

Clinical correlates of common corneal neovascular diseases: a literature review

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Abstract

• **A large subset of corneal pathologies involves the formation of new vessels (neovascularization), leading to compromised visual acuity. This article aims to review the clinical causes and presentations of corneal neovascularization (CNV) by examining the mechanisms behind common CNV-related corneal pathologies, with a particular focus on herpes simplex stromal keratitis, contact lenses-induced keratitis and CNV secondary to keratoplasty. Moreover, we reviewed CNV in the context of different types of corneal transplantation and keratoprosthesis, and summarized the most relevant treatment available so far.**

• **KEYWORDS:** cornea; neovascularization; herpes simplex keratitis; keratoplasty; contact lens; keratoprosthesis

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INTRODUCTION

Corneal Neovascularization: a Growing Global Burden Neovascular and infectious diseases of the cornea and other parts of the eye represent a major public health burden. Although the exact incidence and prevalence rates of corneal neovascularization (CNV) globally is still unknown, the incidence rate was estimated at 1.4 million patients per year based on an extrapolation of the 4.14% prevalence rate at the Massachusetts Eye and Ear Infirmary in 1998^[1].

Moreover, twenty percent of the corneal specimens obtained during corneal transplantation procedures showed histopathologic evidence of neovascularization (NV)^[1-4]. CNV does not only reduce visual acuity, but also worsens the prognosis of subsequent penetrating keratoplasty, keeping the patient in a vicious circle of bad prognosis^[2-5]. As such, there is a tremendous need for developing effective measures to prevent and/or treat CNV based on an understanding of its molecular pathogenesis.

CNV involves the sprouting of new vessels essentially from capillaries and venules of the pericorneal plexus. There are three clinical entities of CNV that can be discerned: 1) deep NV overlying Descemet's membrane seen in herpetic and syphilitic stromal keratitis; 2) stromal NV mainly associated with most forms of stromal keratitis; and 3) vascular pannus which is composed of connective tissue proliferating in the superficial corneal periphery and mainly associated with ocular surface disorders^[2,6]. Differentiating features between superficial and deep CNV are included in Table 1^[7].

Risk Factors and Clinical Causes of Corneal Neovascularization Risk factors for CNV have been assessed in patients' status post penetrating keratoplasty without active inflammation, previous CNV, or persistent epithelial defects. The risk of CNV was increased when suture knots were buried in the host stroma, when active blepharitis was present, or when a large recipient bed was used^[1].

Causes of CNV in ophthalmology patients can be classified as shown in Figure 1 as congenital causes such as aniridia; or acquired causes which can be inflammatory^[2-6], degenerative^[5,7-10], traumatic, iatrogenic, or infectious^[1,11,12].

Localization of New Blood and Lymphatic Vessels CNV can be derived from stroma, which is mainly associated with

Table 1 Types of new blood vessels invading the cornea

Blood vessels	Character	Origin
Conjunctival vessels	Crossing the limbus; run in superficial stromal layers; bright red; well defined; branching; may raise irregular epithelium	Superficial vascularization
Anterior ciliary vessels	Disappearing at the limbus; run in deeper stromal layers; dark red; ill-defined; parallel and radial; cannot raise epithelium	Deep vascularization

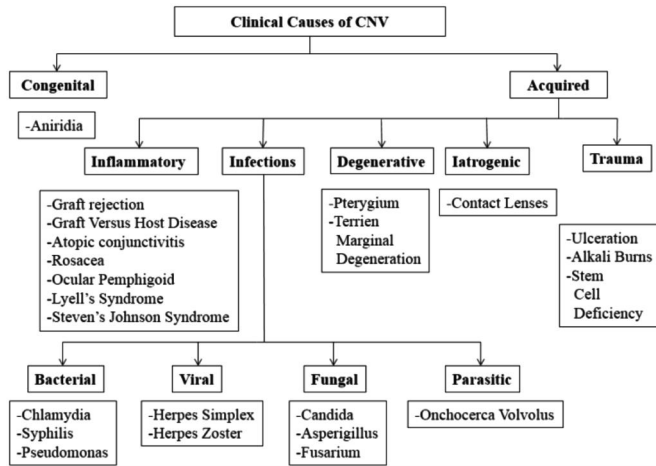


Figure 1 Causes of corneal neovascularization.

stromal keratitis. It can also develop from the superficial corneal periphery, which is mainly associated with ocular surface disorders, such as Stevens-Johnson syndrome, ocular pemphigoid, and thermal or chemical burns [13,14]. Although NV may involve several corneal layers, a study has demonstrated that the main locations of vascularized corneal buttons are in the upper and middle third areas of the anterior stroma [15]. Similarly, induced lymphatic vessels are localized to the corneal subepithelium and stromal layers in the wounded cornea.

Commonest Causes of Clinical Corneal Neovascularization

Herpes simplex stromal keratitis Herpes simplex virus (HSV)-1 is an extraordinarily common human pathogen; the virus infects innervating sensory neurons and establishes a latent infection within the connecting trigeminal ganglion. Periodic reactivation of latent virus within infected ganglia can lead to anterograde transport of the virus back to the periphery causing recrudescence disease, such as the common cold sore or herpetic keratitis [16]. In developed countries, the incidence and prevalence of herpetic ocular surface disease has been estimated to be 5.9-20.7 and 149 per 100 000 person-years, respectively [17].

Although other members of the herpes virus family encode angiogenic proteins [18,19], HSV-1 is not known to directly produce such factors. Nonetheless, proangiogenic cytokines of the vascular endothelial growth factor (VEGF) family are upregulated in the murine cornea following HSV-1 infection [20].

Initial reports indicated the source of VEGF-A to be noninfected corneal epithelial cells neighboring

HSV-infected cells, suggesting that HSV-infected cells were secreting a soluble mediator, acting in a paracrine fashion to induce VEGF production [20,21]. However, a recent report using, perhaps, more sensitive methodology, has identified HSV-infected epithelial cells as the primary source of VEGF-A [22]. Hemangiogenesis is dependent on VEGF-A binding to the VEGF receptor (VEGFR)-1 or 2, while inflammatory lymphangiogenesis is predominantly mediated *via* VEGF-C or D signaling through VEGFR-3 [23]. However, VEGF-A has recently been shown to also mediate lymphangiogenesis following corneal HSV-1 infection [22]. In a murine model of herpes simplex keratitis (HSK), blockade of VEGF-A-mediated signaling through VEGFR-1 and -2 diminished hemangiogenesis and abrogated HSK [20,24]. The effect of inhibiting lymphangiogenesis during corneal HSV-1 infection has yet to be studied. This is an important issue, as selective inhibition of lymphangiogenesis, compared with hemangiogenesis, prevented immune rejection of murine corneal transplants, which is also a CD4 T-cell-mediated immunopathology [25]. The VEGF proteins, as well as certain chemokines, induce angiogenesis by binding to receptors on vascular endothelial cells, causing them to undergo growth and movement. Other molecules influence angiogenesis by breaking down the extracellular matrix, thus facilitating neovessel growth. Matrix-degrading proteases include the collagenases, for example, matrix metalloproteinase-2 (MMP-2) and MMP-9 and heparanases [26]. Some results demonstrate that MMP-9, while undetectable in normal eyes, is produced in the cornea in response to HSV infection. A prominent cell type that produces MMP early after infection is invading neutrophils. When MMP-9 levels were suppressed (as could be achieved by neutrophil depletion) by inhibition with the specific inhibitor tissue inhibitor of metalloproteinase-1 (TIMP-1) or by using MMP-9 knockout mice, HSV-induced angiogenesis was inhibited [27]. It is now known that "matricellular proteins", a group of disparate proteins expressed during development but not in adults are upregulated in sites of tissue re-modelling and act temporally and spatially to provide regulatory signals in cell-cell and cell-matrix interactions [28]. One of the matricellular proteins, extensively studied in the corneal *in vivo* models, is the platelet-derived glycoprotein thrombospondin (TSP). TsPs are a family of five glycoproteins the first two of which TSP 1 and TSP 2 are involved in wound healing and are potent antiangiogenic agents [29,30]. Given that TSP 1 and 2 play an

important role in corneal scarring and vascularisation the next question is their source in the cornea. One mechanism could be by invading blood vessels in the cornea, which have been shown to appear as early as 24h after infection *in vivo*^[27]. Scarring, however, also develops in an avascular cornea, where fibroblasts and not platelets are the predominant cells. This led to the search for a local reservoir of TSP in the cornea. This reservoir was shown to be keratocytes, which express TSP 1 and 2 in an *in vitro* stromal wound repair model^[31]. TSP 1 acts by modulating cellular responses to extra-cellular matrix (ECM) and can also bind and activate TGFb^[29].

Contact lenses induced keratitis It was emphasized that for the general population, the most likely situation in which CNV will be encountered is in association with contact lenses. Around 10% to 30 % of all cases of CNV have contact lens involvement^[32].

Rigid gas permeable (RGP) lenses are much less likely to be associated with CNV than are soft contact lenses (SCL); this is attributed to the fact that typical RGP diameters are in the region of 9 to 10 mm, covering only the central portion of the cornea during wear. Conversely, SCLs are substantially larger, at around 13 to 15 mm, covering the entire cornea, the limbus, and the surrounding peri-limbal conjunctiva. The effect of this additional coverage with SCLs is reducing access of the underlying tissues to oxygen from the atmosphere and that dissolved in the tear film. As a result, the peripheral cornea and limbus are likely to experience some degree of hypoxia^[32]. Although there is debate about whether peripheral hypoxia is a sufficient stimulus in itself to cause CNV, the associated short term vascular changes are apparently identical to those seen where CNV does eventually occur^[32]. An early manifestation of this is hyperaemia within the limbal vessels, a response that has been recognized for many years during SCL wear and one that is directly associated with the hypoxia they produce^[33].

One other key aspect of lens wear that impacts CNV is the mode of use. Wearing lenses for long periods of time, and particularly during periods of sleep, *i.e.* extended or continuous wear, carries a higher risk than the conventional daily wear format where lenses are removed prior to eye closure. Conditions in the closed eye are indicative of a state of subclinical inflammation with huge increases in polymorphonuclear leukocyte recruitment and the upregulation of several factors that have potentially angiogenic properties^[34]; under normal circumstances these are balanced by a complementary upregulation of angiostatic factors, thus maintaining the status quo. Contact lens wear appears to carry the potential to alter this balance and again the increased hypoxic load may be the key factor. During sleep, the oxygen tension at the front surface of the contact lens reduces from the 155 mm Hg available in the

atmosphere to that provided by the vessels of the palpebral conjunctiva, *i.e.* about 55 mm Hg. Placing a contact lens between the closed eyelid and the cornea potentially further restricts this already reduced oxygen supply^[32].

Recently, a substantial new alternative has been provided by the emergence of silicone hydrogel materials. These new polymers allow the fabrication of contact lenses with the same dimensions and comfort levels as traditional SCLs but with very much higher oxygen transmission properties. Use of these lenses makes it possible to reduce hypoxia during wear to levels that, in many cases, approximate the non-wearing situation^[32]. Early indications are that these materials can significantly reduce the occurrence of CNV during SCL wear^[35].

While contact lens associated CNV would benefit greatly from the elimination of hypoxia, it would not be reasonable to assume that this is the only cause. Poorly designed or badly fitted lenses can cause direct mechanical injury to the ocular surface or, if coupled with poor biocompatibility, severe tear film disruption^[32].

There are several steps in the contact-lens-induced NV process: 1) limbal hyperemia, a dilatation of existing limbal capillaries, which is reversible and common with hydrogel soft contact lenses worn overnight, but can also occur with any tightly fitting contact lens; 2) superficial NV (pannus), which is the progression of limbal hyperemia and the penetration of vessels into the superficial cornea; 3) deep stromal NV, which results from chronic hypoxia that may progress to an active inflammatory or fibrovascular deep pannus; and 4) intracorneal hemorrhage, which might occur in some cases^[36].

The prevalence of NV appears to be low with RGP or poly-methyl methacrylate (PMMA) contact lenses, more common with daily-wear soft contact lenses, higher with extended-wear soft contact lenses, and very high with aphakic extended-wear lenses. NV is more common with soft contact lenses than with microcorneal lenses because the soft contact lens covers the entire cornea^[36].

There is no single theory that can account for CNV; rather, several factors may contribute^[37]; proposed theories take the following aspects into account: metabolic factors (hypoxia, lactic acid, edema, stromal softening); angiogenic suppression (necessity of substances that inactivate the normally present angiogenic inhibitors); vasostimulation (locally generated or introduced vasostimulatory factors such as free cellular elements, humoral components, epithelial cell factors, or extrinsic factors); and neural control (mediation of the vascular response to contact lens wear by contact lens-induced changes to corneal neurology). Another way of categorizing stimuli that can promote vessel penetration into the normally avascular cornea includes nutritional, inflammatory, mechanical, traumatic, and toxic factors. One

or all of these stimuli are present during contact lens wear, particularly overnight wear^[36].

Soft contact lens-induced hypoxia has been shown to stimulate the metabolism of arachidonic acid by a nicotinamide adenine dinucleotide phosphate (NADPH), cytochrome P-450 monooxygenase, and 12 hydroxyeicosatrienoic acid (12-HETrE), a proinflammatory and angiogenic factor. Biologic actions of this factor result in an increase in barrier permeability, vasodilatation, polymorphonuclear chemotaxis, and vascular endothelial cell mitogenesis. Routine contact lens wear is associated with inflammatory reactions and, even in asymptomatic patients, can induce release of some proinflammatory cytokines, including interleukins 6 and 8. Hypoxia creates an environment in which epithelial cyclooxygenase activity is severely suppressed, whereas metabolizing activity of cytochrome P-450-arachidonic acid or 12-lipoxygenase is maintained or enhanced. The 12 hydroxyeicosatetraenoic (12-HETE) produced by the corneal epithelium acts intracellularly to promote corneal edema, whereas 12-HETrE acts in a paracrine manner to initiate an inflammatory cascade that can elicit neutrophil chemotaxis and NV of the cornea^[38]. Leukocyte migration into the stroma and release of angiogenic factors from these cells may subsequently promote new corneal vessel growth^[39].

Keratoplasty induced corneal neovascularization Several studies have been published regarding roles of different angiogenic factors and molecular mechanisms of keratoplasty-induced CNV and pathways have been highlighted to make a detailed scheme of how rejection of newly transplanted cornea can take place.

Role of Vascular Endothelial Growth Factor Cursiefen *et al*^[40] analyzed presence and distribution of VEGF, transforming growth factor (TGF)- α , and TGF- β 1 in human corneas with NV. They found that the analyzed angiogenic factors were detectable in corneas in capillary endothelial cells, stromal and intravascular inflammatory cells (T-lymphocytes, and macrophages) and basal corneal epithelial cells in a uniform distribution in different corneal diseases. They concluded hereby that a future antiangiogenic therapy against CNV acting by blockade of angiogenic factors would have to counteract a multitude of angiogenic factors involved in CNV. This therapeutic approach could act either by using a "cocktail" of antiangiogenic factors or, alternatively, by using broad spectrum agents.

On the other hand, Mimura *et al*^[41] examined the expression of VEGF-C, which is the only endogenous lymphangiogenic factor reported so far, and one of its receptors, VEGFR-3, in corneal lymphangiogenesis. Electron microscopy revealed lymphatic vessels in the vascularized rat corneas. Competitive reverse transcription-polymerase chain reaction (RT-PCR) demonstrated that the expression of VEGF-C

mRNA in the rat cornea was normally absent, and was dramatically upregulated 3d after the injury, which gradually decreased. The VEGFR-3 expression in the rat cornea was minimally detected before the injury and was upregulated 3 and 7d after the injury. It was also minimally detected 2 and 4wk after the injury.

In another study, Cursiefen *et al*^[42] declared that lymphangiogenesis, an important initial step in tumor metastasis and transplant sensitization, is mediated by the action of VEGF-C and -D on VEGFR-3. In contrast, VEGF-A binds VEGFR-1 and VEGFR-2 and is an essential hemangiogenic factor. Keeping that in mind, they re-evaluated the potential role of VEGF-A in lymphangiogenesis, and found that administration of VEGF Trap, a receptor-based fusion protein that binds and neutralizes VEGF-A but not VEGF-C or -D, completely inhibited both hemangiogenesis and the outgrowth of LYVE-1+ lymphatic vessels following injury. Because VEGF-A is chemotactic for macrophages and macrophages in inflamed corneas release lymphangiogenic VEGF-C/VEGF-D, the possibility that macrophage recruitment plays a role in VEGF-A-mediated lymphangiogenesis was evaluated. They concluded that VEGF-A recruitment of monocytes/macrophages plays a crucial role in inducing inflammatory NV by supplying/amplifying signals essential for pathological hemangiogenesis and lymphangiogenesis^[42].

Roles of Miscellaneous Molecules and Pathways Here, we are presenting results of diverse studies that discussed different molecules and pathways of corneal immune response in corneal transplantation-induced CNV.

Zhu *et al*^[43] experiments showed that murine recipients grafted with syngenic and allogenic mice corneas showed early expression of proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor- α (TNF- α) by corneal grafts. The principal source of cytokine expression in the transplanted tissue was found to be the recipient rim, with overexpression of both IL-1 α and TNF- α during the first 2 wk after transplantation in both syngenic and allogeneic orthotopic corneal grafts.

Cursiefen *et al*^[44] studied the fine tuning of new capillary formation from blood vessels when vascular endothelial cells become apoptotic if sufficient supply of angiogenic factors is lacking. Morphologically, this period correlates with the absence of pericyte coverage of new vessels. Mature, pericyte covered vessels, in contrast, do not depend on elevated levels of angiogenic factors for survival. They found out that pathological new vessels in human corneal angiogenesis are rapidly covered by pericytes. And hence, treatments aimed at regression of immature, not yet pericyte covered vessels by antagonising angiogenic factors should be most effective if applied very early.

Kuhlmann *et al*^[45] investigated involvement of the potent angiogenic growth factor endothelin (ET)-1 and its receptors, ET_A and ET_B, in CNV. Protein expression was evaluated in nonvascularized and vascularized human corneas; and revealed that ET-1 protein and mRNA expression was significantly increased in the epithelium of vascularized corneas, which may represent an additional target for corneal antiangiogenic therapy.

Lam *et al*^[46] underwent a case-series study on 66 eyes to identify the speed of CNV after penetrating keratoplasty (PK) and to evaluate the influence of surgery-related factors on postkeratoplasty CNV in keratoconus patients. They found that 67% out of 66 corneal grafts developed some degree of CNV after PK. The mean speed of CNV growth was 114 $\mu\text{m}/\text{mo}$ with the fastest growth occurring during the first six weeks after PK. Ninety percent of all CNVs developed with limbus suture distance (LSD)<406 μm and with limbus graft distance (LGD)<1000 μm . As a result, the conclusion was that small LSD, small LGD, and narrow stitching with small inner suture angle were identified as potentially modifiable surgical risk factors for CNV after PK.

CORNEAL NEOVASCULARIZATION AND CORNEAL TRANSPLANTATION: A CLOSER LOOK

Corneal Neovascularization and Keratoplasty There are three main ways in which corneas could be transplanted: PK, anterior lamellar keratoplasty (ALK) and endothelial keratoplasty (EK). PK involves removal of the full thickness of the cornea and replacing it with the donor graft. ALK on the other hand, involves removing the corneal epithelium, Bowman's membrane and parts of the stroma, while leaving Descemet's membrane and corneal endothelium in place. One variant of ALK, deep ALK (DALK) involves removing the full stroma. EK, on the other hand, involves removing the endothelial cell layer and Decemet's membrane only. Variants of EK include Descemet's stripping endothelial keratoplasty (DSEK) and Descemet's membrane endothelial keratoplasty (DMEK)^[47].

As expected, each of the above techniques has its own indications and side effects. PK is the oldest technique and can be used for pathologies involving any or all of the corneal layers while ALK is suitable for corneal pathologies that have not extended to the Decemet's membrane. EK, on the other hand, is a good option in endothelial dystrophies^[47].

The cornea is one of the very few organs that have been known to enjoy "immune privilege", such that corneal transplantation is done without histocompatibility matching. Among the most important factors maintaining this immune privilege is corneal avascularity, or as Azar^[48] pinned it, its "angiogenic privilege". Hence, it should come as no surprise that corneal transplants with vascularized beds have a higher chance of failure (risk ratio=1.35, 95%CI: 1.15-1.49)^[49]. The most common direct cause of corneal graft failure is

"endothelial rejection". That is, failure of the endothelium to maintain the dehydrated state of the cornea due to progressive loss of endothelial cells. This could occur during surgery (if host endothelium is retained as in DALK) or during the transfer of cornea from donor to recipient (loss of donor endothelial cells in PK and EK)^[50-54]. It is for this reason that DALK is superior to PK, since the preservation of host endothelium decreases the chances of late endothelial rejection^[55]. Tan *et al*^[56] reported the results of the Singapore Corneal Transplant Study (over 2700 transplants), where 15% of therapeutic PK allografts were rejected, while none of the DALK allografts did so. On the long term, Tan *et al*^[56] reported significantly better long-term outcomes of ALK, with 67.8% survival at 5y in comparison to 59.2% survival of PK allografts. On the other hand, the 2012 Report of the Australian Corneal Graft Registry, reporting 23 048 grafts from all across Australia reported the survival of PK grafts to be 73% at 5y, compared to 67% survival of lamellar grafts^[54]. Nonetheless, the extra ocular nature of ALK decreases the chances of endophthalmitis and fibrous retrocorneal membrane formation in comparison to PK for therapeutic purposes. This is especially true for DALK, where total stromal removal gets rid of "remains" of past infection, which might lead to recurrence and/or vascularization and rejection. Tan *et al*^[56] reported that 50% of the PK cases that suffered from recurrent infection developed endophthalmitis, while none of the DALK group patients did so. A number of factors increase the odds of endothelial rejection. Among them is recent inflammation and vascularization of the corneal stroma^[54,57,58].

According to the 2012 Report of the Australian Corneal Graft Registry, approximately 31% of PK had a vascularized bed at the time of implantation. Graft NV significantly increases the chances of rejection (hazard ratio=2.27)^[54]. In fact, the chances of rejection of corneal transplants increase with the number of vascularized quadrants of the recipient's cornea before transplantation^[49,54]. So the extent of vascularization, too, is related to the chances of rejection. Moreover, it has been found that keratoplasties performed to treat keratoconus have a significantly increased survival time in comparison to therapeutic transplants for other pathologies^[54,57]. This makes sense, since the absence of vascular or inflammatory pathologies in the corneal beds of keratoconus-treating transplants would decrease the chances of rejection.

Surprisingly, it is lymphatics which seem to have the more important role in this process than blood vessels. The presence of lymphatics in vascularized graft beds and NV of corneal grafts provides a pathway to transfer antigen-presenting cells such as dendritic cells into regional lymph nodes, leading to sensitization of the immune system against the graft. Afterwards, the neovessels act to transport sensitized immune cells to the graft and to provide the

cytokine "environment" that pertains to graft rejection^[59]. Indeed, it has been shown that high-risk vascularized corneal allografts contained CXCL1/KC, which is a T-cell chemokine that increases the production of other chemokines, namely CXCL9/Mig and CXCL10/IP10. These chemokines are found to be absent in non-vascularized corneal beds^[60].

In order to explore ways to minimize the chances of graft failure, Altenburger *et al*^[61] sought to find out the factors that increase the chances of graft NV in high-risk keratoplasties. High-risk keratoplasties are those that are already known to have higher chances of rejection due to vascularized beds, limbal stem cell deficiency or inadequate tear film. They found an inverse correlation between LSD and CNV growth speed. Their results are in agreement with experimental corneal assays, where sutures are used as angiogenic stimuli^[15]. For some reason, Altenburger *et al*^[61] found a significant "preference" of neovessel formation at the superior aspect of corneal implants (at or around 12 o'clock), turning the attention of surgeons to take special care with suture distances in this area of the cornea. Their study also concluded that it was useful to scrape off remnants of herpetic keratitis (virally-transformed cell proteins and HSV DNA fragments) from the graft bed during therapeutic transplants. As mentioned earlier in another section of this review, HSV-1 is pro-angiogenic through a VEGF-A/VEGFR-2 dependent pathway.

Corneal Neovascularization and Keratoprosthesis

Patients who suffer from repeated graft failure may be good candidates for artificial corneal implantation or keratoprosthesis (KPro). KPro are generally classified into two broad categories: those which require a non-inflamed implantation site and a satisfactory tear film (Boston Type 1 KPro and AlphaCorTM) and those which may be implanted in inflamed corneas [Osteo-Odonto-Keartoprosthesis (OOKP) and Boston Type 2 KPro].

Boston Type 1 KPro is the most widely used type of artificial corneas, and is used in situations where there is a high risk of graft rejection or endothelial cell failure following keratoplasty^[62]. AlphaCorTM implants are used for similar indications, although they have more side effects and, thus, are less commonly used than other types^[63]. OOKP is a technically-demanding type of KPro which is only performed in a few centres worldwide. It is used in end-stage inflammatory corneal diseases such as severe chemical injuries and Stevens-Johnson syndrome (SJS). OOKP's solve this by replacing the conjunctiva with buccal mucosa and the cornea with a single-rooted tooth surrounding an optical cylinder^[64].

Repeated graft failure may occur due to a number of reasons, including inflammation and vascularization of the graft bed, as mentioned earlier. Hence, KPro may hold the solution to high-risk graft beds. Nonetheless, KPro have their own set of

complications, including retro-prosthetic membrane formation, sterile corneal stromal necrosis and glaucoma, and should therefore be reserved as a final option^[63-65].

TREATMENT OF CORNEAL NEOVASCULARIZATION: AN OVERVIEW

There are various conditions that may necessitate the use of treatments to inhibit CNV. The most obvious setting is to prevent corneal allograft rejection by decreasing vascularization before implantation (therefore preventing high-risk graft bed formation) and after implantation (to prevent graft NV). Other potential indications of antiangiogenic therapy of the cornea exist as well. For example, antiangiogenic drugs may be used in infective keratitis (whether bacterial, viral, fungal or parasitic), atopic conjunctivitis, SJS and other inflammatory conditions. Moreover, anti-angiogenic therapy may have a role in the management of limbal stem cell deficiency and corneal injuries where there is a loss of limbal barrier function^[66].

Currently, steroids are widely used to suppress inflammatory processes which characterize a large subset of CNV causes^[67-70]. Non-steroidal anti-inflammatory agents may also be used^[70-75]. Nonetheless, their use is limited by their numerous side effects and lack of efficacy in non-inflammatory CNV. Bevacizumab, initially approved for treating metastatic colorectal carcinoma, has shown very promising results in preventing CNV. Several clinical trials and case series reporting the success of bevacizumab in CNV prevention are found in the literature^[76-81]. Surgical treatment of CNV includes argon laser photocoagulation of neovessels, photodynamic therapy, electrocoagulation and stem cell transplants^[82-95].

Table 2 outlines the most clinical-relevant treatments (medical, surgical and gene therapy-based)^[67-98]. Other potential molecules and approaches that have shown experimental success in the fight against CNV are outlined in Table 3^[23,99-138].

CONCLUSION

New blood vessels help combating infections and encourage healing; however, they might compromise corneal clarity. Many of the molecular angiogenic and anti-angiogenic responses are incorporated in this process and a large subset of corneal pathologies involves new vessel formation at one stage or the other. Moreover, vascularization of corneal beds and NV represent significant risk factors in corneal graft failure. Thus the importance of understanding the molecular underpinnings of CNV cannot be over stressed. Different types of corneal transplantation and their relevance to CNV have been discussed in this review.

Recent investigations have focused on understanding the mechanisms maintaining corneal avascularity under homeostatic conditions and in avascular wound healing^[139-143]. To the best of our knowledge, there are few-if any-

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Table 2 Clinically-relevant treatments of CNV

Treatment modality	Type	State of research progress	Rationale	Notes
Steroids ^[73-76]	Medical	Used in clinical practice	Antiangiogenic effect is secondary to the anti-inflammatory action of steroids; anti-inflammatory actions of steroids include: inhibition of vascular endothelial cell proliferation and migration; inhibition of cytokine synthesis; Inhibition of chemotaxis	Steroids have many side effects which limit their use, especially on the long term; limited use in non-inflammatory CNV
Non-steroidal anti-inflammatory (NSAID) agents ^[76-81]	Medical	Used in clinical practice	Inhibition of COX enzymes has an anti-inflammatory effect and antagonizes CNV.	Limited used in non-inflammatory CNV
Bevacizumab and Ranibizumab ^[82-87]	Medical	Promising clinical trial results. FDA-approved for management of metastatic colorectal cancer.	Anti-VEGF antibody binds to and inactivates VEGF	Side effects include corneal thinning and loss of epithelial integrity, probably because VEGF is also responsible for nerve repair (loss of sensation leading to decreased physiologic response to noxious stimuli)
GS-101 antisense oligonucleotide ^[103]	Medical	Clinical Trial	Inhibits the expression of Insulin Receptor Substrate-1 (IRS-1), which is involved in IL-1B signaling	
IL-1 receptor antagonist (IL-1 RA) ^[104]	Medical	Clinical Trial	IL1 RA suppresses the effects of IL-1 and, hence, inflammation	
Argon laser photocoagulation ^[88-89]	Surgical	Used in clinical practice	Light activation of a photosensitized dye, causing localized thrombosis and regression of new vessels	The coagulation process itself may trigger inflammation and upregulation of VEGF's. Combining photocoagulation with medical therapy may solve this problem; not successful in managing extensive CNV; unlike medical therapy, photocoagulation deals with existing new vessels and does not prevent them from forming in the first place
Electrocoagulation (fine needle or electrolysis needle cauterization) ^[91-92]	Surgical	Used in clinical practice	Cauterization of new vessels causes their regression	
Photodynamic therapy ^[93-95]	Surgical	Used in clinical practice	Photosensitive (administered systemically or topically) accumulates in new vessels and activated by a laser beam.	
Limbal stem cell transplantation ^[90, 96]	Surgical	Used in clinical practice	Direct inhibition of vascular endothelial cells; provide stem cells to regenerate the corneal surface in stem cell deficiency, thereby minimizing the chances of corneal ulceration and neovascularization.	
Amniotic membrane transplantation ^[97-99]	Surgical	Used in clinical practice	Regenerating the ocular surface, thereby preventing ulceration and neovascularization	
Conjunctival transplantation ^[100-101]	Surgical	Used in clinical practice	Regenerating the ocular surface, thereby preventing ulceration and neovascularization	

Table 3 Other potential therapeutics against CNV

Potential medical therapeutics	Potential gene therapy-based approaches
VEGF TRAP-Eye ^[105]	VEGF gene knockout/siRNA vector ^[27]
Cyclosporin ^[106-109]	VEGF antisense RNA/Adenovirus vector ^[132]
Methotrexate ^[110-111]	sFlt-1 gene/Naked DNA vector ^[133]
Ocateride ^[112]	sFlt-1 gene/Adeno-associated virus ^[134]
1-25(OH)D3 (Vitamin D) ^[113]	sFlt-1 gene/Adenovirus ^[135]
PAF antagonists ^[114]	Flt23K gene/albumin polyplexes vector ^[136]
Thalidomide ^[115-116]	Flt23K and Flt24K genes/Naked DNA vector ^[137]
Soluble TNF- α receptors ^[117]	Angiopoietin-2 gene/RNA aptamer vector ^[138]
Angiostatin ^[118]	bFGF/Antisense Oligo-Dextro-Nucleotide (ODN) vector ^[139]
Spironolactone ^[119]	K5 gene/Naked DNA vector ^[140]
Curcumin ^[120]	Endostatin: K5 fusion gene/Lentivirus vector ^[141]
Anti-VEGFR-3 antibody ^[121]	IL 12 and IP10/Naked DNA vector ^[142]
Anti-VEGFR-2 antibody (DC101) ^[122]	IL-18/Naked DNA vector ^[143]
Anti-VEGF-A antibody ^[124]	
Soluble VEGFR-3 ^[125]	
VEGFR-3 inhibitors (e.g. E7080) ^[126]	
L-NMMA ^[127]	
Anti-PDGF-R- β antibody ^[128]	
Ethanol extracts of brazilian propolis (EEBP) ^[129]	
Resveratrol ^[130]	
Epigallocatechin-3-gallate (EGCG) ^[131]	

prevalence studies conducted to identify the true extent of the iceberg hiding beneath CNV. Such studies, in our opinion, represent a priority in revealing how much burden CNV holds on public health.

VEGF took most of the clinicians and scientists' attention in the last decade, especially with the development of anti-VEGF molecules and their implications in tumors management, as well as retinal and choroidal new vascular diseases. Moreover, the development of contact lenses materials and further dissemination of its usage precautions have significantly contributed to the decrease in its burden on corneal integrity. Nevertheless, the development of other refractive surgery options has shifted the trend of contact lens wear.

We do hope that as future research further elucidates the molecular underpinnings of CNV, ever improving therapeutic approaches could be pursued in the prevention and treatment of vascular pathologies of the cornea.

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