Invasion of Lymphatic Vessels into the Eye after Open Globe Injuries

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Purpose. We analyzed whether lymphatic vessels can be detected in eyes enucleated after an open globe injury.

Methods. The presence of lymphatic vessels was analyzed immunohistochemically using podoplanin as a specific lymphatic endothelial marker in 21 globes that had been enucleated after open globe injury. The localization of pathologic lymphatic vessels (within the eye wall or inside the eye) was correlated with the mechanism of trauma, anatomic site of perforation or rupture, and time interval between trauma and enucleation.

Results. Pathologic lymphatic vessels were detected in 15 of 21 eyes (71%) enucleated after an open globe injury. In 5 globes (24%) they were found within the eye, located in retrocorneal membranes, underneath the sclera, and adjacent to uveal tissue (ciliary body, iris). No significant association was observed between the presence of pathologic lymphatic vessels and the mechanism of trauma ($P = 0.598$), anatomic site of perforation or rupture ($P = 0.303$), and time interval between trauma and enucleation ($P = 0.145$).

Conclusions. The human eye can be invaded secondarily by lymphatic vessels if the eye wall is opened by trauma. This mechanism could be important for wound healing, immunologic defense against intruding microorganisms, and autoimmune reactions against intraocular antigens. (*Invest Ophthalmol Vis Sci.* 2012;53:3717–3725) DOI:10.1167/iovs.12-9507

Apart from the extraocular conjunctiva and corneal limbus,[1–4] the human eye physiologically is devoid of lymphatic vessels.[5] Although the human choroid has an abundance of blood vessels, it does not contain any typical lymphatic vessels.[6] Therefore, the human eye has been considered as an immune-privileged site.[5,7]

However, under a variety of pathologic conditions, for example inflammation or cancer, the formation of new lymphatic vessels can occur.[8,9] Lymphangiogenesis is mediated by vascular endothelial growth factors (VEGF)-C and -D, which are ligands for the tyrosine-kinase receptor VEGF receptor-3 (VEGFR-3).[10] Whereas VEGFR-3 can be detected on blood endothelial cells as well as on lymphatic endothelial cells during embryogenesis, it becomes specific for lymphatic endothelial cells during post-embryologic development.[11] Lymphangiogenesis arises not only in the developing organism, but also in adults, especially in cases of wound healing and within tumors. It has been shown that transient lymphangiogenesis occurs in the early phase of skin and cornea wound healing.[12–15] These mechanisms also could be of importance for intraocular inflammatory disorders, as well as if lymphatic vessels were capable of surmounting the eye sheath barrier in eye-opening trauma.

It has been shown that lymphatic vessels can invade physiologically alymphatic ocular structures under pathologic conditions. Firstly, blood and lymphatic vessels can grow from the limbus into the normally avascular cornea due to a variety of corneal diseases, such as keratitis, trauma, or chemical burn.[1,12] Secondarily, malignant melanomas of the ciliary body with extraocular extension are capable of inducing secondary lymphangiogenesis into the intraocular space of the eye.[16–18]

The primary role of lymphatic vessels is the transport of extravasated fluid, proteins, and cells. In case of malignant tumors, lymphatic vessels are relevant for the dissemination of tumor cells.[17,18] Furthermore, they take an important part in mediating immunologic responses. In the context of corneal injury or corneal transplantation, invading lymphatic vessels can serve as the "afferent arm of the immune reflex arc" by facilitating the passage of antigen-presenting cells with foreign antigens from the cornea or corneal graft to the regional lymph node. Blood vessels represent the "efferent arm of the immune reflex arc" by allowing invasion of immunologic effector cells into the cornea and, thus, enhancing an immune rejection, for example after corneal transplantation.[19,20]

Based on our observation that lymphatic vessels can invade the eye walls as well as the intraocular space of the eye under pathologic conditions, such as malignant melanomas of the ciliary body with extraocular extension,[16] we were interested in analyzing whether lymphatic vessels can be found in human eyes after open globe injuries. Therefore, in our study 21 eyes enucleated after an open globe injury were analyzed by means of immunohistochemistry.

Methods

We identified a total of 30 globes enucleated after open globe injury between 1993 and 2011 in the archives of the Ophthalmic Pathology Laboratory at the Department of Ophthalmology, Friedrich-Alexander University Erlangen-Nürnberg, Erlangen, Germany. The globes were received consecutively by the ophthalmic pathology laboratory and were not pre-selected for this study. All clinical files as well as histopathology reports were reviewed retrospectively. Inclusion criteria for this retrospective, nonrandomized, clinicopathologic, single-center study were histopathologically confirmed diagnosis of

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Table 1. Clinical Characteristics of 21 Patients Enucleated after Open Eye Injury with and without Pathologic Intraocular Lymphatic Vessels

	Total ($n = 21$)	With Pathologic Lymphatic Vessels ($n = 15$)		Without Pathologic Lymphatic Vessels ($n = 6$)
		In Eye Sheets ($n = 10$)	Inside the Eye ($n = 5$)	
Age at trauma (y)				
Mean ± SD (range)	48 ± 30 (1–89)	48 ± 36 (1–89)	77 ± 2 (75–78)	35 ± 19 (17–70)
Sex				
Male, *n* (%)	14 (77%)	5 (50%)	4 (80%)	5 (83%)
Female, *n* (%)	7 (33%)	5 (50%)	1 (20%)	1 (17%)
Laterality				
Right eye, *n* (%)	13 (62%)	7 (70%)	3 (60%)	3 (50%)
Left eye, *n* (%)	8 (38%)	3 (30%)	2 (40%)	3 (50%)
Eye surgeries before trauma				
Penetrating keratoplasty, *n* (%)	1 (5%)	1 (10%)	–	–
Cataract surgery, *n* (%)	1 (5%)	–	–	1 (17%)
Mechanism of trauma				
Perforation by foreign body, *n* (%)	16 (76%)	9 (90%)	3 (60%)	4 (67%)
Rupture by blunt contusion, *n* (%)	5 (24%)	1 (10%)	2 (40%)	2 (33%)
Anatomic site of perforation or rupture				
Cornea, *n* (%)	13 (62%)	6 (60%)	3 (60%)	4 (67%)
Anterior sclera, *n* (%)	2 (10%)	1 (10%)	1 (20%)	–
Posterior sclera, *n* (%)	3 (14%)	1 (10%)	–	2 (33%)
Cornea + anterior sclera, *n* (%)	–	–	–	–
Cornea + posterior sclera, *n* (%)	2 (10%)	1 (10%)	1 (20%)	–
Anterior + posterior sclera, *n* (%)	–	–	–	–
Cornea + anterior + posterior sclera, *n* (%)	–	–	–	–
N/A, *n* (%)	1 (5%)	1 (10%)	–	–
Therapeutic management				
Primary enucleation, *n* (%)	5 (24%)	4 (40%)	–	1 (17%)
Primary wound closure, *n* (%)	16 (76%)	6 (60%)	5 (100%)	5 (83%)
Posttraumatic cataract surgery, *n* (%)	1 (5%)	–	–	1 (17%)
Posttraumatic vitreoretinal surgery, *n* (%)	2 (10%)	–	1 (20%)	1 (17%)
Secondary enucleation, *n* (%)	16 (76%)	6 (60%)	5 (100%)	5 (83%)
Time interval between trauma and enucleation (y)				
Mean ± SD (range)	7 ± 9 (1 d–29 y)	5 ± 7 (1 d–17 y)	4 ± 6 (5 d–8 y)	10 ± 13 (8 d–29 y)
≤30 d, *n* (%)	7 (33%)	5 (50%)	1 (20%)	1 (17%)
31–365 d, *n* (%)	1 (5%)	–	–	1 (17%)
>365 d, *n* (%)	8 (38%)	3 (30%)	1 (20%)	4 (67%)
N/A, *n* (%)	5 (24%)	2 (20%)	3 (60%)	–
Visual acuity at enucleation				
No light perception, *n* (%)	8 (38%)	4 (40%)	2 (40%)	2 (33%)
Weak light perception, *n* (%)	4 (19%)	2 (20%)	–	2 (33%)
N/A, *n* (%)	9 (43%)	4 (40%)	3 (60%)	2 (33%)
Intraocular pressure at enucleation (mm Hg)				
<10, *n* (%)	10 (48%)	6 (60%)	2 (40%)	2 (33%)
10–21, *n* (%)	2 (10%)	1 (10%)	–	1 (17%)
>21, *n* (%)	2 (10%)	1 (10%)	–	1 (17%)
N/A, *n* (%)	7 (33%)	2 (20%)	3 (60%)	2 (33%)

N/A, not available; y, years; d, days.

globe perforation by foreign body or globe rupture by blunt contusion, and primary enucleation for injuries with severe damage or extensive inflammation without chance for surgical repair, or secondary enucleation for painful blind eyes following primary wound closure. Exclusion criteria were bilateral cases, contusion of the globe without rupture, and evidence for granulomatous inflammation of the choroid indicating sympathetic ophthalmia. Altogether, a total of 21 consecutive patients fulfilled these criteria and were included in the our clinicopathologic study, which was performed in conformance with the tenets of the Declaration of Helsinki. Institutional review board or ethics committee approval was not required in this instance.

Patient Population and Clinical Data

The 21 patients (7 females and 14 males) had a mean age of 48 ± 30 years (range 1–89 years) at the time of trauma (Table 1). Of the patients 2 (10%) had a history of an intraocular surgical procedure (including penetrating keratoplasty in 1 and cataract surgery in 1) before the traumatic event. The mechanism of trauma included perforation of the globe by a foreign body in 16 patients (76%) and rupture of the globe by a blunt contusion in 5 (24%). Perforation was caused by sharp items, such as broken glass, fireworks, a fork, and a hammer/chisel. Contusion of the globe was mediated by a metal bar or in connection with a car

accident. Two patients with autism or dementia, respectively, had an open globe injury in which the exact reason could not be elicited. The anatomic site of globe perforation or rupture included the cornea in 13 patients (62%), anterior sclera in 2 (10%), posterior sclera in 3 (14%), and cornea and posterior sclera in 2 (10%). Five eyes (24%) had to be enucleated primarily after the accident, and 16 (76%) secondarily following primary wound closure, and in some cases following posttraumatic cataract or vitreoretinal surgery. The mean time interval between trauma and enucleation was 4 ± 3 days (range 1–8 days) in cases of primary enucleation, and 12 ± 10 years (range 3 months–29 years) in case of secondary enucleation. Visual acuity at the time of enucleation was no light perception in 8 patients (38%) and weak light perception in 4 (19%). Intraocular pressure was within normal range (10–21 mm Hg) in 2 patients (10%), hypertonic in 2 (10%), and hypotonic in 10 (48%).

Histopathological Data

All enucleated globes were fixed in a buffered 4% formaldehyde-glutaraldehyde solution, dehydrated, and embedded in paraffin. Serial sections in the pupil-to-optic disc plane cut at 5 μm were stained with hematoxylin and eosin, and PAS, and analyzed by 3 independent investigators by microscope (Axiophot; Carl Zeiss, Oberkochen, Germany) to confirm the diagnosis histopathologically. Main criteria of the histologic analyses included traumatic alterations of cornea, anterior chamber, and its angle, lens, uvea, retina, vitreous, optic nerve, and sclera as well as signs of chronic ocular degeneration (calcification, ossification, phthisis) or intraocular foreign body. The anatomic site of perforation or rupture was confirmed histologically, and subdivided into cornea, anterior sclera (i.e., covered by conjunctiva), and/or posterior sclera (i.e., not covered by conjunctiva). All globes were assessed thoroughly for morphologic changes indicative of sympathetic ophthalmia, such as granulomatous inflammation in the choroid, ciliary body or iris, and granuloma between Bruch's membrane and retinal pigment epithelium (Dalen-Fuchs nodules).[21] All histopathologic data are summarized in Table 2.

Immunohistochemical Detection of Lymphatic Vessels

To identify lymphatic vessels, immunohistochemistry was performed in all 21 cases as described previously.[10] For the differentiation between lymphatic and blood vessels, all paraffin-embedded sections were double-stained using a monoclonal antibody against the human lymphatic vascular endothelial-specific glycoprotein podoplanin D2-40 (mouse, 1:40; AbD Serotec, Kidlington, Oxford, UK) as a specific marker for lymphatic vascular endothelium and a monoclonal antibody against the 400 kD alpha- and 220 kD beta-chain of laminin (rabbit, 1:100; Medac, Hamburg, Germany), a basement membrane glycoprotein of blood vessels. Podoplanin D2-40 is considered a highly specific and well-established antibody for lymphatic endothelium,[22] showing expression patterns similar to lymphatic vascular endothelium-specific hyaluronan receptor LYVE-1.[4]

Binding of the primary antibody was visualized using a horseradish peroxidase mediated reaction with the substrates 3-amino-9-ethylcarbozole (AEC) and 3,3′-diaminobenzidine (DAB) as described previously.[12] Lymphatic vessels on corneoscleral rims were marked by the antibody against podoplanin D2-40. Following incubation of the specimen with immunoglobulin G, which serves as negative control, no enzymatic reaction with the substrate could be detected.

Serial sections were evaluated for evidence of lymphatic vessels by 3 independent investigators in a masked fashion using a microscope (Axiophot; Carl Zeiss) combined with a computer-aided image analysis system (Axio-Vision 4.6; Carl Zeiss). We counted as lymphatic vessels only those structures that demonstrated immunopositivity for podoplanin and immunonegativity for laminin, as well as having a clearly identifiable erythrocyte-free lumen. Presence of pathologic lymphatic vessels was defined as detection of at least 5 podoplanin$^+$/laminin$^-$ vessels per section with an erythrocyte-free lumen located within the eye wall (i.e., cornea and sclera) and/or inside the eye. Presence of intraocular lymphatic vessels was defined as detection of at least 3 podoplanin$^+$/laminin$^-$ vessels per section with an erythrocyte-free lumen located below the endothelium-Descemet's membrane layer and/or below the inner scleral layers within the intraocular space of the eye.

Statistical Analyses

Commercial software was used for all statistical analyses (SPSS version 18.0 for Windows; SPSS, Inc., Chicago, IL). Comparisons between the presence of pathologic or intraocular lymphatic vessels and the other clinicopathologic variables were performed using the nonparametric Mann-Whitney U test, Fisher exact test, and Pearson's χ^2 test. $P < 0.05$ was considered statistically significant.

Results

Identification of Lymphatic Vessels

Pathologic podoplanin$^+$/laminin$^-$ lymphatic vessels could be detected in 15 of 21 eyes (71%) enucleated after open globe injury. They were found in the superficial corneal stroma in 7 eyes, the upper and deep corneal stroma in 5, and the superficial and deep sclera in 3. They were most pronounced at the anatomic site of the eye wall defect (Figs. 1, 2), and formed a migration track into the depth in 5 cases (Fig. 2).

Intraocular podoplanin$^+$/laminin$^-$ lymphatic vessels could be observed in 5 of 21 eyes (24%) enucleated after open globe injury. They were located adjacent to the ciliary body and iris in 4 cases (Fig. 2), within retrocorneal membranes in 1 (Fig. 3), and underneath the sclera in 1 (Fig. 3).

In 6 of 21 eyes (29%) enucleated after open globe injury, podoplanin$^+$/laminin$^-$ lymphatic vessels were detectable only at the corneal limbus, but not within the corneal stroma, sclera, or intraocular space of the eye.

Association with Clinical Features of Open Eye Injury

The presence of pathologic podoplanin$^+$/laminin$^-$ lymphatic vessels was not associated significantly with age ($P = 0.151$), sex ($P = 0.613$), history of an intraocular surgical procedure before the traumatic event ($P = 0.500$), mechanism of trauma ($P = 0.598$), anatomic site of perforation or rupture ($P = 0.303$), therapeutic management ($P = 0.550$), time interval between trauma and enucleation ($P = 0.145$), visual acuity ($P = 0.569$), and intraocular pressure ($P = 0.739$).

No significant association was observed between the presence of intraocular podoplanin$^+$/laminin$^-$ lymphatic vessels and age ($P = 0.203$), sex ($P = 0.443$), history of an intraocular surgical procedure before the traumatic event ($P = 0.571$), mechanism of trauma ($P = 0.553$), anatomic site of perforation or rupture ($P = 0.501$), therapeutic management ($P = 0.278$), time interval between trauma and enucleation ($P = 0.700$), visual acuity ($P = 0.426$), and intraocular pressure ($P = 0.435$).

Association with Pathological Features of Open Eye Injury

The presence of pathologic podoplanin$^+$/laminin$^-$ lymphatic vessels was not associated significantly with traumatic alterations of the cornea ($P = 0.598$), anterior chamber and its angle ($P = 0.523$), lens ($P = 0.361$), uvea ($P = 0.523$), retina ($P = 0.354$), vitreous ($P = 0.123$), optic nerve ($P = 0.590$), and sclera

Table 2. Histopathological Characteristics of 21 Globes Enucleated after Open Eye Injury with and without Pathologic Intraocular Lymphatic Vessels

	Total ($n = 21$)	With Pathologic Lymphatic Vessels ($n = 15$)		Without Pathologic Lymphatic Vessels ($n = 6$)
		In Eye Sheets ($n = 10$)	Inside the Eye ($n = 5$)	
Alterations of the cornea				
Band keratopathy, *n* (%)	6 (29%)	2 (20%)	2 (40%)	2 (33%)
Vascularized pannus, *n* (%)	4 (19%)	3 (30%)	1 (20%)	–
Vascularized superficial scar, *n* (%)	6 (29%)	2 (20%)	2 (40%)	2 (33%)
Full-thickness scar, *n* (%)	2 (10%)	–	–	2 (33%)
Edema, *n* (%)	3 (14%)	–	1 (20%)	2 (33%)
Purulent inflammation, *n* (%)	2 (10%)	2 (20%)	–	–
Ulcer, *n* (%)	1 (5%)	–	1 (20%)	–
Retrocorneal membrane, *n* (%)	3 (14%)	–	3 (60%)	–
Full-thickness defect, *n* (%)	7 (33%)	5 (50%)	2 (40%)	–
Alterations of the anterior chamber and its angle				
Anterior synechiae, *n* (%)	6 (29%)	1 (10%)	4 (80%)	1 (17%)
Flat anterior chamber, *n* (%)	4 (19%)	2 (20%)	1 (20%)	1 (17%)
Hyphema, *n* (%)	1 (5%)	–	1 (20%)	–
Secondary angle block, *n* (%)	8 (38%)	2 (20%)	3 (60%)	3 (50%)
Epithelial ingrowth, *n* (%)	–	–	–	–
Alterations of the lens				
Rupture of lens capsule, *n* (%)	1 (5%)	1 (10%)	–	–
Aphakia, *n* (%)	10 (48%)	6 (60%)	2 (40%)	2 (33%)
Alterations of the uvea				
Cyclodialysis, *n* (%)	2 (10%)	–	–	2 (33%)
Aniridia, *n* (%)	4 (19%)	2 (20%)	1 (20%)	1 (17%)
Rubeosis iridis, *n* (%)	2 (10%)	–	1 (20%)	1 (17%)
Posterior synechiae, *n* (%)	2 (10%)	–	1 (20%)	1 (17%)
Occlusion membrane, *n* (%)	1 (5%)	–	1 (20%)	–
Full-thickness iris defect, *n* (%)	1 (5%)	–	1 (20%)	–
Cyclitic membrane, *n* (%)	3 (14%)	1 (10%)	1 (20%)	1 (17%)
Ciliary body detachment, *n* (%)	1 (5%)	1 (10%)	–	–
Choroidal detachment, *n* (%)	2 (10%)	2 (20%)	–	–
Choroidal hemorrhage, *n* (%)	3 (14%)	1 (10%)	1 (20%)	1 (17%)
Uveal prolapse, *n* (%)	2 (10%)	1 (10%)	1 (20%)	–
Sympathetic ophthalmia, *n* (%)	–	–	–	–
Alterations of the retina				
Total detachment, *n* (%)	13 (62%)	8 (80%)	3 (60%)	2 (33%)
Partial loss, *n* (%)	3 (14%)	2 (20%)	–	1 (17%)
Epiretinal membrane, *n* (%)	1 (5%)	–	–	1 (17%)
Proliferative vitreoretinopathy, *n* (%)	3 (14%)	–	1 (20%)	2 (33%)
Alterations of vitreous and optic nerve				
Vitreous hemorrhage, *n* (%)	3 (14%)	1 (10%)	2 (40%)	–
Endophthalmitis, *n* (%)	2 (10%)	1 (10%)	1 (20%)	–
Silicone oil tamponade, *n* (%)	1 (5%)	1 (10%)	–	–
Optic nerve atrophy, *n* (%)	8 (38%)	4 (40%)	2 (40%)	2 (33%)
Alterations of the sclera				
Full-thickness defect of ant. sclera, *n* (%)	1 (5%)	1 (10%)	–	–
Full-thickness scar of anterior sclera, *n* (%)	1 (5%)	–	1 (20%)	–
Full-thickness defect of posterior sclera, *n* (%)	2 (10%)	2 (20%)	–	–
Full-thickness scar of posterior sclera, *n* (%)	3 (14%)	–	1 (20%)	2 (33%)
General alterations				
Phthisis bulbi, *n* (%)	6 (29%)	1 (10%)	2 (40%)	3 (50%)
Intraocular calcification, *n* (%)	1 (5%)	–	–	1 (17%)
Intraocular ossification, *n* (%)	4 (19%)	2 (20%)	1 (20%)	1 (17%)
Intraocular foreign body, *n* (%)	5 (24%)	2 (20%)	–	3 (50%)

($P = 0.686$), as well as signs of chronic ocular degeneration ($P = 0.354$) or intraocular foreign body ($P = 0.115$).

No significant association was observed between the presence of intraocular podoplanin$^+$/laminin$^-$ lymphatic vessels and traumatic alterations of the cornea ($P = 0.662$), anterior chamber and its angle ($P = 0.119$), lens ($P = 0.635$), uvea ($P = 0.647$), retina ($P = 0.557$), vitreous ($P = 0.115$), optic nerve ($P = 0.656$), and sclera ($P = 0.557$), as well as signs of chronic ocular degeneration ($P = 0.557$) or intraocular foreign body ($P = 0.278$).

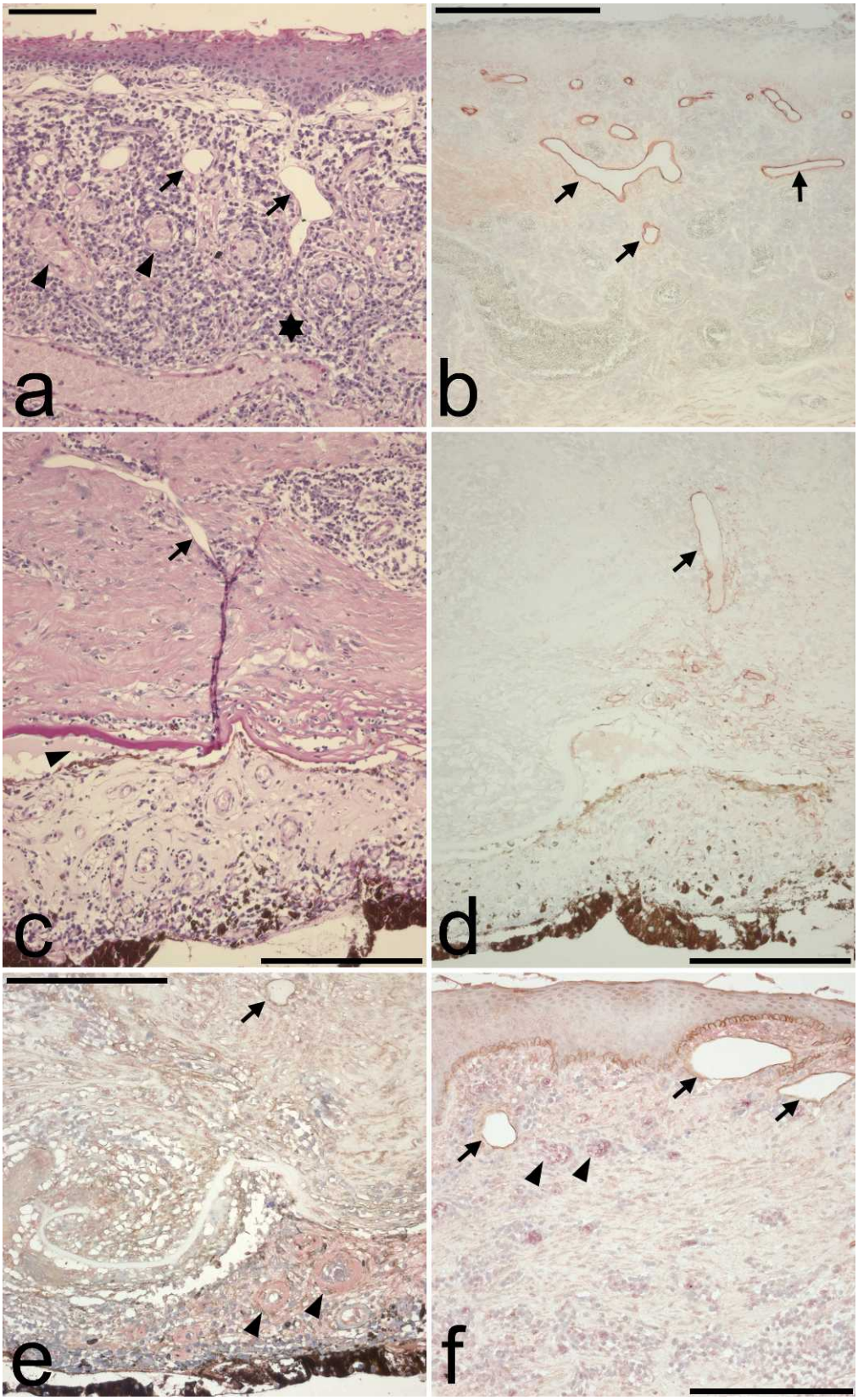

Figure 1. Migration of lymphatic vessels into the perforated cornea in an eye enucleated 3 days after corneal perforation. (**a**) Lymphatic (*arrows*) and blood (*arrowheads*) vessels in the superficial corneal stroma with marked inflammatory infiltration (*asterisk*). PAS stain. (**b**) Podoplanin$^+$ lymphatic vessels (*arrows*) in the superficial corneal stroma (podoplanin, *red*). (**c**) Lymphatic vessel (*arrow*) in the deep corneal stroma and flat anterior chamber (*arrowhead*). PAS stain. (**d**) Podoplanin$^+$ lymphatic vessel (*arrow*) in the deep corneal stroma (podoplanin, *red*). (**e**) Podoplanin$^+$ lymphatic vessel (*arrow*) in the deep corneal stroma, laminin$^+$ blood vessel (*arrowhead*) in the iris and flat anterior chamber (podoplanin, *brown*; laminin, *red*). (**f**) Podoplanin$^+$ lymphatic vessels (*arrows*) and laminin$^+$ blood vessels (*arrowheads*) in the superficial corneal stroma (podoplanin, *brown*; laminin, *red*). Length of scale bar, 50 μm.

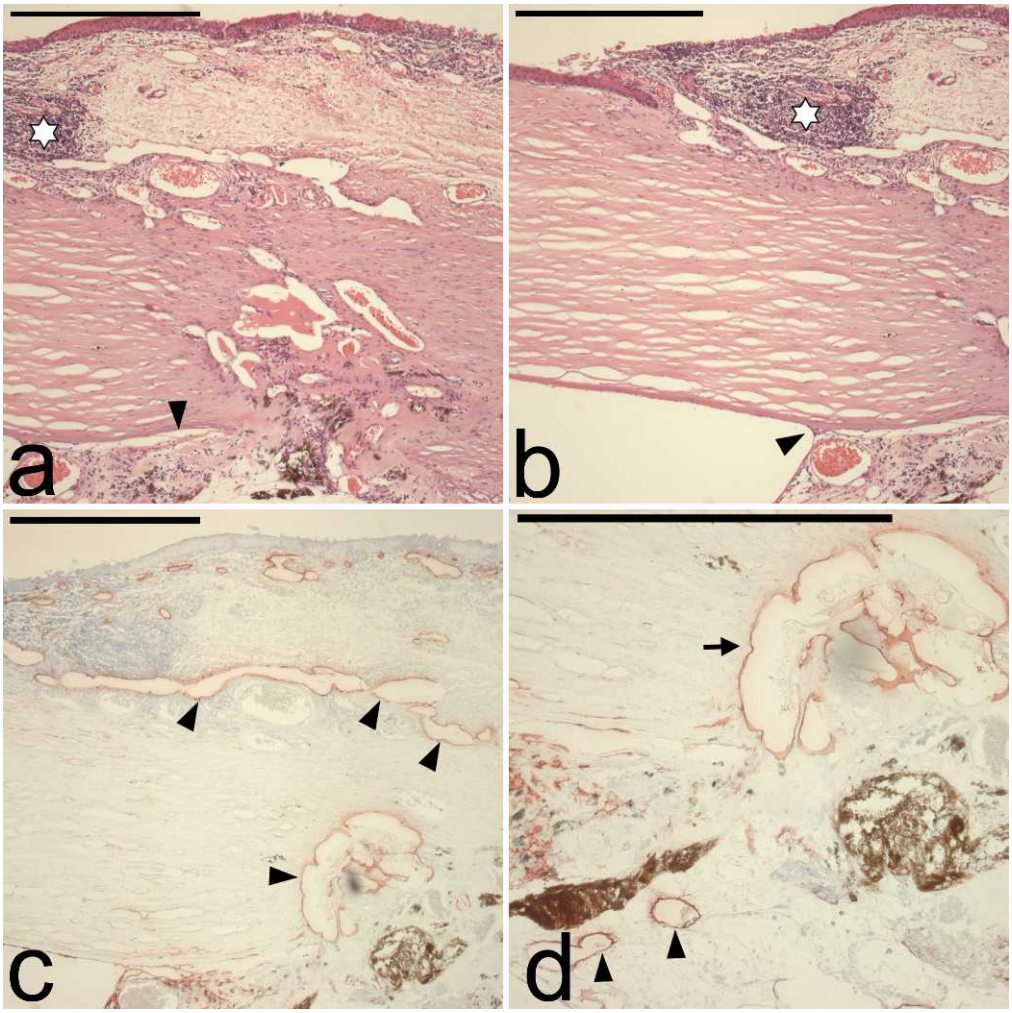

Figure 2. Presence of intraocular lymphatic vessels adjacent to the iris in an eye enucleated 8 years after corneoscleral perforation. (**a**, **b**) Vascularized full-thickness corneal scar at the corneoscleral junction with inflammatory infiltration in the superficial cornea (*asterisk*) and anterior synechia (*arrowhead*). Hematoxylin-eosin stain. (**c**) Dilated podoplanin$^+$ lymphatic vessels (*arrowheads*) with erythrocyte-free lumen, forming a migration track into the depth (podoplanin, *red*). (**d**) Dilated podoplanin$^+$ lymphatic vessels (*arrows*) in the deep corneal stroma and behind the pigment epithelium of the iris (*arrowhead*). Podoplanin, *red*. Length of scale bar, 50 μm.

Discussion

Our clinicopathologic study revealed two novel important findings. First, podoplanin$^+$/laminin$^-$ lymphatic vessels can be detected within the cornea, sclera, and intraocular space of globes enucleated after open globe injury. To our knowledge, following our studies on intraocular malignancies with extraocular extension,[16–18] this is the second evidence of secondary lymphangiogenesis into the physiologically alymphatic intraocular space. Secondly, the presence of these pathologic lymphatic vessels does not seem to be associated with the mechanism of trauma, anatomic site of the eye wall defect, and time interval between trauma and enucleation. This mechanism of secondary ingrowth of lymphatic vessels into the eye after open eye injury may be important for wound healing, immunologic defense against intruding microorganisms, and autoimmune reactions against intraocular antigens.

To our knowledge, intraocular lymphatic vessels after open globe injury were observed for the first time in our study. Apart from the extraocular conjunctiva and corneal limbus,[1–4] the human eye physiologically is devoid of lymphatic vessels.[5] Even the normal human choroid, endowed with a significant number of lymphatic vessel endothelial hyaluronan receptor-1 (LYVE-1)$^+$ macrophages, does not contain typical lymphatic vessels.[6] Recently, new lymphatic channels were suggested for the human and sheep ciliary body.[23] However, the absence of LYVE-1$^+$/podoplanin$^+$ lymphatic vessels with an erythrocyte-free lumen in the ciliary body region of human control eyes devoid of ciliary body melanoma, and eyes with ciliary body melanoma without extraocular extension indicates that the normal eye is thought to be devoid physiologically of true lymphatic vessels.[16]

It has been shown for pathologic conditions that preexisting lymphatic vessels of conjunctiva and limbus are able to grow secondarily into physiologically lymphatic-free ocular structures, such as cornea.[12] Secondary to several inflammatory diseases of the cornea, blood and lymphatic vessels can invade the normally avascular cornea.[1,12,15,19,20,24] Furthermore, malignant melanomas of the ciliary body can attract extraocular lymphatics into the physiologically alymphatic eye when extraocular tumor extension provides routes of entry through the sclera, which otherwise seems to be a natural barrier against the invasion of lymphatic vessels.[16–18] Our data suggested that in a similar manner, preexisting conjunctival and/or limbal lymphatic vessels can outgrow and proliferate into the eye if the eye wall is opened by trauma.

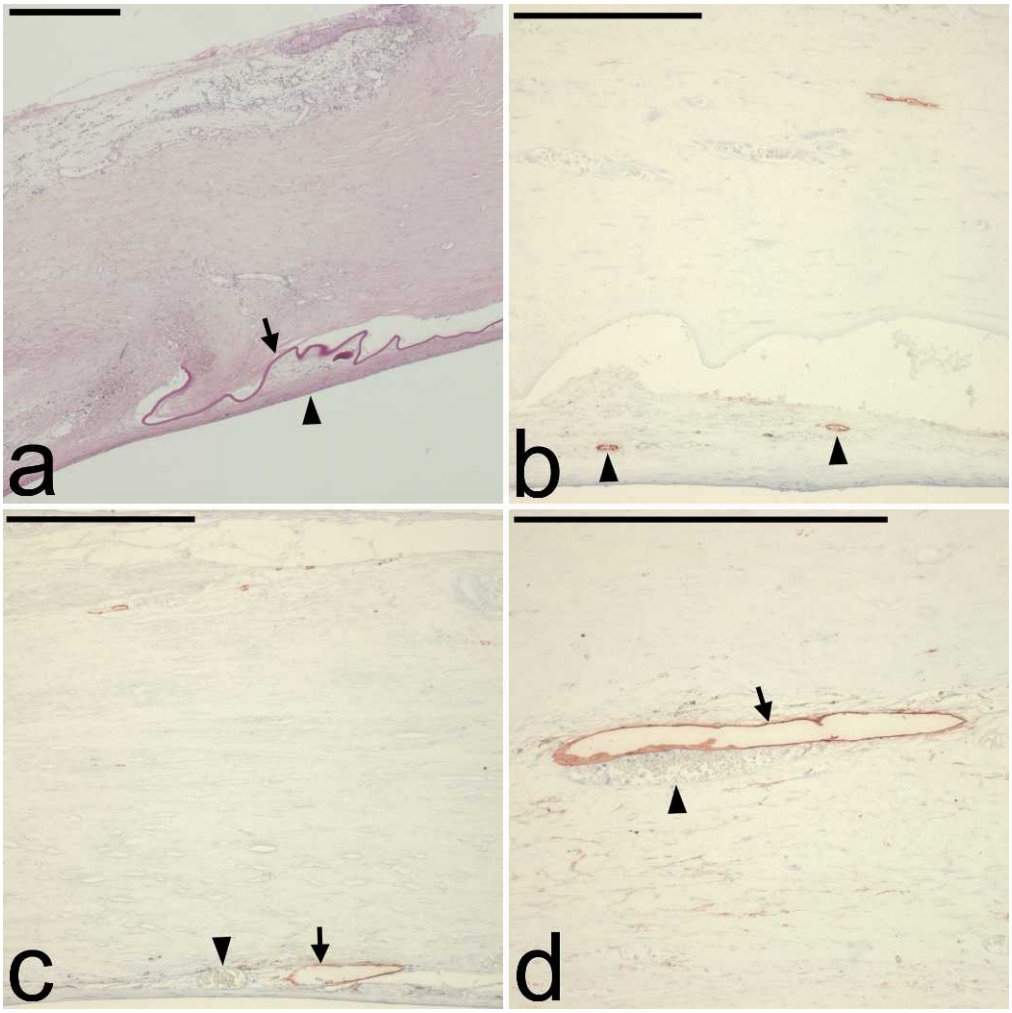

Figure 3. Presence of intraocular lymphatic vessels within a retrocorneal membrane, and underneath the sclera in an eye enucleated many years after corneal and posterior scleral rupture. (**a**) Cornea with crimped Descemet's membrane (*arrow*) and retrocorneal membrane (*arrowhead*). PAS stain. (**b**) Small podoplanin$^+$ lymphatic vessels (*arrowheads*) within the retrocorneal membrane (podoplanin, *red*). (**c**) Podoplanin$^+$ lymphatic vessel (*arrow*) beside podoplanin$^-$ blood vessel (*arrowhead*) on the inner surface of the sclera (podoplanin, *red*). (**d**) Podoplanin$^+$ lymphatic vessel (*arrow*) beside podoplanin$^-$ blood vessel (*arrowhead*) within the sclera (podoplanin, *red*). Length of scale bar, 50 μm.

The hypothesis that the intraocular lymphatics that we detected actually are invading rather than displaced preexisting conjunctival lymphatic vessels is supported by the following observations. First, intraocular lymphatic vessels could be detected not only after perforation of the globe by a foreign body, but also after rupture of the globe by a blunt contusion. Second, in all enucleated globes showing intraocular lymphatic vessels there were no signs of posttraumatic diffuse or cystic epithelial ingrowth. Therefore, we concluded that lymphatic vessels actually can grow into the depth and do not originate from displaced conjunctival tissue. At this stage, we cannot rule out the remote possibility of a de novo formation of intraocular lymphatic vessels. Stimulating factors, such as interleukins, could be secreted in the opened, inflamed eye and, thereby, activate lymphvasculogenesis. Nevertheless, in view of the localization and distribution of the pathologic lymphatic vessels, we favor the concept of secondary ingrowth of conjunctival lymphatic vessels into the eye (secondary intraocular lymphangiogenesis). Our histopathologic analysis of the enucleated globes with intraocular lymphatic vessels revealed mostly a track of podoplanin$^+$/laminin$^-$ lymphatic vessels along the borders of the corneal or scleral defect. In addition, the density of lymphatic vessels in these areas usually diminished in the deeper parts of the sclera or cornea. This kind of active "lymphangiotaxis" may be the result of increased levels of prolymphangiogenic factors, such as interleukin-7,[25] VEGF-C or –D, and decreased levels of antilymphangiogenic inhibitors owing to the corneal or scleral weakness. Further studies, including the biochemical analysis of vitreous and aqueous humor of traumatically harmed eyes, are necessary. However, we did not observe distinct connections between the preexisting conjunctival and limbal lymphatics and the pathologic lymphatic vessels within the intraocular space of the eye, but the immediate vicinity suggests their likely existence.

In our small series, there was no significant association between the presence of these pathologic lymphatic vessels and the mechanism of trauma, anatomic site of the eye wall defect, and time interval between trauma and enucleation. Future larger prospective studies must define that association more closely. Particularly, the time course of lymphatic vessels' ingrowth, and its dependence on the access of the eye wall defect to preexisting limbal, conjunctival, and orbital lymphatic vessels must be defined in detail.

Nevertheless, the main significance of intraocular lymphatic vessels after open globe injury is that they could be important

for wound healing, immunologic defense against intruding microorganisms, and autoimmune reactions against intraocular antigens. It has been shown that transient lymphangiogenesis occurs in the early phase of wound healing. Paavonen et al. could detect upregulation of VEGFR-3$^+$ lymphatic vessels peaked on day 6 in experimental skin wounds in pigs and, to a smaller degree, in chronic wounds in humans.[13] Similarly, after experimental penetrating keratoplasty in the mouse model, a combined outgrowth of blood and lymphatic vessels can be observed starting at day 3 and reaching the donor-host interface at day 7.[15] The same phenomenon of postkeratoplasty neovascularization occurs in patients, albeit to a lesser extent.[12] These data are concordant with the time-course of secondary lymphangiogenesis after open globe injury in our study.

Intraocular lymphatic vessels after open globe injury could form the afferent arm of the immunologic cycle in the defense against intruding microorganisms. Cursiefen et al. analyzed vascularized corneas and ascertained that lymphatic vessels were most pronounced in areas with severe inflammatory infiltration.[12] Our study supports these findings in enucleated eyes after open globe injury. Pathologic podoplanin$^+$/laminin$^-$ lymphatic vessels were observed to be more condensed and dilated within or adjacent to areas of severe posttraumatic inflammation. A possible explanation could be the putative role of cytokines, such as interleukin 7, as prolymphangiogenic factors.[25]

Interestingly, even years after open globe injury lymphatic vessels persist and do not regress, as seems to be the case in the cornea.[1,12,26] The observation that invaded lymphatic vessels persist in some eyes over years and vanish in others is difficult to explain. We hypothesize that internal factors, such as cytokines or persisting local inflammation after trauma, as well as external factors (medical treatment, interval between trauma and treatment, and so forth) may influence whether lymphatic vessels can persist within the eye. These aspects need further evaluation in larger studies. The persistence of lymphatic vessels may be important in eyes with sympathetic ophthalmia. Sympathetic ophthalmia is a bilateral, diffuse, granulomatous, T-cell mediated uveitis that occurs as early as 5 days or as late as 50 years after penetrating or perforating ocular injury.[27] Persisting lymphatic vessels could act as an afferent pathway for immunologic response in these cases, suggesting perhaps a potential for novel antilymphangiogenic therapies in these patients.[28] However, all of our three hypotheses on the functional significance of intraocular lymphatic vessels require further investigation.

In conclusion, secondary invasion of lymphatic vessels into the physiologically alymphatic intraocular space can be found not only in intraocular malignant tumors with extraocular extension,[16–18] but also in traumatically injured eyes with opening of the eye wall. Intraocular lymphatic vessels after open globe injury could be important for wound healing, immunologic defense against intruding microorganisms, and autoimmune reactions against intraocular antigens.

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