasound, optical coherence tomography, etc.) were initiated. Photographs of the anterior segment were taken at first presentation and during the follow-up. Anterior-segment optical coherence tomography (AS-OCT) measurements of the cornea were taken with the device CASIA-2 (*Tomey corporation, Nagoya, Japan*).

2.3. Histological analysis

The corneal material excised during lamellar keratoplasty of three patients (cases #1, 2, and 4) were fixed in buffered 10 % formaldehyde solution (pH 7.2), dehydrated, and embedded in paraffin. Serial sections cut at 5 µm were stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS).

The results of case #1–4 are summarized in Table 1.

3.1. Case #1

A 70-year-old male patient presented with bilateral painless loss of vision and epiphora of the eyes which had started 2 weeks ago. He was in a reduced general state of health, with normal body weight (body mass index 21.6 kg/m^2). According to his relatives, he usually avoided contact to the health system. His general medical history was positive for psoriasis, chronic alcohol consumption (about 1 L of beer per day), and cigarette smoking. Visual acuity was reduced to 20/630 (OD) and light perception (OS). The cornea of the right eye had several small stromal infiltrates, epithelial defects, stromal edema and Descemet folds (Fig. 1 A/B). In the left eye, the cornea was cloudy due to a large ulcer (measuring 5.8 × 6.7 mm) with a diffuse yellowish infiltrate, retrocorneal fibrin and bulging of the inferior corneal hemisphere (Fig. 1 G/H). Besides, stromal vascularizations and edema with Descemet folds in the upper half of the cornea were apparent. The bulbar conjunctiva of both eyes showed marked Bitot's spots with glistening of the keratinized surface (Fig. 1 E/F).

AS-OCT was used to measure the thickness of the cornea (right eye: 809 µm) and the conjunctiva and to visualize the bulging of the left cornea due to advanced keratomalacia (Fig. 1 B/F/H).

Laboratory tests revealed low serum levels of albumin, general protein, vitamin A (<20 ng/ml, normal range: 300–800 ng/ml), vitamin B1 (25 µg/l) vitamin B12 (133 pg/ml), and vitamin D3 (4.3 ng/ml). In abdominal ultrasound, fibrosis with beginning cirrhosis of the liver was diagnosed. Supplementation of vitamin B1, B12, D3 and vitamin A was started orally (200.000 I.E. per day orally for 2 days).

The right eye was treated with topical antibiotics and lubricants leading to fast clearance of the stroma, reduction of stromal thickness, and normalization of the conjunctival appearance (Fig. 1 C/D). In the left eye, an anterior tectonic lamellar keratoplasty with amniotic membrane patch was performed without complications (Fig. 1 I/J).

By AS-OCT the morphological alterations after successful treatment of VAD were monitored and the correct alignment of the lamellar corneal graft in the stromal bed was documented (Fig. 1 D/J). The corneal tissue (anterior stromal lamella), which was removed at preparation of the lamellar stromal bed during keratoplasty, was obtained and sent for histological examination.

3.2. Case #2

A 35-year-old female was referred with a history of ocular irritation for three weeks in her left eye. The medical history of the patient revealed severe liver cirrhosis with ascites due to chronic alcoholism.

At 8 o'clock at the corneal periphery of the left eye, a perforated corneal ulcer with iris prolapse was seen at the slit lamp (Fig. 2 A/B). AS-OCT was used to visualize the perforation of the peripheral cornea with incarceration of the iris (Fig. 2 C). There were no signs of Bitot's spots, no infection, no hypopyon, no vitreous cells. The right eye appeared normal with visual acuity of 20/25.

Laboratory tests revealed a severely reduced vitamin A serum level (<20 ng/ml). A tectonic anterior lamellar keratoplasty was performed (Fig. 2 D), and the removed corneal tissue was examined histologically (Fig. 2 E/F). The corneal graft healed well and the patient was dismissed. Further medical work-up of her liver dysfunction was initiated and a supplementation of vitamin A orally was started.

At the follow-up visits 12 and 15 months after first presentation, the corneal sutures were removed. The corneal graft showed slight vascularizations and both eyes had 1+ corneal staining. Treatment consisted of lubricants including vitamin A ointment (both eyes), topical steroid eye drops once daily (OS), and systemic vitamin A supplementation.

3.3. Case #3

A 65-year-old female patient presented with foreign body sensation and slight pain in her left eye. The right eye had been blind for several years for unknown reason; the patient had not visited an ophthalmologist in this regard. The patient had severe underweight (body mass index 15.8 kg/m^2) without known bowel disease. She reported that she usually eats two meals per day (no special diet or allergies), drinks rarely alcohol, and negated drug consumption.

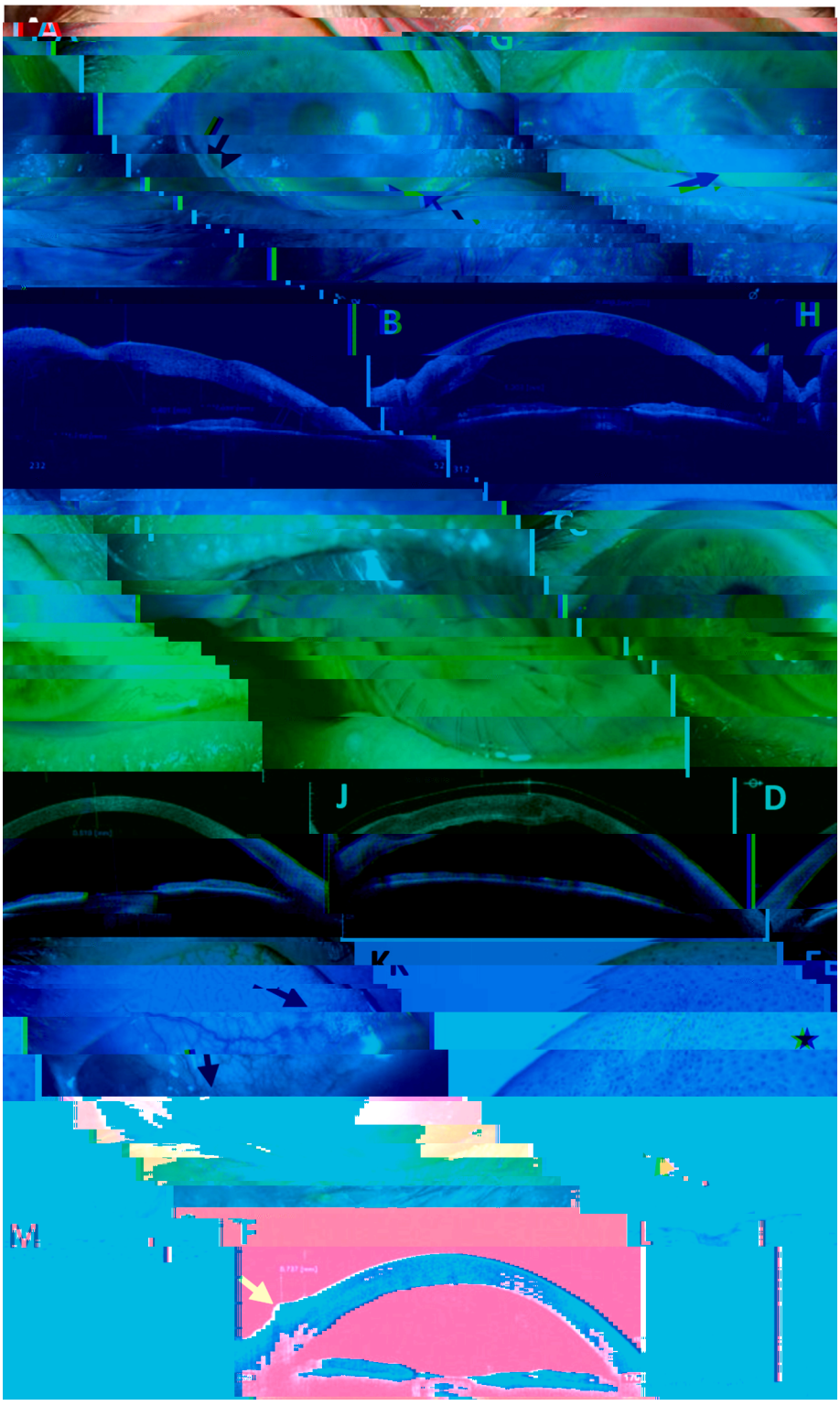
Visual acuity of the right eye was no light perception and a diffuse vascularized scar of the cornea was seen. Because of complete athalamia, previous corneal perforation was suspected (Fig. 3 A/B). AS-OCT revealed bulging of the cornea as in keratectasia (Fig. 3 B). The epithelium could be demarcated as superficial, thickened layer (196 µm) and the iris was shown to be completely attached to the posterior surface of the cornea (Fig. 3 B/D).

In the left eye, BCVA was 40/200, corneal sensitivity was slightly reduced, and posterior blepharitis with conjunctival injection was noticed. In the left eye, there was a peripheral corneal ulcer (2.0 × 1.4 mm) with underlying stromal infiltrate and stromal thinning to 556 µm at the periphery, and anterior chamber cells (Fig. 3 E/F).

Because of the low body mass index, malnutrition was suspected and vitamin A serum levels were measured, revealing a vitamin A level of 205 ng/ml. Further gastrointestinal examination and vitamin A supplementation was initiated. Under treatment of the left eye with topical antibiotics (cefuroxim/tobramycin eye drops hourly, vitamin A ointment 5 times a day), the superficial corneal ulcer healed within 6 days.

3.4. Case #4

A 72-year-old female presented with a paracentral corneal ulcer with a white stromal infiltrate and thinning of the cornea to 397 µm in her left eye (Fig. 4 A/B). Her medical history was positive for many diseases, most notably liver cirrhosis, gastric ulcer, chronic obstructive pulmonary disease (COPD), chronic pancreatitis, and cachexia. Her husband



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Case #1: clinical photos and AS-OCT imaging of a 70-year-old male patient with severe vitamin A deficiency due to chronic liver disease.
A) Photograph, OD, at first presentation: a paracentral stromal white infiltrate (red arrow), massive Bitot's spots in the bulbar conjunctiva (black arrow), Descemet folds, and stromal thickening.
B) AS-OCT, OD, at first presentation: thickening of the bulbar conjunctiva, edema of the corneal stroma.
C) Photograph, OD, after three weeks: clearance of the cornea with focal stromal scar, and a smoother bulbar conjunctiva without hyperemia.
D) AS-OCT, OD, after three weeks: normal corneal thickness.
E) Photograph, OD, at first presentation showing Bitot's spots (arrows) in the temporal and superior bulbar conjunctiva.
F) AS-OCT, OD, at first presentation, Bitot's spots can be detected as wavy folding of the bulbar conjunctiva overlapping the peripheral cornea with thickening of the conjunctiva up to 737 μm (arrow).
G) Photograph, OS, at first presentation: massive conjunctival folds and keratinization (Bitot's spots), keratomalacia with with-yellow bulging of the stroma (arrow).
H) AS-OCT, OS, at first presentation: bulging of the inferior hemisphere of the cornea (asterisk), focal stromal thinning to 401 μm (arrow) beside stromal edema (1039 μm).
I) Photograph, OS, after three weeks: anterior lamellar corneal graft with single sutures, slight hemorrhage in the interface
J) AS-OCT, OS, after three weeks: good adaptation of the lamellar graft, normal anatomy of the anterior chamber
K) Histology, OS: thickened corneal epithelium (asterisk) with focal papillomatosis (arrow), loss of Bowman's layer, adherent irregular stromal lamellae (HE, magnification 50×)
L) Histology, OS: excessive thickening of the epithelium to about 20 cell layers with PAS-positive granular material in the apical layers and slight keratinization (PAS, 100×, the bar indicates the epithelial thickness)
M) Histology, control eye: normal corneal epithelium (about 5 cell layers) with underlying Bowman's layer and stroma for comparison (PAS, 100×, the bar indicates the epithelial thickness).

Case #2: clinical photos and AS-OCT imaging of a 35-year-old female with severe vitamin A deficiency due to chronic liver disease and alcoholism
A) Overview photograph, OS, at first presentation: distortion of the pupil and prolapse of the iris at 7 o'clock without visible signs of inflammation.
B) Slit-lamp photograph, OS, at first presentation: prolapse of the iris through a perforated corneal ulcer (arrow).
C) AS-OCT, OS, at first presentation: thinning of the corneal stroma at the periphery with incarceration of the iris in the stroma (arrow).
D) Photograph, OS, after two months: well-adapted, fusiform, anterior lamellar tectonic graft without signs of inflammation.
E) Histology, OS: thickened corneal epithelium covering the irregular stromal collagen; loss of Bowman's layer (black arrow); steep rim of the corneal ulcer; overlying keratinizing epithelium with adherent iris pigment (red arrow) due to corneal perforation (PAS, 50×)
F) Histology, OS: massively thickened epithelium as in case #1; slight papillomatosis; absence of Bowman's layer (black arrow) (PAS, 100×).

reported that the eyelids did not close completely at night. Laboratory tests showed a reduced serum vitamin A level (231 ng/ml) and increased liver enzymes.

Corneal scraping revealed *Candida albicans* and antimycotic topical treatment with amphotericin B/voriconazole eye drops hourly was initiated. The infiltrate diminished slowly, but the corneal epithelial wound healed very slowly. After 1 month, there was a new, enlarging stromal infiltrate with retrocorneal attachment of fibrin, and further

Case #3: clinical photos and AS-OCT imaging of a 65-year-old female with cachexia

A) Photograph, OD, at first presentation: bulging of the whitish cornea, which is completely covered by conjunctival tissue; athalamia.

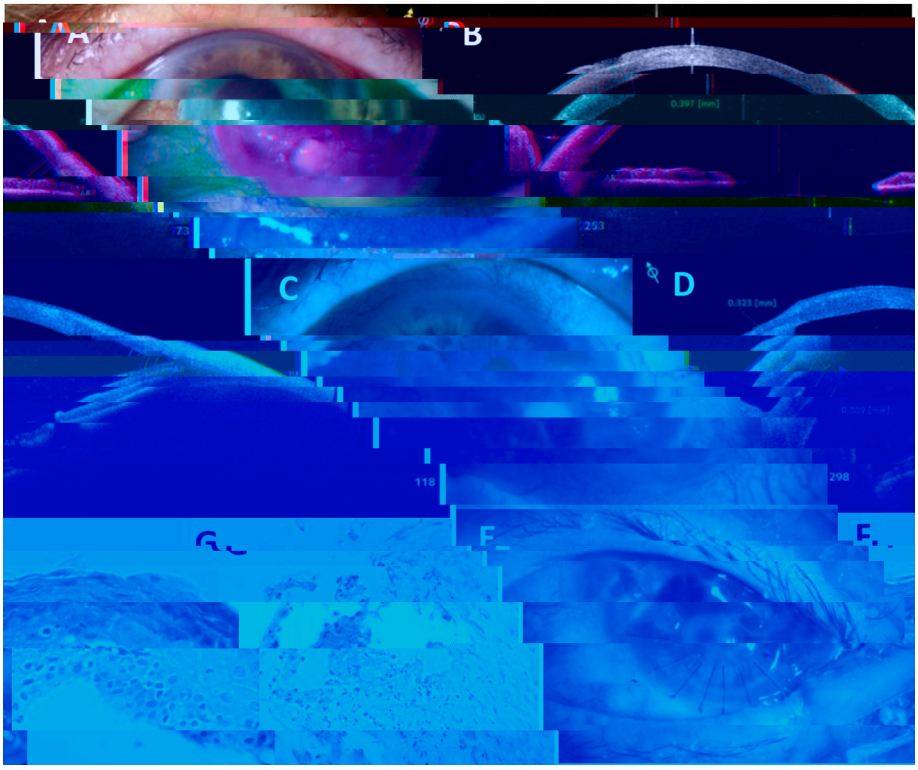
B) AS-OCT, OD, at first presentation: the cornea is thickened to 940 µm at the center and bulges forward due to keratomalacia; focal cleft between stroma and Descemet's membrane; complete attachment of the iris to the cornea (arrow).

C) Photograph, OD, higher magnification: keratinization of the corneal surface similar to conjunctival Bitot's spots (arrow).

D) AS-OCT, OD, higher magnification: massive thickening of the epithelium to 196 µm, attached iris to the cornea (arrow).

E) Photograph, OS, at first presentation: hyperemia of the bulbar conjunctiva, hazy lesion at 7–8 o'clock peripherally (arrow).

F) AS-OCT, OS, at first presentation: slight corneal thinning (556 µm) at 7–8 o'clock (arrow).



– Case #4: clinical photos, AS-OCT imaging, and histological findings of a 72-year-old multimorbid female

A) Photograph, OS, at first presentation: hyperemia of the bulbar conjunctiva, focal white corneal infiltrate with overlying erosion; surrounded by irregular epithelium.

B) AS-OCT, OS, at first presentation: focal corneal thinning to 397 µm

C) Photograph, OS, after 1 month: enlarged infiltrate/retrocorneal fibrin deposit; persisting corneal erosion

D) AS-OCT, OS, after 1 month: progressive corneal thinning (323 µm); retrocorneal attached fibrin (889 µm, arrow).

E) Photograph, OS, after surgery: anterior lamellar tectonic keratoplasty and lateral tarsorrhaphy has been performed for the repair of the focally perforated corneal ulcer.

F) Histology, periodic acid-Schiff/PAS stain, 200 ×: thickening of the corneal epithelium, PAS-positive granular deposits in the superficial corneal layers.

G) Histology, PAS stain, 100 ×: irregular, partially necrotic corneal stromal layers with focal PAS-positive material (presumed non-vital fungal elements), few inflammatory cells.

stromal thinning (Fig. 4 C/D). *Stenotrophomonas maltophilia* was detected by microbiological culture, and the treatment regimen was changed to ofloxacin eye drops hourly. After stabilization of the infection, an amniotic membrane patch was performed for the treatment of persistent corneal erosion. Nevertheless, the cornea perforated within 6 days, and the decision was made for tectonic anterior lamellar keratoplasty with lateral tarsorrhaphy (Fig. 4 E). The graft integration and wound healing was well, but the general condition of the patient deteriorated due to anemia, making erythrocyte transfusion necessary. In this case, AS-OCT helped to measure the residual stromal thickness and to differentiate between the stromal infiltrate and the retrocorneal fibrin clot (Fig. 4 B/D).

The corneal tissue (epithelial and stromal fragments), which had been removed during lamellar keratoplasty, was processed for histological assessment. Histological analysis of the corneal epithelium showed thickening and slight keratinization of the epithelium with PAS-positive granular deposits in the superficial layers (Fig. 4 F). Bowman's layer was absent and the stromal layers were irregular with focal necrosis and few inflammatory cells around PAS-positive material (possibly non-vital fungal cells, Fig. 4 G).

In this case series, we report the clinical, AS-OCT, and histological findings of four patients with keratomalacia in VAD. Serum vitamin A levels were reduced below detection level (<20 ng/ml) in two out of the four cases. The other two patients had subnormal vitamin A levels, but these patients had further risk factors (chronic posterior blepharitis and exposition keratopathy) for corneal ulceration. Typical Bitot's spots were found only in one of our cases (#1). Bitot's spots might be missing even in cases with severe VAD as in case #2. In one patient (#3), the cornea had been overgrown with conjunctival tissue, which showed keratinization (Fig. 3 C) and massive thickening of the epithelium detected by AS-OCT. Thickening of the epithelium of the cornea and conjunctiva was documented and followed by AS-OCT (e.g. Fig. 1 B/F, Fig. 3 D). Interestingly, none of our patients had severe pain. Instead they

clinicians should be aware that thickness measurements in keratomalacia must be interpreted differently than in normal corneas. The study is limited by the small number of cases and the relatively short follow-up time. Furthermore, the case series was inhomogenous since two of the four patients had only subnormal vitamin A levels.

In conclusion, ocular signs of VAD are seen rarely in countries with sufficient general food supply, but can lead to severe corneal complications up to perforation. It is important to consider VAD as cause of delayed corneal wound healing or melting even if obvious corneal or conjunctival xerosis (Bitot's spots) are not always present. Chronic alcoholism and cachexia were the most common causes of VAD in our case series. AS-OCT can help to detect thickened corneal epithelium consistent with reduced epithelial turnover. Taking a detailed medical history and awareness of the possibility of VAD is mandatory in the treatment of patients with corneal ulcers.

Consent to publish these case reports has been obtained from the patients in writing.

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