Biosustitutos para el tratamiento de la patología de la córnea y la superficie ocular: epidemiología, aplicaciones clínicas y controles microbiológicos

Noelia Sabater Cruz

BIOSUSTITUTOS PARA EL TRATAMIENTO DE LA PATOLOGÍA DE LA CÓRNEA Y LA SUPERFICIE OCULAR: EPIDEMIOLOGÍA, APLICACIONES CLÍNICAS Y CONTROLES MICROBIOLÓGICOS

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Ricardo-Pedro Casaroli-Marano, Catedrático de Universidad del Departamento de Cirugía y Especialidades Médico-quirúrgicas de la *Facultat de Medicina i Ciències de la Salut* de la *Universitat de Barcelona*,

CERTIFICO:

Que la tesis doctoral titulada **BIOSUSTITUTOS PARA EL TRATAMIENTO DE LA PATOLOGÍA DE LA CÓRNEA Y LA SUPERFICIE OCULAR: EPIDEMIOLOGÍA, APLICACIONES CLÍNICAS Y CONTROLES MICROBIOLÓGICOS,** presentada por NOELIA SABATER CRUZ ha sido realizada en el *Institut Clínic d'Oftalmologia* (ICOF) de *l'Hospital Clínic de Barcelona*, en el *Banc de Sang i Teixits* de Barcelona i en el Departamento de Cirugía y Especialidades Médico-quirúrgicas de la *Facultat de Medicina i Ciències de la Salut* de la *Universitat de Barcelona* bajo mi dirección y cumple con todos los requisitos necesarios para su tramitación y posterior defensa ante el tribunal correspondiente. La tesis doctoral es original, fruto de investigación de la doctoranda, habiéndose cumplido los códigos éticos y de buenas prácticas, no conteniendo plagios, y se consiente que esta memoria sea sometida a procedimientos para comprobar su originalidad.

Ricardo Pedro Casaroli-Marano
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LISTADO DE ABREVIATURAS

bFGF: *basic Fibroblast Growth Factor*
BST: *Banc de Sang i Teixits*
DEWS II: *Dry Eye Workshop* II
DMEK: *Descemet Membrane Endothelial Keratoplasty*
DSAEK: *Descemet Stripping Automated Endothelial Keratoplasty*
EBAA: *Eye Bank Association of America*
ECCTR*:* *European Cornea and Cell Transplant Registry*
EEBA: *European Eye Bank Association*
EGF: *Epidermal Growth Factor*
ESCRS: *European Society of Cataract and Refractive Surgeons*
GMP: *Good Manufacturing Practices*
HLA: *Human Leukocyte Antigen*
IGF-1: *Insulin-like Growth Factor* 1
IgG: inmunoglobulina G
IL: Interleucina
IMO: Instituto de Microcirugía Ocular
INF-γ: Interferón-gamma
NGF: *Nerve Growth Factor*
OCATT: *Organització Catalana de Trasplantaments*
OSDI: *Ocular Surface Disease Index*
PDGF*:* *Platelet Derived Growth Factor*
pmp: por millón de población
PNT: Procedimientos Normalizados de Trabajo
SoHO: *Substances of Human Origin*
TBUT: *Tear Break-Up Time*
TFOS: *Tear Film and Ocular Surface Society*
TGF-β: *Transforming Growth Factor beta*
TNF: *Tumoral Necrosis Factor*
VEGF: *Vascular Endothelial Growth Factor*

LISTA DE ARTÍCULOS

La presente tesis doctoral ha sido presentada por compendio de artículos. Esta memoria consta de seis objetivos – uno principal y cinco secundarios –, cinco artículos y dos manuscritos.

Artículo 1: **Sabater-Cruz N**, Figueras-Roca M, Padró-Pitarch L, Tort J, Casaroli-Marano RP. Corneal transplantation activity in Catalonia, Spain, from 2011 to 2018: Evolution of indications and surgical techniques. **PLOS ONE**. 2021 Apr 8;16(4):e0249946. doi: 10.1371/journal.pone.0249946. PMID: 33831081. Factor de impacto (2019, JCR): 2,740; **Q2** del área de conocimiento *Multidisciplinary Sciences* (JCR) y **Q1** del área de conocimiento *Multidisciplinary* (Scimago). Objetivo principal y secundario 1.

Artículo 2: **Sabater-Cruz N**, Figueras-Roca M, González Ventosa A, Padró-Pitarch L, Tort J, Casaroli-Marano RP. Current clinical application of sclera and amniotic membrane for ocular tissue bio-replacement. **Cell Tissue Bank**. 2020 Dec;21(4):597-603. doi: 10.1007/s10561-020-09848-x. PMID: 32661595. Factor de impacto (2019, JCR): 1,149; **Q4** de las áreas de conocimiento *Cell Biology* y *Engineering Biomedical* (JCR); **Q3** de las áreas de conocimiento *Biomedical Engineering*, *Biomaterials* y *Transplantation* y **Q4** del área de conocimiento *Cell Biology* (Scimago)**.** Objetivo principal y secundario 2.

Artículo 3: **Sabater-Cruz N**, Otero N, Dotti-Boada M, Ríos J, Gris O, Güell JL, Vilarrodona A, Casaroli-Marano RP. Eye bank and theatre factors for positive microbiological culture of corneoscleral rim and cornea storage medium in the real-world. **Eye (Lond)**. 2021 Jan 19. Online ahead of print. doi: 10.1038/s41433-020-01342-8. PMID: 33469128. Factor de impacto (2019, JCR): 2,455; **Q2** del área de conocimiento O*phthalmology* (JCR) y **Q1** del área de conocimiento O*phthalmology* (Scimago). Objetivo secundario 3.

Artículo 4: **Sabater-Cruz N**, Dotti-Boada M, Rios J, Carrion MT, Chamorro L, Sánchez-Dalmau BF, Casaroli-Marano RP. Postoperative treatment compliance rate and complications with two different protocols after pterygium excision and conjunctival autografting. **Eur J Ophthalmol**. 2020 Apr 27:1120672120917335. Online ahead of print. doi: 10.1177/1120672120917335. PMID: 32338523. Factor de impacto

(2019, JCR): 1,642; **Q3** del área de conocimiento *Ophthalmology* (JCR) y **Q2** del área de conocimiento *Ophthalmology* (Scimago). Objetivo secundario 4.

Manuscrito 1: **Sabater-Cruz N**, Martínez-Conesa E, Vilarrodona A, Casaroli-Marano RP. Lyophilized amniotic membrane graft for primary pterygium surgery: preliminary results. En revisión (R1) en la revista *Cell and Tissue Banking*. Objetivo secundario 5.

Manuscrito 2: Pérez ML, Barreales Soto S, **Sabater-Cruz N**, Martinez-Conesa EM, Vilarrodona A, Casaroli-Marano RP, on behalf of the AMEED Research Group. Amniotic membrane extract eye drops: a new approach to severe ocular surface pathologies. En revisión (R1) en la revista *Cell and Tissue Banking*. Objetivo secundario 5.

Artículo 5: **Sabater-Cruz N**, Figueras-Roca M, Ferrán-Fuertes M, Agustí E, Martínez-Conesa EM, Pérez-Rodríguez ML, Vilarrodona A, Casaroli-Marano RP, *on behalf of AMEED Study Group. Amniotic membrane extract eye drops for ocular surface diseases: use and clinical outcome in real-world practice.* ***International Ophthalmology***. 2021 Apr 17. Online ahead of print. doi: 10.1007/s10792-021-01856-4. PMID: 33864578. Factor de impacto (2019, JCR): 1,314; **Q4** del área de conocimiento *Ophthalmology* (JCR); **Q2** del área de conocimiento *Ophthalmology* (Scimago). Objetivo secundario 5.

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INTRODUCCIÓN

INTRODUCCIÓN

La patología de la superficie ocular puede requerir, cuando es grave o no responde al tratamiento médico y según la patología a tratar, de cirugía reparadora asociada al implante de diferentes tipos de tejido. Se han descrito diversos biosustitutos de la superficie ocular para la rehabilitación funcional y visual, usados tanto como tratamiento quirúrgico o médico tópico. Entre estos biosustitutos se encuentran el tejido corneal, esclera, membrana amniótica, conjuntiva y hemoderivados autólogos o alogénicos, entre otros (Malhotra & Jain 2014; Clearfield et al. 2017; Hodge et al. 2017; Sabater-Cruz & Casaroli-Marano 2019). A continuación, se analizan los principales aspectos de los biosustitutos de la córnea y superficie ocular en relación a aspectos epidemiológicos, evolución, indicaciones, calidad tisular y controles microbiológicos.

1.1. BIOSUSTITUTOS DE LA SUPERFICIE OCULAR:

Las sustancias de origen humano, conocidas como SoHO por su terminología en inglés - *Substances of Human Origin* – incluyen órganos, tejidos, células y hemoderivados destinados al tratamiento, mediante biosustitución, de diferentes patologías humanas.

Se entiende por biosustituto de la córnea y la superficie ocular aquel tratamiento de origen biológico, tisular humano – SoHO autólogo o alogénico -, encaminado a la rehabilitación anatómica, estructural y/o visual tanto de la córnea (en grosor total o parcial) como del resto de la superficie ocular, incluyendo la conjuntiva y esclera.

1.1.1. BIOSUSTITUTOS AUTÓLOGOS

1.1.1.1. Conjuntiva

La conjuntiva es un tejido altamente vascularizado capaz de reclutar fibroblastos y leucocitos hacia la córnea, favoreciendo su curación y cierre epitelial. Se usa como injerto (colgajo o *flap*) por sus propiedades terapéuticas y su capacidad de disminuir la inflamación de la superficie ocular, restaurar el epitelio, mejorar la calidad de la película lagrimal - mejorando la sintomatología de los pacientes - y disminuir el riesgo de *ptisis bulbi* (Lim et al. 2009; Sun et al. 2018). Se usa también como coadyuvante de

diferentes cirugías (Figura 1). Una de ellas, importante por su prevalencia – que puede llegar al 22% en algunas zonas -, es la cirugía de exéresis del pterigión (Liu et al. 2013; Clearfield et al. 2017).

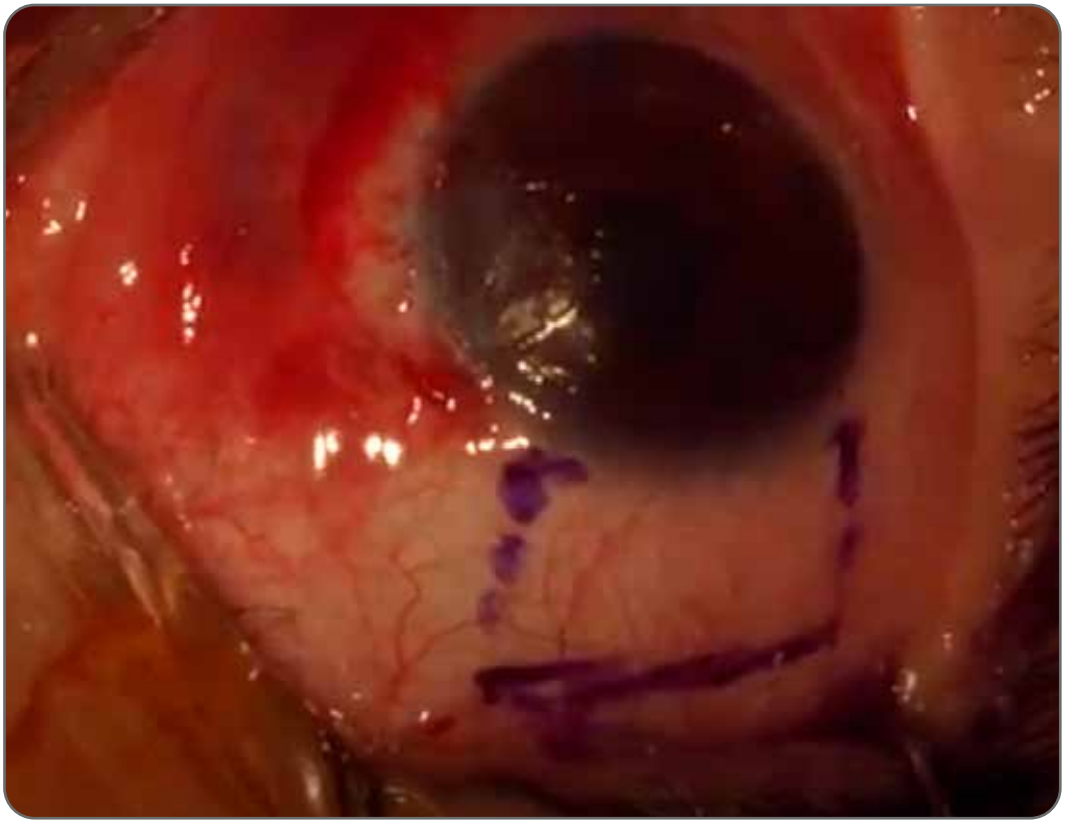

Figura 1: Cirugía de la exéresis de pterigión con autoinjerto conjuntival. La conjuntiva bulbar superior ha sido medida y está preparada para su disección y posterior implante en la zona de esclera que ha quedado desnuda tras la exéresis del pterigión. Imagen original de Noelia Sabater Cruz.

1.1.1.2. Biosustitutos autólogos de la lágrima natural (hemoderivados autólogos)

Se entiende por hemoderivados aquellos productos derivados de la sangre o sus fracciones destinados a uso terapéutico, y para sustitución de la lágrima natural ante patologías de la superficie ocular y córnea. Estos SoHO incluyen suero de la sangre y derivados de plaquetas, además de la propia sangre sin procesar. Los hemoderivados no solo protegen el epitelio del trauma biomecánico sino que además contienen algunos de los mismos factores de crecimiento – *Epidermal Growth Factor* (EGF), *Transforming Growth Factor beta* (TGF-β), *Platelet Derived Growth Factor* (PDGF),

Insulin-like Growth Factor 1 (IGF-1), *Nerve Growth Factor* (NGF), *basic Fibroblast Growth Factor* (bFGF), citoquinas, vitaminas – vitamina A - y nutrientes que la lágrima natural (Anitua et al. 2015; Giannaccare et al. 2017; Sabater-Cruz & Casaroli-Marano 2019).

1.1.1.2.1. *Suero autólogo*

El suero autólogo es el producto resultante del procesamiento de una muestra de sangre periférica que se deja coagular a temperatura ambiente durante varias horas y cuyo sobrenadante se centrifuga para separarlo de los elementos formes. El suero resultante puede aplicarse directamente o tras diluirlo a diferentes concentraciones, del 20 hasta el 100% (Figura 2). Se conserva a -20ºC un máximo de 3 meses hasta su uso y el vial abierto debe mantenerse a 4ºC. La posología es variable, desde 3 veces al día hasta aplicación horaria. La eficacia del suero autólogo en el tratamiento de diversas patologías de la córnea y superficie ocular ha estado ampliamente estudiada, con diferentes ensayos clínicos prospectivos y aleatorizados que comparan su eficacia con placebo, lágrima artificial e incluso otros biosustitutos de la lágrima, autólogos y alogénicos (Noble et al. 2004; Kojima et al. 2005; Yoon et al. 2007; Urzua et al. 2012; Pan et al. 2017; Yilmaz et al. 2017; Wang et al. 2020).

Figura 2: Preparación del suero autólogo. Tras el centrifugado (imagen superior) se procede a su dilución (imagen inferior). Extraída de *Actualización en hemoderivados para el manejo de alteraciones de la superficie ocular. N. Sabater Cruz, RP Casaroli-Marano. Annals d'Oftalmologia 2019;27(1):17-23.*

1.1.1.2.2. *Derivados de plaquetas*

Las plaquetas, esenciales en la formación del tapón plaquetario y activación de la coagulación, contienen gránulos-α que liberan PDGF, TGF-β y factor IV plaquetario. Cada factor de crecimiento está implicado en una fase del proceso de curación – inflamación, síntesis del colágeno, angiogénesis y granulación tisular – que en conjunto promueve el restablecimiento tisular. Diferentes preparados de derivados de plaquetas se han usado en diferentes especialidades médicas y quirúrgicas como medicina regenerativa, cirugía maxilofacial, traumatología y oftalmología. Según el protocolo de preparación, se dispone de diferentes tipos de derivados de plaquetas, aunque sus indicaciones y aplicabilidad pueden ser imprecisas ya que cada método de obtención resulta en un producto diferente con composición y usos potenciales diversos (Alio et al. 2015; Giannaccare et al. 2017; European Committee on Organ Transplantation & Keitel 2019; Sabater-Cruz & Casaroli-Marano 2019):

1.1.1.2.2.1. *Plasma rico en plaquetas*

El plasma rico en plaquetas es una fuente de plaquetas autólogas concentradas, que contiene diferentes factores de crecimiento y otras citoquinas en concentración de 5 a 10 veces mayor que en el plasma. Para su preparación, se obtiene una muestra de sangre periférica a la que se le añade el anticoagulante citrato de sodio al 3,2% y se centrifuga, obteniéndose tres fracciones: elementos formes, plasma pobre y plasma rico en plaquetas. Este último, contiene de 1,6 a 2,5 veces más plaquetas que la sangre (Kim et al. 2012; Alio et al. 2015; Solans-Perez de Larraya et al. 2015; European Committee on Organ Transplantation & Keitel 2019).

1.1.1.2.2.2. *Lisado de plaquetas*

Este SoHO se produce a partir del protocolo de obtención del plasma rico en plaquetas, que una vez centrifugado y separada la fracción de plasma rico en plaquetas se diluye al 30%, se congela a -80ºC durante una hora y luego se descongela a 4,1ºC, cosa que produce la lisis de las plaquetas y liberación del PDGF (Sandri et al. 2016; Pezzotta et al. 2017). Algunos autores han explorado el uso de dispositivos de liberación sostenida de este hemoderivado, como lentes de contacto cargadas con condroitín

sulfato y lisado de plaquetas, para el tratamiento de lesiones de la superficie ocular (Sandri et al. 2016).

1.1.1.2.2.3. *Plasma rico en factores de crecimiento*

Para su obtención se requiere de una muestra de sangre periférica sin coagular que se centrifuga, obteniéndose tres fracciones: plasma rico en plaquetas, banda de leucocitos y un depósito de células sanguíneas. El plasma sobrenadante es aspirado y se incuba primero a 37ºC durante una hora seguido de incubación a 56ºC una hora más. Como ventaja sobre los otros protocolos, presenta una mayor estandarización –por disponibilidad de todo el aparataje necesario para el proceso de forma comercial (Endoret®) -, menor presencia de leucocitos, ausencia de dilución y mayor versatilidad en sus indicaciones y aplicaciones (Anitua et al. 2015; Sanchez-Avila et al. 2017; Sanchez-Avila et al. 2018).

1.1.1.2.3. *Sangre autóloga*

Se ha descrito el uso de una gota de sangre autóloga (obtenida mediante una lanceta) aplicada varias veces al día en el *fórnix* palpebral inferior como tratamiento para el ojo seco y el defecto epitelial persistente (Than et al. 2017; Balal et al. 2020). Aunque el mecanismo de acción no parece estar totalmente establecido, parece que el traumatismo producido por la lanceta y el contacto persistente del coágulo con la superficie ocular activaría las plaquetas presentes en la gota de sangre. Los efectos antiangiogénicos y antiinflamatorios asociados a los factores de crecimiento se explicarían por las altas concentraciones de éstos en los gránulos α de las plaquetas (Than et al. 2017).

1.1.2. BIOSUSTITUTOS ALOGÉNICOS

1.1.2.1. Córnea

La córnea es una estructura de la superficie ocular cuya transparencia la hace un tejido único en el cuerpo. La patología corneal se asocia a una pérdida de dicha transparencia y en consecuencia a disminución de la agudeza visual. Las patologías de la

córnea causantes de opacidad pueden requerir de biosustitución que, para mantener su característica transparencia, debe ser mediante un alotrasplante del mismo tejido.

La cirugía de trasplante de córnea - o queratoplastia – puede realizarse de espesor corneal total (queratoplastia penetrante) o de manera selectiva de ciertas capas de la córnea (queratoplastia lamelar). El objetivo de las diferentes técnicas es trasplantar el mínimo tejido posible para evitar su rechazo, reducir complicaciones postoperatorias y aumentar la tasa de supervivencia de la córnea. En la actualidad, las técnicas lamelares incluyen queratoplastias lamelares anteriores y posteriores. De entre las últimas, cabe destacar dos técnicas que difieren en el grosor y capas de tejido trasplantado: la *Descemet Membrane Endothelial Keratoplasty* (DMEK) trasplanta un grosor de córnea que incluye solamente membrana de Descemet y endotelio. Se prepara mediante pelado, por el lado endotelial, de la córnea donante. La *Descemet Stripping Automated Endothelial Keratoplasty* (DSAEK) trasplanta un grosor mayor de córnea que además incluye una porción variable de estroma corneal (Figura 3). Para su preparación se requiere de un microqueratomo para realizar un corte de alta precisión en el estroma de la córnea (Patel 2012; Röck et al. 2017; Singh et al. 2019).

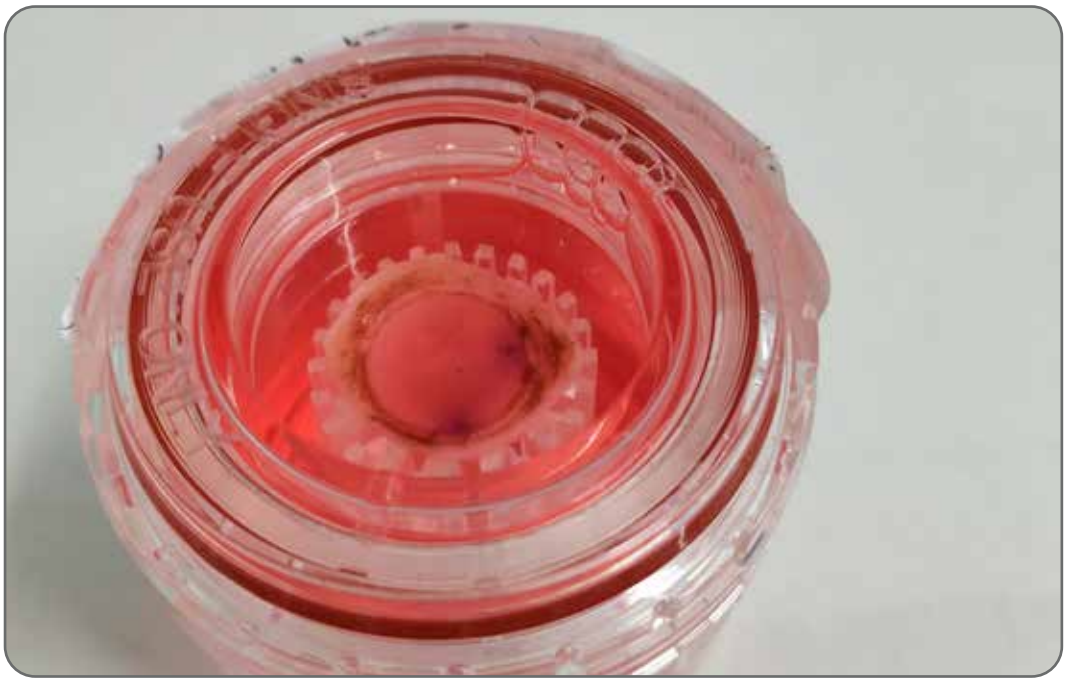

Figura 3: Córnea precortada para *Descemet Stripping Automated Endothelial Keratoplasty* (DSAEK) preservada en hipotermia. Imagen original de Noelia Sabater Cruz.

Las córneas son obtenidas de donantes seronegativos para patologías transmisibles (virus de la inmunodeficiencia humana, COVID-SARS2, virus de la hepatitis B y C, *Treponema pallidum*, virus linfotrópico de células T humanas I y II y virus Epstein Barr) por equipos extractores en medio hospitalario. Para su extracción, se realiza un lavado con yodo y se extraen ambos discos esclerocorneales en condiciones de esterilidad. Luego son enviados en medio Optisol GS® (Bausch & Lomb Surgical Inc., San Dimas, California) a 4ºC al banco de tejidos, donde se realizan estudios de calidad y son procesados (European Committee on Organ Transplantation & Keitel 2019).

En nuestro medio, hay dos métodos para la preservación de las córneas: el cultivo organotípico – o córnea cultivada – y la preservación en frío o hipotermia. El primer tipo de preservación, el más utilizado por nuestro banco regional - el *Banc de Sang i Teixits de Barcelona*, BST -, preserva las córneas en el medio CorneaMax® (Eurobio, Les Ulis, Francia) a 31ºC durante un máximo de tres semanas. El segundo, preserva las córneas a 4ºC en medio Optisol GS® un máximo de 5 días. El cultivo organotípico es también el más usado por los bancos de tejidos europeos – que forman parte de la *European Eye Bank Association* (EEBA) – llegando a usarse hasta en el 75% de las córneas (Zanetti et al. 2005; Linke et al. 2013; European Eye Bank Association 2020).

Además, el banco puede customizar o preparar la córnea antes de su distribución para personalizarla al tipo de cirugía programada. La adecuación del tejido corneal para su implante puede realizarse íntegramente en el quirófano o solicitarse "precortado" al banco de tejidos. En las técnicas lamelares, algunos cirujanos preparan la córnea previamente a la cirugía, en ocasiones mediante dispositivos específicos como el microqueratomo. Otros cirujanos adquieren el tejido ya precortado, con lo que se ahorra tiempo quirúrgico, inversiones de aparataje y se disminuye el riesgo de dañar o estropear el tejido y cancelar la cirugía programada. Debe destacarse que el BST empezó hace unos años a suministrar tejido corneal precortado para diferentes tipos de queratoplastia lamelar posterior: así, en 2013 se empezó a preparar y subministrar tejido precortado para DSAEK y en 2017 para DMEK. La disponibilidad del tejido corneal precortado ha facilitado que muchos cirujanos que solo realizaban la técnica penetrante hayan ido usando con más frecuencia las técnicas lamelares (Terry 2012; Palma-Carvajal et al. 2020).

Los trasplantes permiten tratar ciertas patologías de órganos o tejidos que no tienen capacidad de regeneración, como es el caso de la córnea. Para ello, los programas para incentivar las donaciones altruistas y la creación de bancos especializados para su preservación, optimizan el uso de estos recursos tan limitados. Los bancos de tejidos valoran la calidad, viabilidad y seguridad de las córneas con estrictos controles funcionales y microbiológicos durante su procesamiento, almacenamiento y tras su implantación (Lambert & Chamberlain 2017; European Committee on Organ Transplantation & Keitel 2019; EURO GTP II 2019).

1.1.2.2. Esclera

La esclera es un tejido relativamente avascular, rígido y compuesto por tejido conectivo denso cuya función principal es la protección de las estructuras intraoculares. Se obtiene por enucleación de los donantes en condiciones estériles, y se conserva en etanol al 70% a temperatura ambiente. Durante los años cincuenta se empezó a utilizar esclera de donante para dar soporte a complicaciones quirúrgicas, popularizándose por su mínimo riesgo de transmisión de enfermedades, incluyendo priones (Mentha & WA 2002). Con posterioridad se han añadido otras indicaciones como la reconstrucción de la superficie ocular - escleromalacia, perforación corneal - y como coadyuvante de diferentes cirugías como la de glaucoma, reconstrucción palpebral e implantes orbitarios, entre otros (Barman et al. 2012; Dubey et al. 2017; Hodge et al. 2017; Park et al. 2017; Wen et al. 2018) (Figura 4).

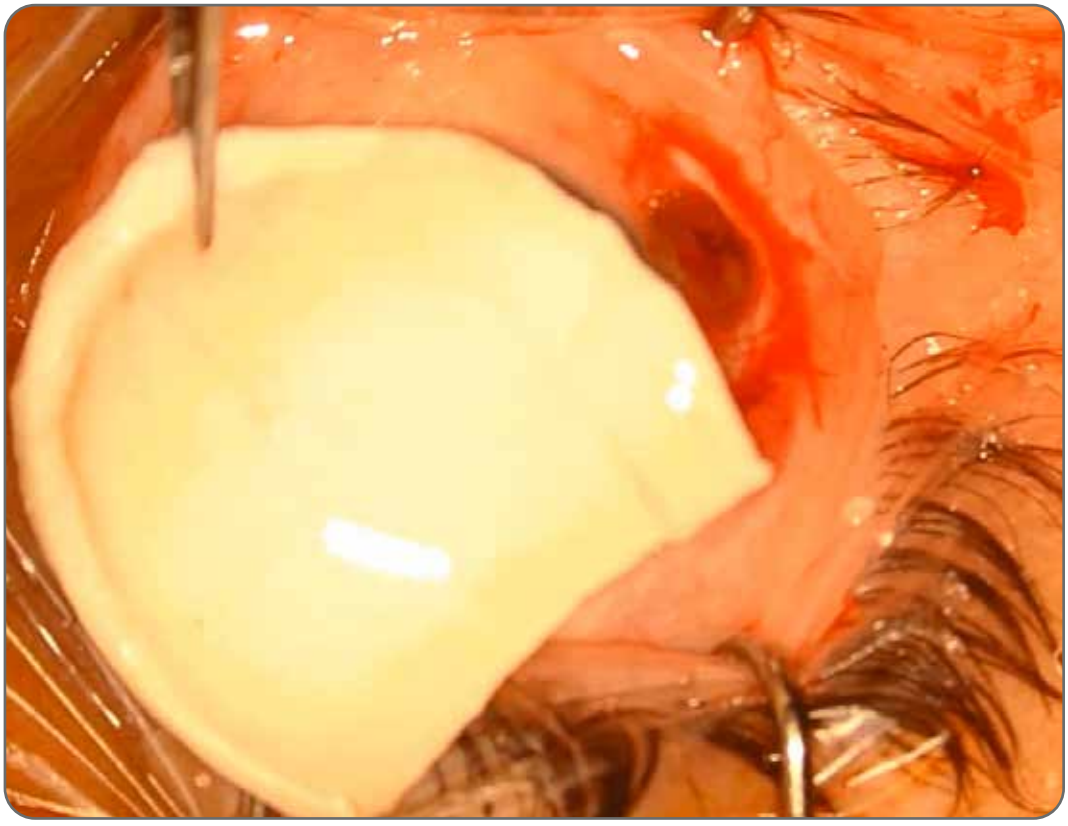

Figura 4: Esclera de donante que va a ser implantada en una cirugía de reparación de una lesión escleral. Imagen original de Noelia Sabater Cruz.

1.1.2.3. Membrana amniótica criopreservada y liofilizada

La membrana amniótica es un biosustituto ampliamente usado en oftalmología para la reconstrucción de la superficie ocular en casos de defectos epiteliales persistentes, úlceras corneales, perforaciones corneales y descematoceles, queratopatía bullosa, deficiencia de células madres límbicas, *melting* corneal, neoplasia intraepitelial conjuntival, cirugía del glaucoma y/o cirugía del pterigión (Rahman et al. 2009; Meller et al. 2011; Malhotra & Jain 2014; Jirsova & Jones 2017; Walkden 2020). La exéresis del pterigión con injerto de membrana amniótica está especialmente indicada en aquellos casos en los que la conjuntiva debe reservarse (para una eventual cirugía de glaucoma, por ejemplo) o es insuficiente (Prabhasawat et al. 1997; Clearfield et al. 2017).

La membrana amniótica presenta en su matriz estromal un alto contenido de ácido hialurónico fetal que está implicado en mecanismos de señalización de varios factores de crecimiento, como el factor transformador de crecimiento β (TGF-β), que regula en parte la proliferación de los fibroblastos en la superficie ocular. A través de sus efectos antifibróticos, la diferenciación de los fibroblastos en miofibroblastos queda inhibida, reduciendo la formación de tejido cicatricial. Otra propiedad de la membrana amniótica consiste en la inhibición de la expresión de algunas interleucinas (IL) inflamatorias como IL-1, IL-2; el interferón-gamma (INF-γ) y el factor de necrosis tumoral (TNF). la membrana amniótica tiene además propiedades antiangiogénicas, retrasando la formación de neovasos mediante la estimulación de la producción de trombospondina-1 e inhibidores tisulares de las metaloproteinasas (TIMP-1,2,3 y 4) (Malhotra & Jain 2014; Li et al. 2015; Jirsova & Jones 2017; Walkden 2020).

Las membranas amnióticas proceden de gestantes controladas que donan su placenta de manera voluntaria y libremente, sin compensación económica alguna, las cuales han dado su consentimiento para la donación y a las que se les realiza una evaluación médica y un cribado serológico (RD 1301/2006; European Committee on Organ Transplantation & Keitel 2019; EURO GTP II 2019).

La membrana amniótica puede implantarse fresca o bajo algún tipo de preservación. La membrana suministrada por el *Banc de Sang i Teixits* puede ser criopreservada (*Cryopreserved Amniotic Membrane*; CAM) (Figura 5) o, de suministro más reciente, liofilizada (*Lyophilized Amniotic Membrane;* LAM) (Figura 6). La liofilización de la membrana amniótica es un tipo de preservación que deja muy poca concentración de agua (<10%), lo que permite su almacenamiento con menos requerimientos - a temperatura ambiente y con stock en el área quirúrgica -, mientras que la criopreservada debe conservarse a -80ºC (Thomasen et al. 2009; Jirsova & Jones 2017; Walkden 2020).

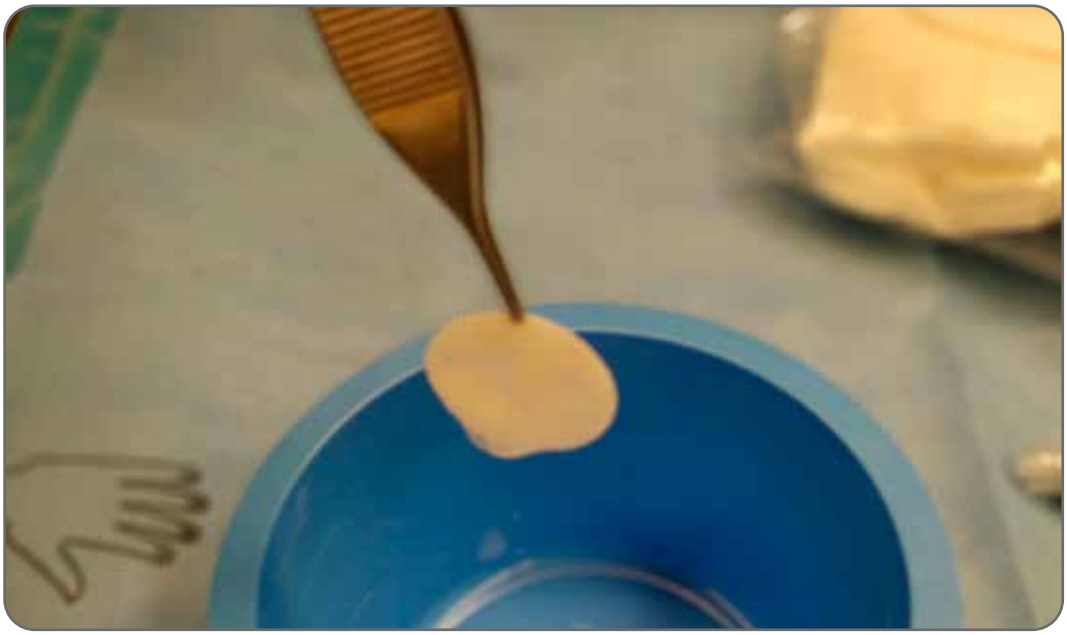

Figura 5: Apariencia de la membrana criopreservada (CAM) tras la descongelación y el lavado con suero fisiológico, previo a su uso. Imagen original de Noelia Sabater Cruz.

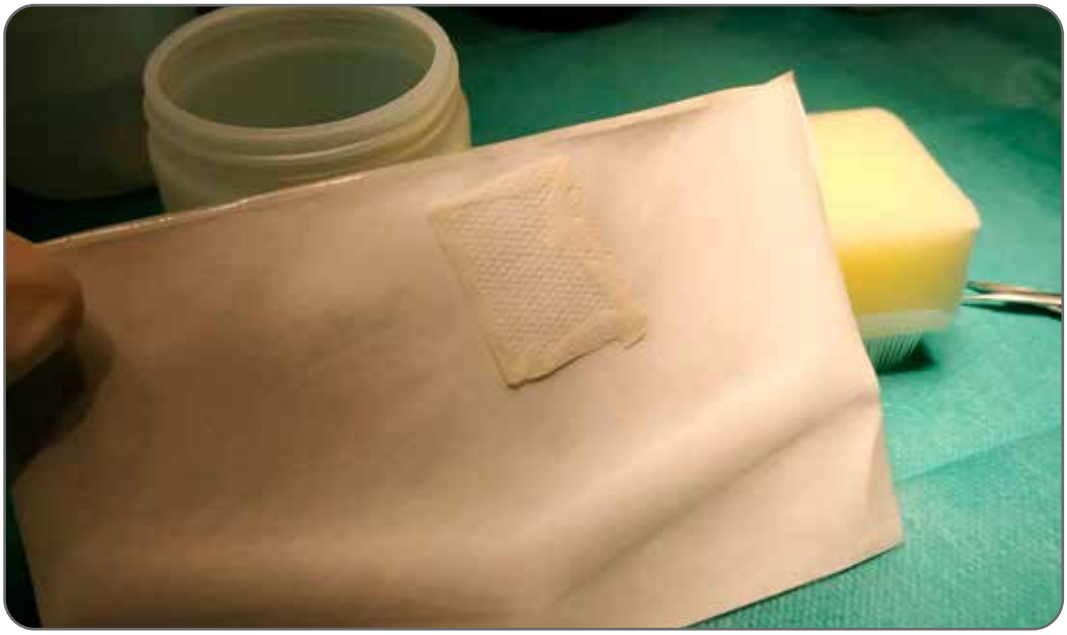

Figura 6: Presentación de la membrana liofilizada (LAM) antes de la rehidratación necesaria previa a su implante. Imagen original de Noelia Sabater Cruz.

1.1.2.4. Biosustitutos alogénicos de la lágrima natural

Diferentes biosustitutos de la lágrima alogénicos se han propuesto para el tratamiento de diferentes patologías de la superficie ocular como el ojo seco (Rauz & Saw 2010), causticaciones (Liang et al. 2009) y defectos epiteliales persistentes (Bonci et al. 2005).

1.1.2.4.1. *Colirio de extracto de membrana amniótica (AMEED)*

El colirio de extracto de membrana amniótica (*Amniotic Membrane Extract Eye Drops*; AMEED) favorece la reepitelización y contribuye a reducir la inflamación y la neovascularización corneal por las propiedades atribuidas a las diversas citoquinas y factores de crecimiento presentes en la membrana amniótica (Hopkinson et al. 2006; Kim et al. 2012; Mamede et al. 2012; Walkden 2020).

El AMEED se procesa a partir de membrana amniótica previamente congelada que se pulveriza y centrifuga - a 4500 rpm - a temperatura ambiente. El sobrenadante es alicuotado en viales en fracciones de 1 mL y liofilizado, manteniéndolo en forma pulverizada a temperatura ambiente hasta su uso. Cada vial es envasado junto el material necesario para reconstituir el colirio: jeringa, aguja, botella de 10 mL y 4 mL de agua estéril. Una vez reconstituido debe desecharse a los 15 días.

El AMEED ha sido estudiado tanto experimentalmente (Shahriari et al. 2008; Choi et al. 2011; Guo et al. 2011; Tighe et al. 2017), como clínicamente para tratamiento de quemaduras químicas, retraso de la epitelización (Bonci et al. 2005), úlceras neurotróficas (Bonci et al. 2005) y ojo seco (Yeu et al. 2019). Algunos autores además plantean el tratamiento con AMEED como una alternativa al implante quirúrgico de membrana amniótica en patologías como el defecto epitelial persistente o la úlcera corneal (Murri et al. 2018).

1.1.2.4.2. *Hemoderivados alogénicos*

1.1.2.4.2.1. *Suero alogénico*

El suero alogénico (o heterólogo) presenta las mismas indicaciones que el suero autólogo; se reserva para aquellos pacientes que no pueden usar colirio de su propia sangre por motivos tales como serologías positivas, venas inaccesibles, anemia, discrasia sanguínea o disminución de la capacidad epiteliotrófica de su sangre - como en la artritis reumatoide o la insuficiencia renal crónica - (Sabater-Cruz & Casaroli-Marano 2019). En estos casos se ha observado una menor concentración de TGF-β, fibronectina, EGF o PDGF asociado a elevados niveles de mediadores proinflamatorios, como también ocurre en el síndrome de Sjögren o la enfermedad de injerto contra huésped (Harloff et al. 2008; Kang et al. 2015; Giannaccare et al. 2017). El proceso de preparación del suero alogénico es equivalente al autólogo (véase apartado 1.1.1.2.1), tras lo cual se mantiene en cuarentena durante 4 meses. Se obtiene de donantes emparentados o de *pools* de donantes con un perfil de citoquinas o factores de crecimiento concreto, prefiriéndose aquellos de grupo sanguíneo AB para evitar anticuerpos anti-B y anti-A (Giannaccare et al. 2017; Campos et al. 2020).

1.1.2.4.2.2. *Suero de cordón umbilical*

El suero de cordón umbilical se obtiene tras recoger una muestra de sangre de cordón tras el parto, vía vaginal o cesárea, sin añadir anticoagulantes. Tras centrifugarla se diluye el sobrenadante al 20% y se almacena a -20ºC un máximo de 3 meses. Su alto contenido en IgG, lisozima y complemento lo convierten en un buen bacteriostático. Característicamente, contiene más EGF, TGF-β, *Vascular Endothelial Growth Factor* (VEGF) y NGF que otros hemoderivados y menor concentración de IGF-1 y vitamina A (Yoon 2014; Versura et al. 2014; Giannaccare et al. 2017; Campos et al. 2020). Algunos estudios comparativos concluyen que el suero de cordón umbilical es más efectivo que el suero autólogo en el tratamiento patologías como el síndrome de Sjögren y la enfermedad de injerto contra huésped (Yoon et al. 2007).

1.2. SITUACIÓN DE CATALUNYA EN LA DONACIÓN, PROCESAMIENTO E IMPLANTE DE TEJIDOS OCULARES Y DE USO OCULAR

Catalunya tiene una población de 7,5 millones de personas (https://web.gencat.cat/ca/temes/catalunya/ - última entrada 16/01/2021) y una actividad implantadora de SoHO para la biosustitución de la superficie ocular y córnea relevante: en Cataluña se realizan una media 190,2 trasplantes corneales por millón de población (pmp) y año; cifra muy superior a la media nacional de 80,4 pmp. En esta comunidad autónoma se implantan más del 6% de las escleras y del 5% de las membranas amnióticas implantadas en Europa y subministradas por bancos miembros de la EEBA (Organización Nacional de Trasplantes 2018; European Eye Bank Association 2020).

En Catalunya hay actualmente un banco público de tejidos (el BST) y un banco privado de ojos (*Banc d'Ulls per al Tractament de la Ceguesa*, Centro de Oftalmología Barraquer) que trabaja de manera coordinada con el BST. El *Banc d'Ulls per al Tractament de la Ceguesa*, fue fundado por el Profesor Barraquer en 1962 y es el banco de ojos en funcionamiento más antiguo en la Europa continental (https://www.bancsang.net) (http://www.bancdulls.org/?i=4&si=0).

El BST evalúa, procesa, almacena y suministra diversos tejidos y hemoderivados de interés para la biosustitución de la córnea y/o superficie ocular, como son córnea, esclera, membrana amniótica y AMEED, suero autólogo, suero alogénico y suero de cordón umbilical. El BST suministra más de 1000 córneas al año y fue pionero en España en subministrar tejido corneal precortado para diferentes tipos de queratoplastia lamelar posterior: así, en 2013 se empezó a preparar y subministrar tejido precortado para DSAEK y en 2017 para DMEK. En cuanto a la membrana amniótica, el BST suministra membrana amniótica criopreservada y desde enero de 2019, membrana amniótica liofilizada. Desde abril de 2018 está disponible el AMEED.

La historia de los trasplantes en España empieza en 1965 cuando se realizan los primeros trasplantes renales con éxito en Catalunya y España (Dr. Gil-Vernet y Dr. Caralps). Las primeras leyes sobre trasplantes datan de 1979, ley 30/1979 y Real Decreto 426/1980, regulando la certificación de muerte por médicos independientes (al equipo de trasplantes) y por muerte encefálica, el respeto a la voluntad del fallecido,

el carácter altruista de la donación – prohibiendo la comercialización -, garantía del anonimato y prevaleciendo los criterios médicos en la distribución de los órganos (http://www.ont.es/home/Paginas/HistoriadelaONT.aspx).

Este modelo, conocido internacionalmente como "el modelo español" se basa en un sistema *opting-out* (todos son donantes excepto cuando se expresa el deseo contrario) con una coordinación de donaciones a tres niveles – nacional, regional y hospitalario – y un sistema de cobertura sanitaria universal (sanidad pública). Este *modelo español* ha sido trasladado a otros países del mundo por su éxito (European Committee on Organ Transplantation & Keitel 2019) (http://www.ont.es/home/Paginas/ElModeloEspanol.aspx).

En el año 1989 entró en funcionamiento la Organización Nacional de Trasplantes (ONT) que coordina todas las donaciones de órganos registradas en España, mientras que desde Catalunya se coordinan los intercambios internacionales. La ONT actúa, entre otras funciones, promocionando la donación de órganos, tejidos y células y garantizando su correcta distribución según conocimientos técnicos y principios éticos.

En Catalunya existía un programa de atención a la insuficiencia renal crónica desde 1982 que se amplió con la creación de un registro de enfermos renales, la implantación del coordinador hospitalario de trasplantes y la creación de un programa de trasplantes de órganos en el 1984. Diez años más tarde, en el 1994, se creó la *Organització Catalana de Trasplantaments* (OCATT), para ser la cobertura legal del programa de trasplantes de órganos. Por tanto, la OCATT es la organización responsable de planificar, ordenar y coordinar las actividades relacionadas con la extracción, conservación, distribución, trasplante e intercambio de órganos y tejidos para su uso con finalidades terapéuticas en Catalunya (http://trasplantaments.gencat.cat/ca/ocatt/historia).

Los SoHO provienen del cuerpo de un ser humano – vivo o fallecido - por lo que se derivan aspectos éticos asociados a su uso. Los estándares éticos de los diferentes aspectos de la donación y el trasplante de los tejidos deben adherirse a la Convención en Derechos Humanos y Biomedicina de Oviedo (1997), la Declaración de Estambul sobre Turismo y Tráfico de Trasplantes (2008) y los Principios de Barcelona en el uso de Tejido Humano Donado para Trasplante Ocular (2018). Los tejidos no deben ex-

traerse del cuerpo de una persona fallecida hasta que no se ha certificado su muerte acorde a la ley vigente y se haya obtenido consentimiento y autorización. No debe extraerse ningún SoHO si la persona expresó objeción, ya que la donación debe ser siempre voluntaria. Las directivas de la Unión Europea dictan que los estados miembros deben animar las donaciones voluntarias y sin compensación económica de SoHO y deben asegurarse de que la obtención de dichos tejidos se realiza sin provecho económico (European Committee on Organ Transplantation & Keitel 2019).

Un programa de donaciones exitoso debe incluir unas adecuadas estrategias para promoción de la donación de tejidos, órganos y células. Estas estrategias incluyen sistemas que faciliten el reclutamiento de donantes vivos, como en el caso de las donantes de membrana amniótica, preservando su seguridad y bienestar; y la identificación y remisión de los potenciales donantes fallecidos a la organización apropiada. Finalmente, para garantizar el éxito de los programas de donación es imprescindible la adecuada formación del equipo extractor. Una vez el donante es identificado y/o reclutado, la obtención del consentimiento informado antes de que se produzca la donación es obligatoria. Puede ser obtenido del propio donante o de sus representantes legales, si está vivo; en el caso de donantes fallecidos, el consentimiento puede provenir del propio donante antes de su muerte - registros de donantes, cartilla de donantes, voluntades anticipadas - o de sus parientes (ley 30/1979; RD 426/1980; Directiva de la Comisión Europea 2006/17/EC; European Committee on Organ Transplantation & Keitel 2019; http://www.ont.es/home/Paginas/default.aspx).

1.3. IMPORTANCIA DEL CONTROL DE CALIDAD DEL TEJIDO

La ley española define trazabilidad como la capacidad de localizar e identificar un órgano o tejido en cualquier momento entre la donación y su implante o eliminación (Real Decreto-ley 9/2014 y Orden SSI/2396/2014). Este proceso, mediante un sistema de biovigilancia, incluye la localización e identificación de todos los datos no personales relativos a los productos y materiales en contacto con el tejido en que pudiera eventualmente afectar a la calidad o seguridad. Por tanto, la trazabilidad permite una rápida respuesta ante una reacción adversa.

La Comisión Directiva de la Unión Europea 2004/23/EC define como efecto, o evento, adverso grave cualquier hecho desfavorable vinculado a la obtención, evaluación, procesamiento, almacenamiento y distribución de células y tejidos que pueda conducir a la transmisión de una enfermedad, a la muerte del paciente, o a estados que hagan peligrar su vida, a minusvalías o incapacidades o que puedan dar lugar a hospitalización o enfermedad o la pueda prolongar. Se define como reacción adversa grave aquella respuesta inesperada del donante o del receptor, incluida una enfermedad transmisible, asociada a la obtención o aplicación en el ser humano de tejidos y/o células que resulte mortal, potencialmente mortal, que produzca invalidez o incapacidad, o que dé lugar a hospitalización o enfermedad o que las prolongue (http://www.ont.es/infesp/Paginas/default.aspx; European Committee on Organ Transplantation & Keitel 2019). Los bancos de tejidos tienen la obligación de facilitar a los centros implantadores de biosustitutos instrucciones claras de cómo notificar efectos y/o reacciones adversas graves mediante documentación estandarizada (European Committee on Organ Transplantation & Keitel 2019).

Se entiende por biovigilancia la monitorización sistemática de reacciones y efectos adversos severos desde la selección del donante hasta el seguimiento del receptor, con el objetivo de mantener la seguridad en el uso de SoHO. Hay diferentes fases en el Sistema de biovigilancia: primero es detectar e identificar un caso que pudiera ser descrito como reacción o evento adverso que luego se notifica a la autoridad sanitaria incluso antes de cerrar la investigación. Si hay sospecha de otro centro implantador o banco de tejidos afectado, son alertados rápidamente por la autoridad sanitaria para evitar complicaciones adicionales. Una vez la investigación ha finalizado, se decide el manejo y en el cierre del caso se incluye un informe con medidas correctoras y preventivas detallando cómo actuar en un futuro ante un caso similar (European Committee on Organ Transplantation & Keitel 2019; European Eye Bank Association 2020).

La Directiva de la Unión Europea 2006/86/EC exige a los estados miembros la supervisión, autorización e inspección de los bancos de tejidos, a introducir sistemas de trazabilidad tisular, mantener un registro de bancos de tejidos y un sistema de notificaciones de reacciones y efectos adversos graves. El sistema de biovigilancia de reacciones y efectos adversos de Catalunya entró en funcionamiento en el año 2008. Los bancos de tejidos deben tener unos estándares de calidad, basados en los

principios de buenas prácticas de manufactura (*Good Manufacturing Practices* - GMP). Los bancos adheridos a la EEBA cada vez más están cumpliendo con dichas regulaciones (Association 2020). Un proyecto conjunto de la Unión Europea y la *European Society of Cataract and Refractive Surgeons* (ESCRS) está ya en marcha para establecer el *European Cornea and Cell Transplant Registry* (ECCTR) - www.ecctr.org, que permitirá a los cirujanos enviar su actividad y resultados clínicos y será una herramienta más para la biovigilancia.

El BST se rige por los estándares comunes para los bancos de tejidos adheridos a la EEBA y dispuestos por la Unión Europea, el Consejo Europeo y la propia EEBA. La directiva europea incluye la Directiva 2004/23/EC, obligatoria desde abril de 2004, cuyo primer y segundo anexo - 2006/17/EC y 2006/86/EC - tratan sobre los requerimientos técnicos para la donación, codificación, obtención, evaluación, conservación, almacenamiento y distribución de tejidos humanos y células; con sus enmiendas de la Comisión Directiva 2012/39/EU, 2015/565 y 2015/566. El Consejo Europeo defiende los estándares incluidos en la *Guide to the Quality and Safety of Tissues and Cells for Human Application, 4th edition* (European Committee on Organ Transplantation & Keitel 2019). Finalmente, el BST se rige por la EEBA a través de diferentes grupos de trabajo - *Medical Special Interest Group* y *Technical Special Interest Group* – que han definido unos estándares médicos mínimos y guías técnicas.

1.3.1. Control de calidad y seguridad del tejido previo a la llegada al banco

La Directiva de la Comisión Europea 2006/17/EC establece los requerimientos para la obtención de tejidos humanos y células y criterios de selección de donantes. Un paso inicial en el control de calidad y seguridad es la evaluación del donante. Hay dos tipos de donantes, con diferentes riesgos y beneficios resultantes de la donación: alogénicos – vivos o fallecidos – y autólogos. La evaluación de los donantes autólogos es una situación especial dado que el donante es el propio receptor del tratamiento para una patología, dato a tener en cuenta en los criterios de aceptación como donante. Los dos objetivos principales de la evaluación de donantes son, por un lado, la obtención de información del donante que permita identificar contraindicaciones absolutas y relativas al uso de SoHO y que pudieran poner en riesgo al receptor; y por el otro, asegurarse de que la donación no causa daño a un donante vivo sano. Para

garantizar la recogida de información necesaria para la correcta evaluación de un donante, debe valorarse la historia médica – incluyendo enfermedades genéticas e historia familiar de enfermedades -, historia social referente a actividades personales y de comportamiento como historia de viajes, examen físico, examen psicológico en algunos casos de donación en vivo y test para descartar enfermedades transmisibles (European Committee on Organ Transplantation & Keitel 2019).

La Directiva de la Comisión Europea 2006/19/EC, así como el Real Decreto español RD1301/2006 marcan la necesidad de serologías negativas para enfermedades transmisibles como virus de la inmunodeficiencia humana, virus de la hepatitis B y C y *Treponema pallidum* para todo donante de tejidos. Las córneas se obtienen mediante extracción, bajo protocolos quirúrgicos y técnicas asépticas, del disco esclerocorneal que ha sido desinfectado previamente con povidona yodada. En el caso de la obtención de tejido escleral, se realiza enucleación del globo ocular en condiciones quirúrgicas y previa desinfección. La membrana amniótica se obtiene mediante disección de la placenta de donantes sanas. Previamente se lava con una solución de antibióticos y antifúngicos durante unas horas, tras lo cual se inspecciona el tejido y se procesa. Para el procesamiento de hemoderivados, equipos extractores del BST obtienen la sangre de los donantes en condiciones estandarizadas.

La extracción del SoHO es una actividad crítica. Los bancos de tejidos deben tener definidos protocolos para guardar registros de la obtención que incluyan relación de los pasos realizados, materiales e instrumental usado e identificación del personal implicado en la extracción. Estos registros deben ser claros, legibles, estar protegidos de enmiendas no autorizadas y cumplir con la Ley Orgánica 3/2018 de Protección de Datos Personales y garantía de los derechos digitales. Los registros de la obtención de tejidos deben ser suficientemente detallados para facilitar la trazabilidad, siendo ésta capaz de enlazar los registros con un donante en concreto (European Committee on Organ Transplantation & Keitel 2019).

1.3.2. Control de calidad en el banco de tejidos

Los SoHO deben procesarse y almacenarse en unas instalaciones destinadas específicamente a ese uso y que cumplan una serie de requisitos: estar localizadas en un área

segura cuyo acceso esté limitado a personal autorizado, tener suficiente capacidad de almacenamiento ordenado para diferentes tipos de tejido – en cuarentena, aptos para el procesamiento, descartados para uso en humanos, para investigación -; estar, limpias, secas y con adecuada climatización y ventilación (European Committee on Organ Transplantation & Keitel 2019).

Todo procesamiento de SoHO – lavado, centrifugado, corte, remojo en antibiótico, esterilización, descelularización, liofilización, criopreservación - debe realizarse siguiendo procedimientos normalizados de trabajo (PNT) y Guías de Buenas Prácticas (European Committee on Organ Transplantation & Keitel 2019).

Cuando un SoHO llega a las instalaciones del banco de tejidos, antes de iniciar cualquier procesamiento, el primer control de calidad consiste en la comprobación de que la documentación del envío, condiciones del transporte, correcto empaquetado y etiquetado de las muestras se corresponde a los requerimientos marcados por la Directiva Europea 2006/17/EC. Todos los tejidos deben conservarse en un estatus de cuarentena hasta que los test de control de calidad han sido revisados por el responsable. La Directiva de la Comisión Europea 2006/17/EC establece los requerimientos técnicos para cada paso en el proceso de preparación de los SoHO, desde los criterios de selección y obtención hasta los procedimientos de recepción y requerimientos para su distribución hasta llegar al receptor.

Como parte del control de calidad, el control microbiológico empieza con la descontaminación ya sea mediante desinfección (inmersión del SoHO en povidona yodada, clorhexidina y/o antibióticos) o esterilización (por irradiación en tejidos descelularizados como esclera o membrana amniótica). Además, se inspecciona regularmente la apariencia del medio de preservación del tejido para comprobar la esterilidad: un aumento de la turbidez o cambio de coloración en el medio de conservación - por cambio en el pH - es indicativo de contaminación (European Committee on Organ Transplantation & Keitel 2019). Además, se realiza un test microbiológico rutinario, tal como requieren las guías técnicas de la EEBA, ya que algunos tipos de contaminación tisular pueden aparecer sin que haya un cambio de coloración en el medio de preservación (Association 2020).

A parte del control microbiológico, en el caso del tejido corneal hay controles de calidad adicionales que incluyen la evaluación de las características morfológicas e integridad de las capas corneales – buscando signos de cirugías previas oculares que pudieran inutilizar la córnea (queratoplastias o láseres previos) – evaluación del tamaño del diámetro del área central de córnea clara y el contaje endotelial (European Eye Bank Association 2020).

En el caso de la membrana amniótica, el control de calidad empieza por la inspección visual de la membrana para asegurar la integridad y descartar cambios patológicos macroscópicos. Además, se toman muestras para cultivo antes y después de la descontaminación con antibióticos. Si en las muestras tomadas previas al antibiótico crece algún tipo de microorganismo considerado de alta virulencia, el tejido se descarta para uso clínico (European Committee on Organ Transplantation & Keitel 2019).

1.3.3. Control de calidad tras la salida del banco de ojos y perioperatorio

De las complicaciones asociadas al uso de biosustitutos alogénicos, las infecciosas son especialmente importantes: son reacciones adversas que requieren de trazabilidad por el sistema de biovigilancia.

Se entiende por control microbiológico de donantes, el cultivo de restos del tejido implantado en el receptor para el estudio de microorganismos con potencial infeccioso que pudieran crecer en dicho cultivo. De esta manera se puede anticipar un tratamiento profiláctico al receptor que, además, suele estar facilitado por el conocimiento del microorganismo cultivado y del antibiograma realizados de forma rutinaria ante la positividad de un cultivo (Vislisel et al. 2017; Tsui et al. 2019; Das et al. 2020).

La realización de controles microbiológicos de tejidos alogénicos es recomendable, pero no tiene carácter obligatorio según la ley española. La realización o no de estos controles recae en la voluntariedad del cirujano implantador, limitación que también tienen otros países (Aldave et al. 2013). A pesar del interés en la trazabilidad tisular, la utilidad de los cultivos microbiológicos ha sido cuestionada dada la baja tasa de complicaciones infecciosas (Everts et al. 2001; Wilhelmus & Hassan 2007), puesto que

las principales complicaciones infecciosas asociadas al implante de tejido corneal – la queratitis infecciosa y la endoftalmitis – tienen baja incidencia (inferior al 0,3%), no siempre se correlacionan con la contaminación del donante y pueden aparecer con cultivos negativos (Leveilleet al. 1983; Kloess et al. 1993; Everts et al. 2001; Kitzmann et al. 2009). Otros autores, sin embargo, defienden su utilidad y deber de realización (Kiatos et al. 2017; Mian et al. 2018).

Los cultivos microbiológicos tras el implante de tejidos alogénicos para la biosustitución han sido estudiados sobre todo en relación al tejido corneal, analizando especialmente el anillo esclerocorneal restante (McMullan & Cavanagh 1983; Leveille et al. 1991; Kloess et al. 1993; Keyhani et al. 2005; Fontana et al. 2007; Matsumoto et al. 2011; Rauen et al. 2012; Sharma et al. 2018; Hajjar Sesé et al. 2019; Chen et al. 2019; Tsui et al. 2019). En algunos países, como el Reino Unido, para el estudio microbiológico se cultiva el líquido de preservación en lugar del propio tejido (Chen et al. 2015). Algunos autores incluso han sugerido el cultivo de frotis conjuntival del donante (Matsumoto et al. 2011).

El origen de cualquier contaminación del tejido esclerocorneal permanece como la cuestión clave. La transmisión de donante a receptor está descrita (Linke et al. 2013; Gruenert et al. 2017; Röck et al. 2017), pero la contaminación puede también estar relacionada con la manipulación del tejido tanto en el banco como en el quirófano (Rauen et al. 2012; Röck et al. 2017).

1.4. ASPECTOS CLÍNICOS DE LA PATOLOGÍA DE LA SUPERFICIE OCULAR

1.4.1. Patologías de la superficie ocular

La alteración de la superficie ocular ocurre asociada a un gran número de condiciones clínicas como traumatismos, infecciones, inflamación, alteraciones genéticas y disfunción lagrimal, entre otros. Las siguientes patologías son representativas del uso de SoHO de la córnea y superficie ocular.

1.4.1.1. Alteraciones de la superficie ocular: disfunción lagrimal (ojo seco), defecto epitelial persistente y úlcera corneal

La *Tear Film and Ocular Surface Society* (TFOS) a través de su grupo de trabajo *Dry Eye Workshop* II (DEWS II) definió el ojo seco como una enfermedad multifactorial de la superficie ocular que se caracteriza por una pérdida de la homeostasis de la película lagrimal, se acompaña de síntomas oculares y en cuya etiología intervienen la inestabilidad e hiperosmolaridad de la película lagrimal, la inflamación, daño de la superficie ocular y alteraciones neurosensoriales (Craig et al. 2017). Clásicamente, el ojo seco se ha clasificado en acuodeficiente – por disminución de la secreción lagrimal – y evaporativo – por alteración de la composición lagrimal (Figura 7). El informe DEWS II incorporó un tercer tipo – ojo seco mixto – con características de ambos tipos. Otras clasificaciones siguen criterios de gravedad. En los casos más graves asociados al ojo seco (pero no únicamente causados por él) la superficie ocular puede verse agravada por defectos epiteliales persistentes – definidos como daño en el epitelio corneal que no sana en dos semanas (Tsubota et al. 1999) – y úlceras corneales, con pérdida de tejido estromal y llegando incluso a la perforación ocular (Figura 8).

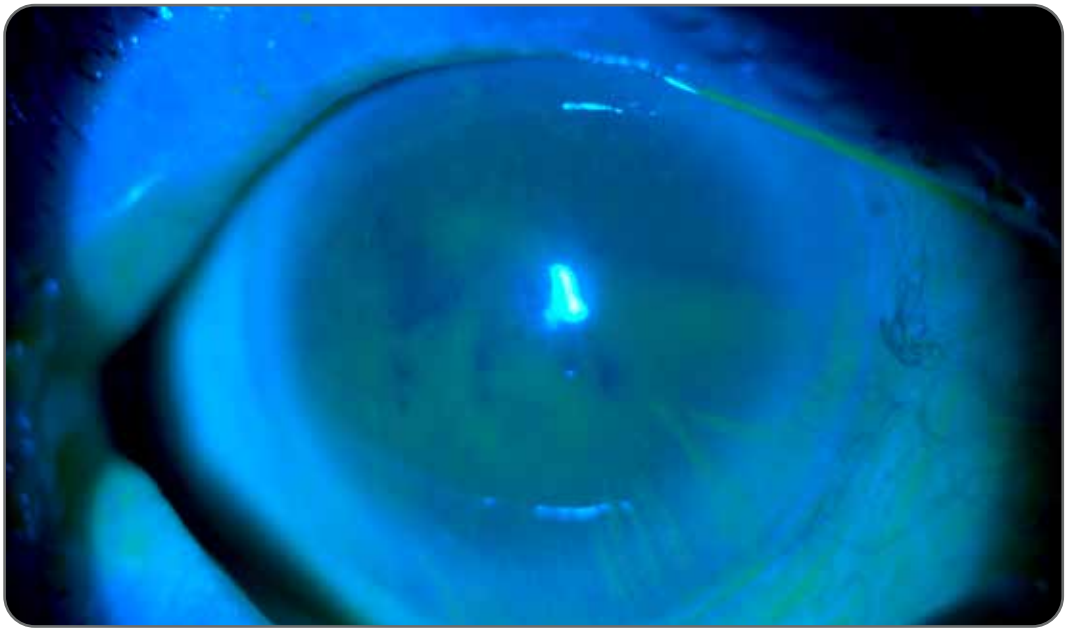

Figura 7: Ojo seco grave. En la imagen puede apreciarse queratopatía punteada superficial, más evidente en la zona perilímbica entre las IV y las VI horas. También se observa la rotura lagrimal (zonas más oscuras de la córnea) asociadas a un menor tiempo de rotura lagrimal (TBUT). Imagen original de Noelia Sabater Cruz.

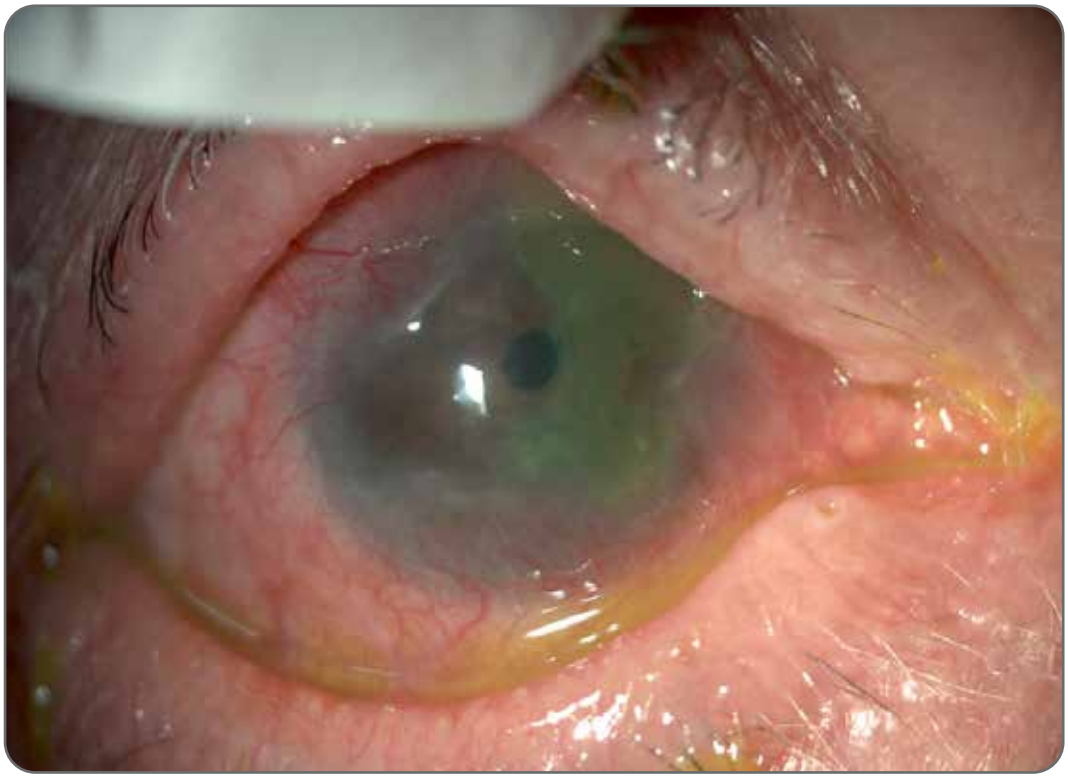

Figura 8: Defecto epitelial persistente asociado a una úlcera corneal neurotrófica en un paciente con queratoconjuntivitis herpética insuficiencia límbica y disminución de la sensibilidad corneal. Imagen original de Noelia Sabater Cruz.

El tratamiento del ojo seco depende de su grado de gravedad y suele ser escalonado. Así en casos leves y leve-moderados, suele ser suficiente con tratamientos de primera línea: lubricantes oculares (lágrimas artificiales) solos o asociados a suplementos de ácidos grasos poliinsaturados omega 3, inmunomoduladores (ciclosporina A), antiinflamatorios (corticoides tópicos), luz pulsada, y/o tapones lagrimales. En casos más graves y/o cuando se acompaña de otras alteraciones de la superficie ocular – defecto epitelial persistente, úlcera corneal – son necesarias opciones terapéuticas tales como tarsorrafia, toxina botulínica, lente de contacto terapéutica o biosustitutos de la superficie ocular instilados (biosustitutos de la lágrima) o implantados (Şimşek et al. 2018; Bernabei et al. 2019; O'Neil et al. 2019; Sabater-Cruz & Casaroli-Marano 2019).

Así en alteraciones de la superficie ocular como las que se producen en la enfermedad de injerto contra huésped, ojo seco grave, defecto epitelial persistente, úlcera neurotrófica, erosión corneal recurrente y quemadura química, diferentes estudios han demostrado la utilidad del lisado de plaquetas (Sandri et al. 2016; Pezzotta et al.

2017), suero autólogo (Fox et al. 1984; Tsubota et al. 1999; Ogawa et al. 2003; Urzua et al. 2012; Pan et al. 2017), suero de cordón umbilical (Yoon 2014; Giannaccare et al. 2020), AMEED (Bonci et al. 2005; Liang et al. 2009; Yeu et al. 2019) y sangre autóloga (Than et al. 2017; Balal et al. 2018; Balal et al. 2020). El defecto epitelial persistente puede tratarse también con implante de membrana amniótica (Seitz et al. 2009; Dhillon et al. 2019) o colgajo conjuntival (Zemba et al. 2020).

Una perforación ocular – corneal o escleral – requiere de tratamiento inmediato por tratarse de una urgencia. Cuando es consecuencia de un traumatismo penetrante sin pérdida de tejido, puede ser suficiente el cierre mediante suturas, pero ante la pérdida de tejido – queratitis ulcerativas periféricas, traumatismo con pérdida de tejido o *melting* y descematocele, entre otros – puede ser necesario un implante tectónico con tejido corneal y/o escleral (Sangwan et al. 2001; Jain & Gupta 2007; Sharma et al. 2018; Pant et al. 2020).

1.4.1.2. Alteraciones corneales causantes de disminución de visión

1.4.1.2.1. *Distrofia de Fuchs y queratopatía bullosa*

La distrofia endotelial de Fuchs es una enfermedad del endotelio corneal que se caracteriza por una pérdida acelerada bilateral de células endoteliales con aparición de cambios en la membrana de Descemet. Estas alteraciones incluyen la acumulación de matriz extracelular, la formación de unas excrecencias llamadas *guttae* o guttas y la evolución progresiva hacia una descompensación endotelial (Figura 9). La disminución de agudeza visual resulta de estos cambios en la membrana de Descemet, así como de la disrupción en los estadios finales de la función de bomba del endotelio, causando edema corneal, formación de bullas y fibrosis subepitelial (Matthaei et al. 2019; Price et al. 2020).

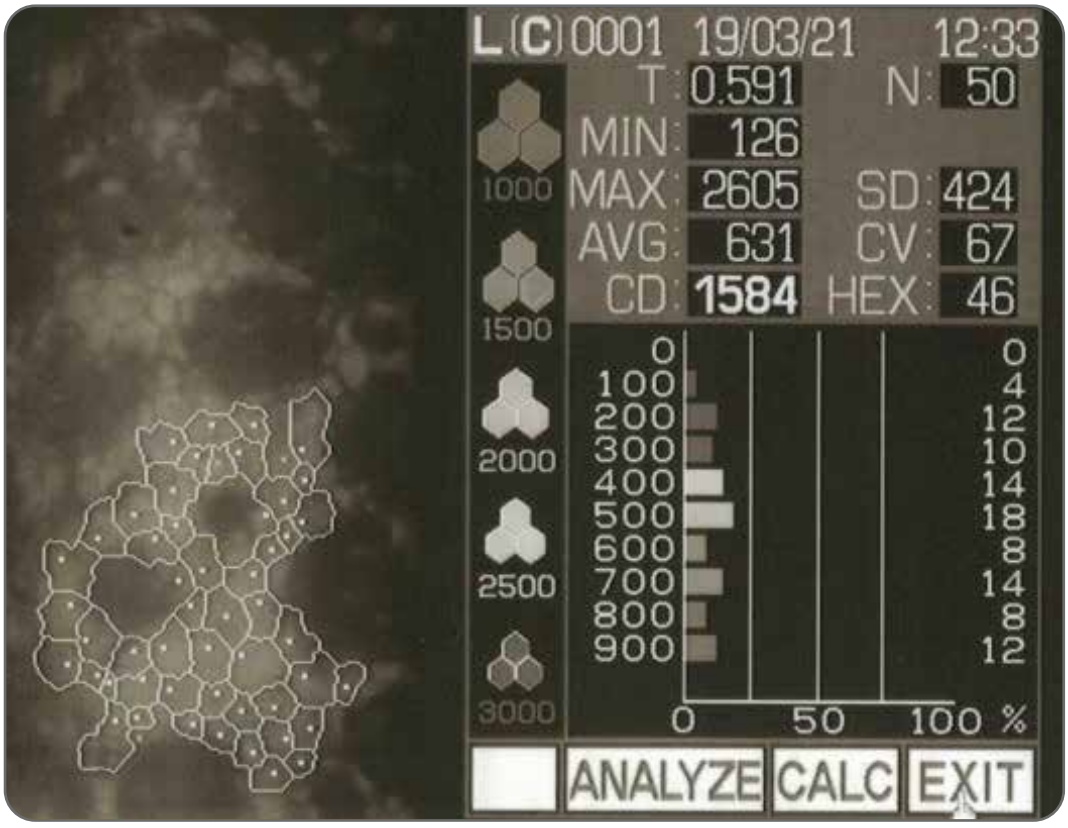

Figura 9: Imagen de microscopia especular del ojo izquierdo de una paciente con distrofia de Fuchs. Se aprecia la presencia de guttas y una densidad endotelial suficiente para evitar la descompensación corneal. Imagen original de Noelia Sabater Cruz.

La queratopatía bullosa es una condición en la que la córnea sufre un edema a causa de la disfunción de las células endoteliales con posterior desarrollo de bullas epiteliales (Siu et al. 2019). La descompensación del endotelio corneal puede ser secundario a cirugía intraocular, distrofia endotelial de Fuchs u otras enfermedades del endotelio corneal (Gonçalves et al. 2008; Stefan et al. 2017; Soh et al. 2020).

La opacidad corneal producida por la descompensación corneal puede requerir de biosustitución corneal mediante queratoplastia (Stefan et al. 2017; Palma-Carvajal et al. 2020; Price et al. 2020; Soh et al. 2020). El manejo de las bullas incluye también el tratamiento con implante de un colgajo de conjuntiva (Lim et al. 2009), membrana amniótica (Stefan et al. 2017; Siu et al. 2019) o ambos (Güell et al. 2012).

1.4.1.2.2 *Ectasias corneales*

La ectasia corneal es una patología no inflamatoria generalmente bilateral, en la que hay un adelgazamiento progresivo del espesor corneal y un aumento de su curvatura. Hay diferentes tipos de ectasia que se distinguen por sus característicos patrones de adelgazamiento corneal: queratocono, degeneración marginal pelúcida, queratoglobo y ectasia post cirugía - refractiva y queratoplastia. Las ectasias corneales están asociadas a una disminución de la agudeza visual sin corrección – y con corrección en los grados más graves - y un incremento de las aberraciones visuales. La etiología de las ectasias corneales incluye tanto factores genéticos como mecánicos (Sridhar et al. 2004; Jin, Dackowski & Chuck 2020).

El queratocono es un tipo de ectasia corneal progresiva bilateral asimétrica en la que la córnea se adelgaza y protruye, adquiriendo la característica forma cónica a la que debe su nombre. Afecta a pacientes jóvenes – suele iniciarse en la adolescencia - y su diagnóstico es topográfico y clínico, siendo la disminución de agudeza visual el principal síntoma (Buzzonetti et al. 2020; Feu-Basilio et al. 2020; Zhang et al. 2021) (Figura 10).

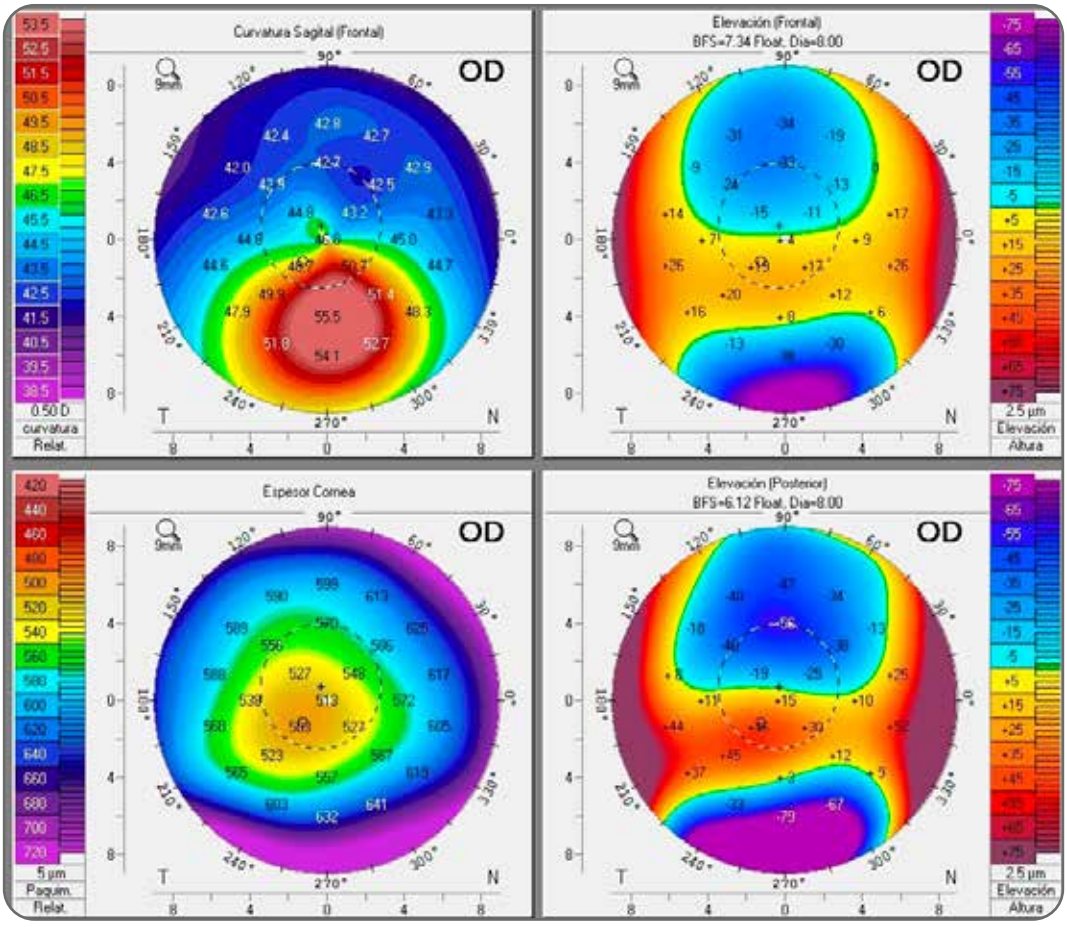

Figura 10: Imagen topográfica del ojo derecho de un paciente con queratocono. Se aprecia adelgazamiento corneal paracentral inferior y temporal a expensas, sobre todo, de la cara posterior de la córnea. Imagen original de Noelia Sabater Cruz.

El manejo de las ectasias depende del grado de gravedad: evitar el frotamiento ocular, corrección de la refracción con gafas o lentes de contacto – reservando las de contacto rígidas gas-permeables para casos más avanzados -, *cross-linking* del colágeno para detener la progresión, implante de segmentos intracorneales y biosustitución con queratoplastia penetrante o lamelar anterior profunda para los casos de patología más avanzada (Saad et al. 2020; Yu, Mattioli & Busin 2020).

1.4.1.3. Pterigión

El pterigión (o *pterigium*) es una lesión proliferativa de tejido fibrovascular sobre la córnea que causa sintomatología ocular como lagrimeo, sensación de cuerpo extraño, disminución de la agudeza visual e incluso restricción ocular (Liu et al. 2013; Cárde-

nas-Cantú et al. 2016) (Figura 11). El tratamiento puede ser médico en casos leves - lubricación ocular - o quirúrgico. En cuanto a técnicas quirúrgicas, se ha demostrado que la exéresis asociada o no a cierre simple de la conjuntiva conlleva una alta tasa de recidivas, por lo que en la actualidad se implanta un SoHO de la conjuntiva resecada que actúe como barrera (Alpay et al. 2009; Hovanesian et al. 2017): la exéresis con autoinjerto conjuntival está considerada el *gold standard* por tener la menor tasa de recidivas (Küçükerdönmez et al. 2007; Akbari et al. 2017; Clearfield et al. 2017). El implante de membrana amniótica es una alternativa efectiva por sus propiedades antiinflamatorias en esos casos en que la conjuntiva deba reservarse (Nakamura et al. 2006; Rahman et al. 2009; Toker & Eraslan 2016; Walkden 2020).

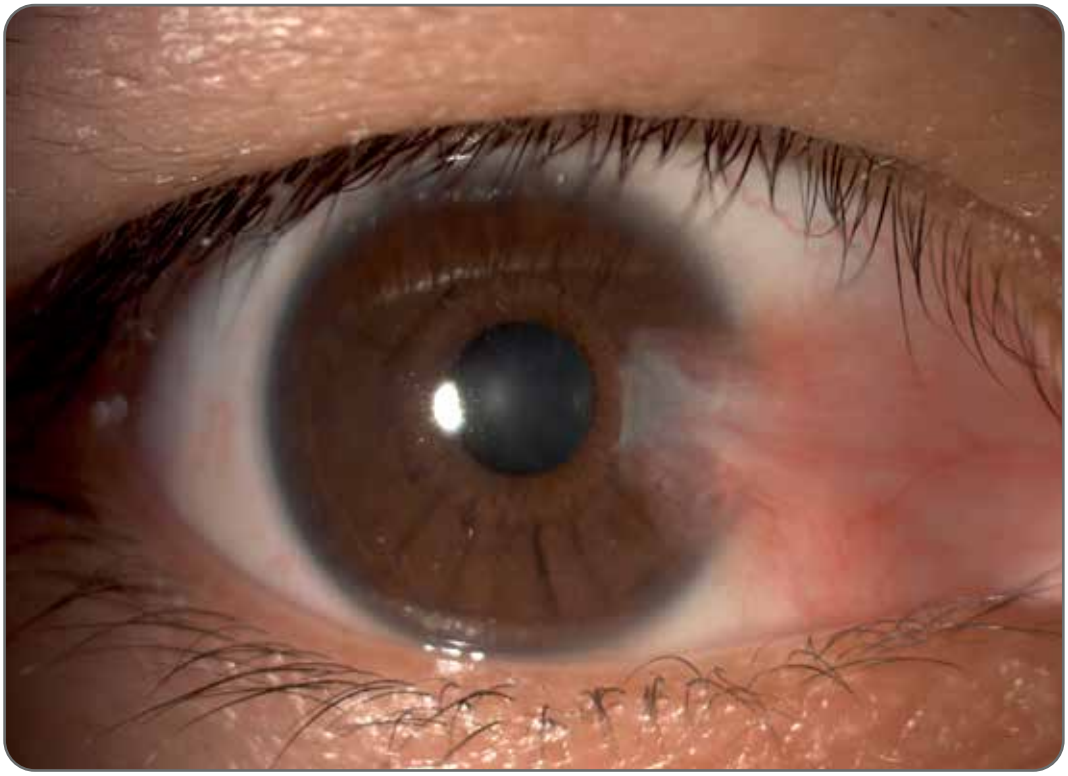

Figura 11: Pterigión primario nasal en ojo derecho. Imagen original de Noelia Sabater Cruz.

1.4.2. Epidemiología, evolución de las indicaciones y técnicas

La queratoplastia ha evolucionado tanto en su técnica como en sus indicaciones desde el primer trasplante realizado con éxito por Eduard Zirm en 1905 (Moffatt et al. 2005; Singh et al. 2019). La evolución en la técnica viene dada por el interés centrado en la personalización de los trasplantes de córnea, permitiendo la separación selectiva del tejido corneal enfermo y su sustitución por tejido corneal donante sano en la queratoplastia lamelar, en contraposición a la queratoplastia penetrante, la técnica clásica aún vigente en la que se sustituye todo el grosor corneal. En las últimas dos décadas se ha detectado un cambio de tendencia en el tipo de técnica usada para queratoplastia, pasando de la realización solamente queratoplastia penetrante a las queratoplastias lamelares anteriores o posteriores, además de la técnica penetrante. La aparición de las diferentes técnicas lamelares ha modificado también la tendencia en las indicaciones de la queratoplastia tanto dentro como fuera de nuestra región (Chen et al. 2001; Zare et al. 2012, Zhang et al. 2013; Tan et al. 2014; Park et al. 2015; Singh et al. 2019). Las nuevas técnicas lamelares han mejorado los resultados visuales y de supervivencia del injerto a largo plazo. De esta forma, pacientes a los que hace unos años se les hubiera retrasado o descartado la indicación de una queratoplastia penetrante, actualmente tienen indicación para las técnicas lamelares y de una manera más precoz. Además, la reciente disponibilidad de tejido precortado por parte del BST puede haber favorecido que algunos cirujanos que solo realizaban técnica penetrante hayan empezado a realizar técnicas lamelares, influyendo así en el cambio de tendencia no solo en las técnicas de queratoplastia sino también de indicaciones (Tan et al. 2014; Park et al. 2015; Palma-Carvajal et al. 2020). La evolución del manejo médico y quirúrgico de diferentes patologías oculares (manejo quirúrgico del glaucoma, del pterigión, de la patología palpebral y orbitaria y manejo médico de las escleritis, entre otros), puede haber producido cambios en las indicaciones de implante de tejido escleral y membrana amniótica (Sainz de la Maza et al. 1989; Harizman et al. 2005; Gawdat & Ahmed 2014; Dubey et al. 2017; Hodge et al. 2017; Jirsova & Jones 2017; Park et al. 2017; Wen et al. 2018). La reciente disponibilidad de diferentes presentaciones de la membrana amniótica y, especialmente AMEED como biosustituto de la lágrima, puede haber aumentado las indicaciones para el uso de membrana amniótica.

2

HIPÓTESIS

HIPÓTESIS

De acuerdo con los antecedentes anteriormente descritos, nuestra hipótesis de trabajo se centra en:

1. La aplicación clínica de biosustitutos de origen humano y la existencia de un cambio de tendencias en las indicaciones y formulaciones y/o técnicas quirúrgicas para el manejo y tratamiento de la patología de la córnea y superficie ocular.

2. Se considera que el control de la calidad de estos biosustitutos es de suma importancia para evitar efectos adversos y complicaciones para el receptor, siendo característico como ejemplo, el control microbiológico en los trasplantes de córnea.

3. La investigación y desarrollo constantes de los biosustitutos de origen humano, ha permitido recientemente disponer de nuevos formatos y formulaciones para la aplicación clínica en el tratamiento de diferentes patologías de la superficie ocular.

3

OBJETIVOS

OBJETIVOS

Con base a nuestra hipótesis de trabajo el *objetivo principal* de nuestra investigación es el de *conocer las indicaciones de biosustitutos de origen humano - córnea, esclera, membrana amniótica - para el tratamiento de las patologías de la córnea y superficie ocular en Catalunya.*

Los *objetivos secundarios* son:

- Evaluar los cambios en las indicaciones y tendencias en la utilización de tejido corneal y de las técnicas de queratoplastia (penetrante versus lamelares) en Catalunya.

- Evaluar las indicaciones y tendencias de la utilización del tejido escleral y de la membrana amniótica como coadyuvante para el tratamiento de las patologías de la superficie ocular.

- Evaluar el uso de los cultivos microbiológicos de anillos esclerocorneales y medios de preservación corneal como control perioperatorio del tejido donante en el mundo real. Con ello, se destaca analizar el valor añadido y la frecuencia de la utilización del control microbiológico en un entorno real y sus factores asociados a la contaminación del tejido corneal.

- Evaluar los resultados clínicos de los biosustitutos autólogos, tomando como biosustituto representativo la conjuntiva y analizando factores de riesgo, como la tasa de adherencia al tratamiento postoperatorio, asociados al resultado clínico de la cirugía del pterigión.

- Evaluar los resultados clínicos de los recientes formatos de la membrana amniótica disponibles en Cataluña – liofilizada y colirio de extracto de membrana amniótica – desde un punto de vista de validación y estudio en la práctica clínica.

4

MATERIALES Y MÉTODOS Y RESULTADOS (RELACIÓN DE ARTÍCULOS)

MATERIALES Y MÉTODOS Y RESULTADOS (RELACIÓN DE ARTÍCULOS)

Artículo 1

Objetivo:
Conocer las indicaciones de biosustitutos de origen humano - córnea, esclera, membrana amniótica - para el tratamiento de las patologías de la córnea y superficie ocular en Catalunya. Y evaluar los cambios en las indicaciones y tendencias en la utilización de tejido corneal y de las técnicas de queratoplastia (penetrante versus lamelares) en Catalunya.

Título:
Corneal transplantation activity in Catalonia, Spain, from 2011 to 2018: evolution of indications and surgical techniques

Autores:
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Cuartil y área de conocimiento:
Q2 *Multidisciplinary Sciences* (JCR)
Q1 *Multidisciplinary* (Scimago)

RESUMEN

Propósito: describir la actividad de trasplante corneal llevada a cabo en Catalunya (España) y la evolución en las indicaciones de queratoplastia durante un periodo de ocho años.

Métodos: se revisaron las memorias anuales de la *Organització Catalana de Trasplantaments* (OCATT) sobre indicaciones y técnicas de queratoplastia entre los años 2011 a 2018.

Resultados: se realizó en Catalunya un total de 9457 queratoplastias entre enero de 2011 y diciembre de 2018. Las indicaciones más frecuentes fueron la queratopatía bullosa (20,5%), la distrofia endotelial de Fuchs (17,9%), el retrasplante (13,7%) y el queratocono (11,3%). La queratoplastia penetrante representó el 63,4% de todos los trasplantes corneales. Desde que el banco de ojos introdujo el tejido precortado para *Descemet Stripping Automated Endothelial Keratoplasty* (DSAEK) en 2013 y para *Descemet Membrane Endothelial Keratoplasty* (DMEK) en 2017 el número de queratoplastias endoteliales ha aumentado de manera drástica. Se ha encontrado una tendencia creciente de las técnicas lamelares posteriores sobre el total de queratoplastias ($p<0,001$). Las queratoplastias endoteliales para diferentes enfermedades endoteliales (queratopatía bullosa, distrofia endotelial de Fuchs y retrasplante) han mostrado también una tendencia al alza ($p<0,001$). La DMEK es la técnica con el mayor incremento (estadísticamente diferente de la linealidad) sobre otras técnicas lamelares en distrofia endotelial de Fuchs ($p<0,001$), pero no en queratopatía bullosa ($p=0,67$) o retrasplantes ($p=0,067$).

Conclusión: las enfermedades endoteliales representan las principales indicaciones de queratoplastia durante el periodo de ocho años estudiado. La queratoplastia penetrante es todavía la técnica más usada en Catalunya, a pesar de que las queratoplastias endoteliales, y especialmente la DMEK han mostrado una significativa tendencia ascendente durante los últimos años. Esto es congruente con la tendencia actual en las queratoplastias: personalizar y trasplantar el menor tejido posible. Por lo tanto, la disponibilidad del tejido precortado puede haber reforzado esta aproximación.

PLOS ONE

RESEARCH ARTICLE

Corneal transplantation activity in Catalonia, Spain, from 2011 to 2018: Evolution of indications and surgical techniques

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Abstract

Purpose

To report corneal transplant activity carried out in Catalonia (Spain) and the evolving indications for keratoplasty over an 8-year period.

Methods

Annual reports from the Catalan Transplant Organization, Spain, on corneal graft indications and techniques from 2011 to 2018 were reviewed.

Results

A total of 9457 keratoplasties were performed in Catalonia, from January 2011 to December 2018. The most frequent indications were bullous keratopathy (BK; 20.5%), Fuchs endothelial dystrophy (FED; 17.9%), re-graft (13.7%), and keratoconus (11.3%). Penetrating keratoplasty (PKP) accounted for 63.4% of all performed keratoplasties. Since the introduction of eye bank precut tissue for Descemet stripping automated endothelial keratoplasty (DSAEK) in 2013 and for Descemet membrane endothelial keratoplasty (DMEK) in 2017 the number of endothelial keratoplasties has drastically increased. An increasing trend of posterior lamellar techniques over the total of keratoplasties was found ($p<0.001$). Endothelial keratoplasties for different endothelial diseases indications (BK, FED, and re-graft), also showed and increasing trend ($p<0.001$). DMEK is the technique with the highest increase (statistically significantly different from linearity) over other endothelial keratoplasties in FED ($p<0.001$) but not in BK ($p = 0.67$) or re-grafts ($p = 0.067$).

Conclusion

Endothelial diseases represented the top indication for keratoplasty over the 8-year period. PKP is still the most used technique in Catalonia, but endothelial keratoplasties and especially DMEK showed a significant increasing trend over the last years. This is congruent with

Competing interests: The authors have declared that no competing interests exist.

the main rationale nowadays for keratoplasties: to customize and transplant as less tissue as possible. Therefore, the availability of precut tissue could have definitely enforced such approach.

Introduction

Corneal grafts are one of the most frequent transplanted tissues in the world [1]. In addition, keratoplasty techniques, and therefore indications, have majorly changed these last years, thanks to the upraise of selective corneal surgeries that have vastly improved visual recovery results. However, epidemiology on such surgeries is not always easy and mostly depends on regional eye bank report services. Moreover, several studies suggest most surgeons tend to customize indications and even perform more posterior lamellar techniques when eye bank precut tissue is available [2,3].

In 1989 the Spanish National Transplant Organization (*Organización Nacional de Trasplantes*–ONT) started its activity coordinating organ and tissue donations in Spain. In 1994, the autonomous region of Catalonia, started its own organization (*Organització Catalana de Trasplantaments*–OCATT), and joined efforts with ONT [4]. However, keratoplasty indications, techniques and trends have not been studied in depth since the introduction of precut corneal tissue supply by the main regional eye bank in such a population with a high volume of transplantations, 1000 keratoplasties/year on average, representing 25% to 30% of total keratoplasties in Spain. Over the last few years, the number of keratoplasties has increased in Spain (over 4000 keratoplasties/year since 2016, and even more in Catalonia [5].

In Catalonia, retrieval of ocular tissue is mainly performed by transplant coordinator teams and centralized by an eye bank (Barcelona Tissue Bank, BTB) [6]. Then, the ocular tissue is processed and distributed. The OCATT collects information on tissue viability and distribution from the different transplant centres, and surgical and clinic details and keratoplasty technique indications from standardized questionnaires sent to surgeons. Moreover, OCATT has a biovigilance system to detect adverse events and adverse reactions.

Being a centralized eye bank in Catalonia, BTB supplies corneal grafts an offers precut tissue for posterior lamellar techniques. In 2013, it started to supply precut tissue for Descemet Stripping Automated Endothelial Keratoplasty (DSAEK), and from 2017, precut tissue for Descemet Membrane Endothelial Keratoplasty (DMEK) was distributed [6]. Despite few surgeons started to perform endothelial keratoplasties (EK) before 2013, from that date, more and more surgeons dared to switch to posterior lamellar techniques due to the availability of precut tissue [3].

This study investigates the evolving trends of corneal transplantation from 2011 to 2018 in Catalonia centres, and its relationship with precut tissue supply.

Materials and methods

This study is a retrospective review of corneal tissue activity records of the OCATT in Catalonia, between January of 2011 and December of 2018. The included data corresponded to corneal tissue retrieved and implanted in Catalonia. ONT website was consulted to obtain data regarding the Spanish registries [5]. Institutional Ethics Committee Board approval was obtained for donor data revision (approval number HCB/2015/0879, Hospital Clinic de Barcelona, amended on 14th November of 2018). Research methods and analysis plan adhered to the Tenets of the Declaration of Helsinki. Data related to ocular tissue, its traceability, and

potential adverse events were treated in accordance with the appropriate European Union directives (2004/23/EC, 2006/17/EC, and 2006/86/EC). Patient data were encoded for management in accordance with the Spanish legislation on personal data protection (RD05/2018).

Reported data outcomes included, as per every year, number of cases in all surgical techniques as well as number of cases in each approach by their main clinical indication for keratoplasty. Descriptive results are presented as absolute frequencies and percentages for categorical variables. Linear trends (Mantel-Haenszel statistic) and deviation from linearity were obtained. All tests were performed with a two-sided type I error of 5% with the statistical package STATA v.15.1 (StataCorp, College Station, Texas, USA).

Results

Between 2011 and 2018, 9457 keratoplasties were performed in Catalonia, that is 1182 keratoplasties/year on average (ranging 989–1456 per year) (Fig 1A). To report how prevalent or frequent are transplants among regions or countries, the unit transplants per million population (pmp) is commonly used. In this regard, 190.2 corneal transplants per million population and year were performed on average in Catalonia (Fig 1A), while this figure was 80.4 pmp/year on average in Spain (Fig 1B).

Together with the corneal tissue sample, a follow-up form is sent to the surgeon, asking for adverse events, surgical technique, and surgery indication, among others. Despite the high

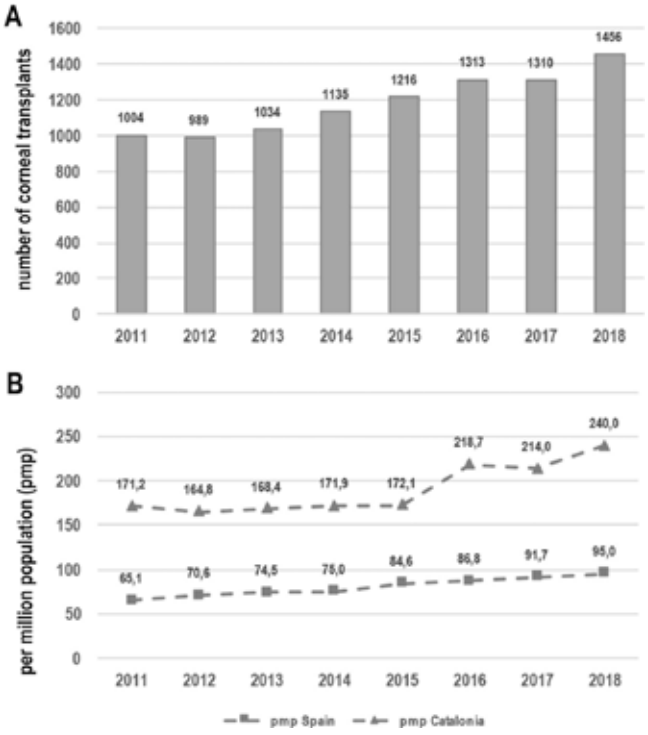

Fig 1. Number of keratoplasties per year carried out in Catalonia (1A) and corneal transplants per million inhabitants in Catalonia and Spain (1B).

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Table 1. Indications reported for corneal transplantation from January 2011 to December 2018 in Catalonia (Spain).

Diagnosis	Number of transplants (%)
Bullous keratopathy secondary to surgery (any)	1574 (20.5)
Fuchs endothelial dystrophy	1373 (17.9)
Re-graft due to endothelial failure	1051 (13.7)
Keratoconus/ectasia (other than post-refractive)	865 (11.3)
Infection (viral, fungal and bacterial)	604 (7.9)
Corneal degeneration	563 (7.3)
Chemical injury/trauma	381 (5.0)
Congenital opacity	225 (2.9)
Ulcerative keratitis (non-infectious)	221 (2.9)
Stromal corneal dystrophy	213 (2.8)
Refractive surgical complication	50 (0.7)
Other*	540 (7.0)
Total of transplants with diagnosis informed	7660 (100)
Total of transplants	9457

*Other included: Rejection (134; 1.75%), regraft for a reason other than endothelial failure (78; 1%), oedema of unknown origin (67; 0.9%), irregular astigmatism (22; 0.3%), opacification (14; 0.18%), perforation (9; 0.12%), toxic epidermal necrolysis (7; 0.09%), descemetocele (5; 0.06%), pemphigoid (4; 0.05%), Peters anomaly (2; 0.03%), congenital glaucoma (1; 0.01%), bacterial keratitis and perforation (1; 0.01%), epithelial ingrowth (1; 0.01%), hematocornea (1; 0.01%), unknown (194; 2.5%).

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recommendation to send it back, the response rate per year was heterogeneous, ranging from 59.4% to 96%. Information about surgical indication and keratoplasty technique was obtained from 7660 out of 9457 procedures (80.9%). The indications for corneal transplantation are listed in Table 1. The most frequent indications were bullous keratopathy (BK; 1574; 20.5%), Fuchs endothelial dystrophy (FED) and other endothelial dystrophies (1373; 17.9%), re-graft due to endothelial failure (1051; 13.7%), and keratoconus (865; 11.3%).

Corneal transplantation techniques performed from 2011 to 2018 were: penetrating keratoplasty (PKP; 4848; 63.4%), deep anterior lamellar keratoplasty (DALK; 827; 10.7%), and endothelial keratoplasty (1985; 25.9%). During the years 2011 and 2012, due to the low number of posterior lamellar keratoplasties performed, no distinction between DMEK and DSAEK was formally made. Evolving trends in keratoplasty techniques over years are shown in Fig 2A.

The evolving trend of lamellar techniques was statistically analysed and resumed in Table 2. It showed statistically significant increasing trends of endothelial and anterior lamellar keratoplasties, which differ from linearity in almost all reported associations but for DMEK performed on BK, given the low number of cases. In addition, although DMEK showed an increasing trend in re-grafts, it did not reach statistical significance ($p = 0.067$) (Fig 2B).

Discussion

Catalonia is an autonomous region of Spain with 32108.2 km^2 and a population of 7.5 million people at end of 2018 [7]. The number of corneal transplants performed in Catalonia from 2011 to 2018 has been 9457 (average of 1050.7 keratoplasties/year), corresponding to 190.2 corneal transplants per million population and year performed on average, while this figure is 80.4 on average in Spain. In other words, 32.7% of all keratoplasties performed in Spain were performed in Catalonia [5]. Currently, the BTB is the only bank in Spain that processes corneas for precut tissue delivery [8].

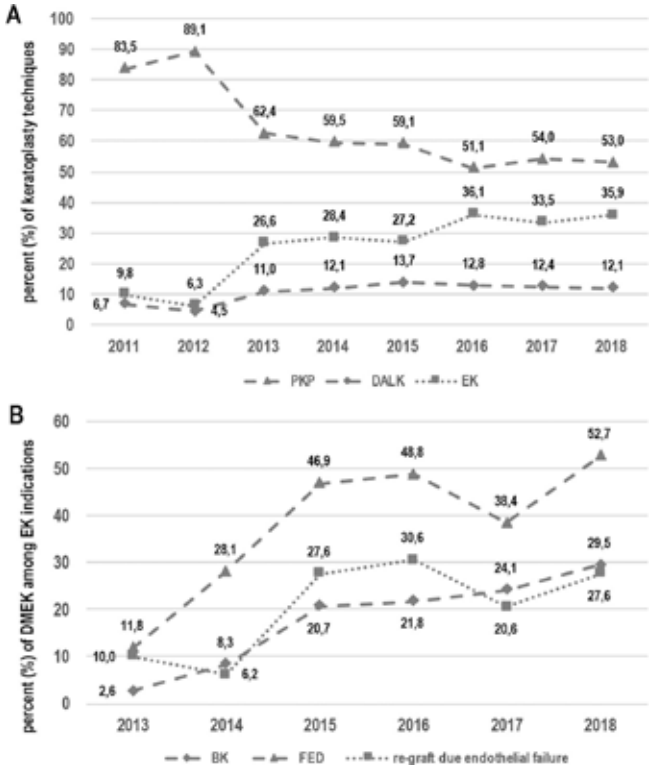

Fig 2. Corneal transplantation techniques: Evolving trends in the period of 2001–2018 (2A). Trends of DMEK in endothelial keratoplasties from 2013 to 2018 for different indications–BK, FED, and re-grafting due to endothelial failure (2B). DMEK: Descemet Membrane Endothelial Keratoplasty. PKP: Penetrating Keratoplasty, DALK: Deep Anterior Lamellar Keratoplasty, EK: Endothelial Keratoplasty. BK bullous keratopathy. FED: Fuchs Endothelial Dystrophy.

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As observed, the most usual indication for corneal transplantation during the period of study was BK followed by FED. The most prevalent technique was PKP, which accounted for 63.4% of all performed keratoplasties. A statistically significantly increasing trend of posterior lamellar techniques over the total of keratoplasties was found. In addition, DMEK was indeed the technique with the highest increase over other endothelial keratoplasties in FED but not in BK or re-grafts due to endothelial failure. That increase could be associated to the start of pre-cut tissue supply for EK by the regional corneal tissue bank. Therefore, a predominance of the lamellar techniques is expected to be found in the next years. Interestingly, such a shift has already happened in other countries several years earlier than in the studied area: PK replaced by lamellar keratoplasties was first reported in 2015 in the United States [9] and 2017 in Germany [10] and a significant shifting trend was stated in 2014 in Canada [11,12] and 2015 in Italy; [13] while in other areas this shift had not been detected so far [14].

Indications for corneal transplantation are still different from one region to another, despite overall trends are indeed changing and converging: BK and FED have been seen to be most common indications for transplant in developed countries whereas infective keratitis and scars are more common in developing countries [11,15–17]. However, BK may represented

Table 2. Increasing trends of different techniques globally and for different pathologies (over total of keratoplasties with available information).

Technique analysed	n/total analysed	% in 2011 (2013 for DMEK, in italics)	% in 2018	Increasing trend	Different from linearity
Overall keratoplasties (2011–2018)					
EK over total of keratoplasties	1985/7660	9.8	36.0	p<0.001	p<0.001
EK in endothelial diseases*	1825/4003	2.0	54.9	p<0.001	p<0.001
EK in BK	588/1576	19.0	29.9	p<0.001	p<0.001
EK in FED	999/1377	43.2	92.0	p<0.001	p<0.001
EK in re-grafts	228/1030	4.1	36.7	p<0.001	p<0.001
DALK over total of keratoplasties	827/7660	6.8	12.2	p<0.001	p<0.001
DALK in ectasia and KC	359/865	23.6	59.4	p<0.001	p = 0.0129
Overall EK (2013–2018)					
DMEK over EK	563/1835	*7.4*	43.8	p<0.001	p = 0.02
DMEK over EK in BK	96/513	*2.6*	29.5	p<0.001	p = 0.67
DMEK over EK in FED	376/938	*11.8*	52.7	p<0.001	p<0.001
DMEK over EK in re-grafts	50/214	*10.0*	27.6	p = 0.067	NA

EK: Endothelial keratoplasty; DMEK: Descemet membrane endothelial keratoplasty; BK: Bullous keratopathy; FED: Fuchs endothelial dystrophy; NA: Non-applicable; KC: Keratoconus.

*Endothelial diseases: BK, FED, and re-graft due to endothelial failure.

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the second indication—after FED [9,11,12,18] or keratoconus [19,20]—and even the third one [10]. Intrinsic methodological issues such as questionnaire codification—for example FED with oedematous cornea could be eventually codified as BK—could explain that finding over the sole fact of a lower prevalence of FED in that geographical area per se.

Keratoplasty nowadays sticks to the rationale of transplanting as less tissue as possible in order to avoid allograft rejection, reduce postoperative complications and increase graft survival rates. Among endothelial techniques, DSAEK receptors often have a hyperopic shift and, in some cases, suboptimal visual recovery [21–23] while DMEK is a more anatomically accurate procedure that just replaces Descemet membrane and endothelium, potentially leading to a faster and better visual recovery with minimal refractive change [24–27]. Therefore, DMEK could be considered as the intended common-practice technique for corneal endothelial disease but, however, its widespread adoption is limited due to the overall challenging surgical technique in addition to the relative difficulty of donor tissue preparation [2,25].

The availability of precut tissue for DSAEK started in Catalonia in 2013, which representing a breakthrough in EK surgical indication [6]. This shift was even more important after the introduction of precut tissue for DMEK in 2017 [6]. Some studies [3] have already highlighted an increased trend to perform EK after precut tissue introduction. It is commonly thought that surgeons are more prone to perform posterior lamellar techniques when the risk to damage corneal tissue—due to manual preparation—is very low [28,29]. Previous reports have shown no differences in best corrected visual acuity, central corneal pachymetry or complications—dislocations, primary graft failure—found between corneas prepared by the surgeon in the operating room and precut tissue for DSAEK [29–31] or even DMEK [32]. Other advantages of precut tissue are the lower rate of microbiological culture positivity and lower risk of receptor infection [33,34]. Despite that, some areas still favour DMEK technique regarding indications over DSAEK even without precut tissue [10]. Taking into consideration all these facts and figures we are prone to presume than in the next few years DMEK trend in Catalonia will continue its increase thanks to precut tissue, among other reasons. Despite that, the increasing trend in lamellar techniques used in Catalonia has turn up late compared to other countries: for example, in 2011 in Catalonia only 19% of BK were treated with EK whereas in Columbia

(Canada) this proportion corresponded to 57.7%; [11] in the United States of America, about 50% of all corneal transplants are indeed EK according to the Eye Bank Association of America [9,35] while in this study that figure only accounted for 9.7%. Moreover, in Italy, an increasing trend of posterior lamellar techniques was found between 2002 and 2008 whereas in our area it happened after 2013 [13]. Other authors have investigated the keratoplasty activity in different centers in Spain [36,37]. As in their conclusions, we found than PKP was still the most prevalent technique used globally during the period of study despite the advantages described in literature of EK over PKP [21,23,25].

Even though being based on solid and official data on tissue transplantation, this study has still some limitations to disclose. First, as it is based on the OCATT's annual reports, data is kept subject to surgeons' responses accuracy. In other studies, preoperative diagnosis reported by surgeons ranged from 50% to 97% [9,38]. Surgeons must be aware of the importance of returning follow-up forms in order to increase exactness of reports. In addition, starting of any supply procedure, such as corneal precut tissue, can present with logistic problems such as bureaucracy issues or temporal shortage; therefore, data on usage of such supply could have been underestimated.

Generally, EK have shown several determinant advantages over PKP such as better visual acuity outcomes, less rejection rate and better postoperative recovery, despite being technically more difficult and having a higher learning curve [15,39,40]. These proven benefits in many prevalent indications and the extended use of eye bank precut tissue are thought to be the main causes of the increasing trend of both EK as a whole and DMEK in particular in our area. Given these characteristics in the next few years we could expect EK to drastically outperform PKP in endothelial corneal disease. On the other hand, and for similar reasons, we could also presume an overall EK major shift towards DMEK accordingly. In-depth epidemiological studies as the presented one would be mandatory in the years to come to follow-up and evaluate tissue transplantation trends.

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Writing – review & editing: Noelia Sabater-Cruz, Marc Figueras-Roca, Lydia Padró-Pitarch, Jaume Tort, Ricardo P. Casaroli-Marano.

References

1. Gadhvi KA, Coco G, Pagano L, Kaye SB, Ferrari S, Levis HJ, et al. Eye Banking: One Cornea for Multiple Recipients. Cornea. 2020; 39:1599–1603. https://doi.org/10.1097/ICO.0000000000002476 PMID: 32947412

2. Terry MA. Endothelial Keratoplasty: Why Aren't We All Doing Descemet Membrane Endothelial Keratoplasty? Cornea. 2012; 31:469–471. https://doi.org/10.1097/ICO.0b013e31823f8ee2 PMID: 22367047

3. Palma-Carvajal F, Morales P, Salazar-Villegas A, Figueroa-Vercellino JP, Spencer F, Peraza-Nieves J, et al. Trends in corneal transplantation in a single center in Barcelona, Spain. Transitioning to DMEK. J Fr Ophtalmol. 2020; 43:1–6. https://doi.org/10.1016/j.jfo.2019.06.026 PMID: 31831273

4. Organització Catalana de Trasplantaments. History of transplants in Catalonia [Internet]. Available from: http://trasplantaments.gencat.cat/ca/ocatt/historia/.

5. Organización Nacional de Trasplantes. Specialized information and reports about donation and transplantation [downloadable pdf]. Available from: http://www.ont.es/infesp/Memorias.

6. Sabater-Cruz N, Otero N, Dotti-Boada M, Ríos J, Gris O, Güell JL, et al. Eye bank and theatre factors for positive microbiological culture of corneoscleral rim and cornea storage medium in the real-world. Eye. 2021. https://doi.org/10.1038/s41433-020-01342-8 PMID: 33469128

7. Gencat: Generalitat de Catalunya. Informació, Tràmits i serveis de la Generalitat de Catalunya. Topics regarding Catalonia [accessed on October 26, 2019]. Available from: https://web.gencat.cat/ca/temes/catalunya/.

8. Banc de Sang i Teixits. Professionals area [Internet]. Available from: https://www.bancsang.net/professionals/teixits/58/lamel·la.

9. Park CY, Lee JK, Gore PK, Lim C, Chuck RS. Keratoplasty in the United States A 10-Year Review from 2005 through 2014. Ophthalmology. 2015; 122:2432–2442. https://doi.org/10.1016/j.ophtha.2015.08.017 PMID: 26386848

10. Röck T, Landenberger J, Bramkamp M, Bartz-schmidt KU, Röck D. The Evolution of Corneal Transplantation. Ann Transplant. 2017; 22:749–754. https://doi.org/10.12659/aot.905498 PMID: 29242495

11. Tan JCH, Holland SP, Dubord PJ, Moloney G, Mccarthy M, Yeung SN. Evolving Indications for and Trends in Keratoplasty in British Columbia, Canada, from 2002 to 2011: A 10-year Review. Cornea. 2014; 33:252–256. https://doi.org/10.1097/ICO.0000000000000066 PMID: 24457452

12. Zhang AQ, Rubenstein D, Price AJ, Elie C, Levitt M, Sharpen L, et al. Evolving surgical techniques of and indications for corneal transplantation in Ontario: 2000–2012. Can J Ophthalmol. 2013; 48:153–159. https://doi.org/10.1016/j.jcjo.2012.12.008 PMID: 23769775

13. Frigo AC, Fasolo A, Capuzzo C, Fornea M, Bellucci R, Busin M, et al. Corneal Transplantation Activity Over 7 Years: Changing Trends for Indications, Patient Demographics and Surgical Techniques From the Corneal Transplant Epidemiological Study (CORTES). Transplant Proc. 2015; 47:528–535. https://doi.org/10.1016/j.transproceed.2014.10.040 PMID: 25769602

14. Wang TW, Chi YC, Hsu PS, Kuo NW, Chen JL. Changing Indications for Corneal Transplantations in Southern Taiwan From 2008 to 2018. Eye Contact Lens. 2020; 46:301–305. https://doi.org/10.1097/ICL.0000000000000638 PMID: 31313699

15. Singh R, Gupta N, Vanathi M, Tandon R. Corneal transplantation in the mordern era. Indian J Med Res. 2019; 150:7–22. https://doi.org/10.4103/ijmr.IJMR_141_19 PMID: 31571625

16. Gupta N, Vashist P, Tandon R, Gupta SK, Dwivedi S, Mani K. Prevalence of corneal diseases in the rural Indian population: the Corneal Opacity Rural Epidemiological (CORE) study. Br J Opthtalmology. 2015; 99:147–152. https://doi.org/10.1136/bjophthalmol-2014-305945 PMID: 25395684

17. Chen W, Hu F, Wang I. Changing Indications for Penetrating Keratoplasty in Taiwan from 1987 to 1999. Cornea. 2001; 20:141–144. https://doi.org/10.1097/00003226-200103000-00004 PMID: 11248815

18. Le R, Yucel N, Khattak S, Yucel YH, Prud'homme GJ, Gupta N. Current indications and surgical approaches to corneal transplants at the University of Toronto: A clinical-pathological study. Can J Ophthalmol. 2017; 52:74–79. https://doi.org/10.1016/j.jcjo.2016.07.005 PMID: 28237153

19. Fasolo A, Frigo AC, Böhm E, Genisi C, Rama P, Spadea L, et al. The CORTES Study: Corneal Transplant Indications and Graft Survival in an Italian Cohort of Patients. Cornea. 2006; 25:507–515. https://doi.org/10.1097/01.ico.0000214211.60317.1f PMID: 16783137

20. Zare M, Javadi MA, Einollahi B, Karimian F, Rafie AR, Feizi S, et al. Changing Indications and Surgical Techniques for Corneal Transplantation between 2004 and 2009 at a Tertiary Referral Center. Middle East Afr J Ophthalmol. 2012; 19:323–329. https://doi.org/10.4103/0974-9233.97941 PMID: 22837628

21. Ang M, Wilkins MR, Mehta JS, Tan D. Descemet membrane endothelial keratoplasty. Br J Opthtalmology. 2016; 100:15–21. https://doi.org/10.1136/bjophthalmol-2015-306837 PMID: 25990654

22. Ang M, Li L, Chua D, Wong C, Htoon HM, Mehta JS, et al. Descemet's stripping automated endothelial keratoplasty with anterior chamber intraocular lenses: complications and 3-year outcomes. Br J Opthtalmology. 2014; 98:1028–1032. https://doi.org/10.1136/bjophthalmol-2013-304622 PMID: 24676725

23. Ang M, Mehta JS, Lim F, Bose S, Htoon HM, Tan D. Endothelial Cell Loss and Graft Survival after Descemet's Stripping Automated Endothelial Keratoplasty and Penetrating keratoplasty. Ophthalmology. 2012; 119:2239–2244. https://doi.org/10.1016/j.ophtha.2012.06.012 PMID: 22885122

24. Patel S V. Graft survival and endothelial outcomes in the new era of endothelial keratoplasty. Exp Eye Res. 2012; 95:40–47. https://doi.org/10.1016/j.exer.2011.05.013 PMID: 21689649

25. Singh NP, Said DG, Dua HS. Lamellar keratoplasty techniques. Indian J Ophthalmol. 2018; 66:1239–1250. https://doi.org/10.4103/ijo.IJO_95_18 PMID: 30127133

26. Tourtas T, Laaser K, Bachmann BO, Cursiefen C, Kruse FE. Descemet Membrane Endothelial Keratoplasty Versus Descemet Stripping Automated Endothelial Keratoplasty. Am J Ophthalmol. 2012; 153:1082–1090. https://doi.org/10.1016/j.ajo.2011.12.012 PMID: 22397955

27. Guerra FP, Anshu A, Price MO, Price FW. Endothelial Keratoplasty: Fellow Eyes Comparison of Descemet Stripping Automated Endothelial Keratoplasty and Descemet Membrane Endothelial Keratoplasty. Cornea. 2011; 30:1382–1386. https://doi.org/10.1097/ICO.0b013e31821ddd25 PMID: 21993468

28. Woodward MA, Titus M, Mavin K, Shtein RM. Corneal Donor Tissue Preparation for Endothelial Keratoplasty. J Vis Exp. 2012; 64:e3847. https://doi.org/10.3791/3847 PMID: 22733178

29. Kitzmann AS, Goins KM, Reed C, Padnick-Silver L, Macsai MS, Sutphin JE. Eye Bank Survey of Surgeons Using Precut Donor Tissue for Descemet Stripping Automated Endothelial Keratoplasty. Cornea. 2008; 27:634–639. https://doi.org/10.1097/QAI.0b013e31815e4011 PMID: 18580252

30. Ragunathan S, Ivarsen A, Nielsen K, Hjortdal J. Comparison of organ cultured precut corneas versus surgeon-cut corneas for Descemet's stripping automated endothelial keratoplasty. Cell Tissue Bank. 2014; 15:573–578. https://doi.org/10.1007/s10561-014-9429-x PMID: 24526412

31. Terry MA. Endothelial keratoplasty: a comparison of complication rates and endothelial survival between precut tissue and surgeon-cut tissue by a single dsaek surgeon. Trans Am Ophthalmol Soc. 2009; 107:184–193. PMID: 20126494

32. Regnier M, Auxenfans C, Maucort-boulch D, Marty A, Damour O, Burillon C, et al. Eye bank prepared versus surgeon cut endothelial graft tissue for Descemet membrane endothelial keratoplasty. Med. 2017; 96:e6885. https://doi.org/10.1097/MD.0000000000006885 PMID: 28489792

33. Mathes KJ, Tran KD, Mayko ZM, Stoeger CG, Straiko MD, Terry MA. Reports of Post-keratoplasty Infections for Eye Bank-prepared and Non–Eye Bank-prepared Corneas: 12 Years of Data From a Single Eye Bank. Cornea. 2019; 38:263–267. https://doi.org/10.1097/ICO.0000000000001839 PMID: 30601289

34. Rauen MP, Goins KM, Sutphin JE, Kitzmann AS, Schmidt GA, Wagoner MD. Impact of eye bank lamellar tissue cutting for endothelial keratoplasty on bacterial and fungal corneoscleral donor rim cultures after corneal transplantation. Cornea. 2012; 31:376–379. https://doi.org/10.1097/ICO.0b013e31823cbee3 PMID: 22410614

35. Ple-plakon PA, Shtein RM. Trends in corneal transplantation: indications and techniques. Curr Opin Ophthalmol. 2014; 25:300–305. https://doi.org/10.1097/ICU.0000000000000080 PMID: 24865170

36. Montesel A, Alió del Barrio JL, Yébana Rubio P, Alió JL. Corneal graft surgery: A monocentric long-term analysis. Eur J Ophthalmol. 2020. https://doi.org/10.1177/1120672120947592 PMID: 32757624

37. Gómez-Benlloch A, Montesel A, Pareja-Aricò L, Mingo-Botín D, Michael R, Barraquer RI, et al. Causes of corneal transplant failure: a multicentric study. Acta Ophthalmol. 2021;1–7. https://doi.org/10.1111/aos.14708 PMID: 33421330

38. Melles GRJ, Lander F, van Dooren BTH, Pels E, Beekhuis WH. Preliminary Clinical Results of Posterior Lamellar Keratoplasty through a Sclerocorneal Pocket Incision. Ophthalmology. 2000; 107:1850–1856. https://doi.org/10.1016/s0161-6420(00)00253-0 PMID: 11013184

39. Kymionis GD, Mikropoulos DG, Portaliou DM, Boboridis KG, Voudouragkaki IC, Dragoumis ND, et al. New Perspectives on Lamellar Keratoplasty. Adv Ther. 2014; 31:494–511. https://doi.org/10.1007/s12325-014-0121-0 PMID: 24846543

40. Maier P, Reinhard T, Cursiefen C. Descemet Stripping Endothelial Keratoplasty—Rapid Recovery of Visual Acuity. Dtsch Arztebl Int. 2013; 110:365–371. https://doi.org/10.3238/arztebl.2013.0365 PMID: 23795211

Artículo 2

Objetivo:
Conocer las indicaciones de biosustitutos de origen humano - córnea, esclera, membrana amniótica - para el tratamiento de las patologías de la córnea y superficie ocular en Catalunya. Y evaluar las indicaciones y tendencias de la utilización del tejido escleral y de la membrana amniótica como coadyuvante para el tratamiento de las patologías de la superficie ocular.

Título:
Current clinical application of sclera and amniotic membrane for ocular tissue bioreplacement.

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Cuartil y área de conocimiento:
Q4 *Cell Biology* y *Engineering Biomedical* (JCR)
Q3 *Biomedical Engineering*, **Q3** *Biomaterials*, **Q3** *Transplantation*
y **Q4** *Cell Biology* (Scimago)

RESUMEN

Propósito: describir las aplicaciones clínicas actuales y tendencias del uso de la esclera y la membrana amniótica en oftalmología.

Métodos: se revisaron las memorias anuales de la *Organització Catalana de Trasplantaments* (OCATT) sobre las indicaciones de parches de esclera y membrana amniótica en Catalunya, España, entre 2013 y 2018.

Resultados: entre enero de 2013 y diciembre de 2018 se implantaron un total de 874 parches de esclera y 1665 membranas amnióticas. La indicación más frecuente para el parche de esclera durante el periodo de estudio fue cirugía de glaucoma (77,5%), reconstrucción palpebral (5,2%) y úlcera de córnea o esclera (5%). En referencia a la membrana amniótica, las indicaciones más frecuentes fueron úlcera corneal (26,9%), reconstrucción conjuntival (23,8%) y defecto corneal epitelial (22,7%). Durante el periodo de estudio, se encontró una tendencia ascendente en el uso de parches de esclera para la reconstrucción palpebral (p=0,0032) y del uso de membrana amniótica para el manejo de la inflamación (p=0,0198).

Conclusión: la cirugía del glaucoma y las úlceras corneales representan las indicaciones principales para el uso de parches de esclera y membrana amniótica respectivamente, durante el periodo estudiado. Se ha hallado también una tendencia ascendente significativa en el uso de esclera para la reconstrucción palpebral y en el de membrana amniótica para el manejo de la inflamación del segmento anterior. Este escenario cambiante en el uso de los tejidos para uso ocular debe ser tenido en consideración, especialmente por los bancos de ojos, encarando cambios presentes y futuros en la preservación, almacenamiento e indicaciones del tejido.

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Current clinical application of sclera and amniotic membrane for ocular tissue bio-replacement

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Abstract To report the current clinical applications and trends of scleral and amniotic membrane use in ophthalmology. Review of annual reports from the Catalan Transplant Organization (OCATT), on scleral patch and amniotic membrane eye indications in Catalonia region (Spain) over a 6-year period from 2013 to 2018. A total of 874 scleral and 1665 amniotic membranes patches were implanted, from January 2013 to December 2018. The most frequent indication over the 6-year period for scleral patch was glaucoma surgery (77.5%), eyelid reconstruction (5.2%) and corneal or scleral ulcer (5%). Regarding amniotic membrane, corneal ulcer (26.9%), conjunctival reconstruction (23.8%) and corneal epithelial defect (22.7%) were the most common indications. During the study period, an increasing trend was found on sclera patches for eyelid reconstruction ($p = 0.0032$) and amniotic membrane for inflammation management ($p = 0.0198$). Glaucoma surgery and corneal ulcers have represented the top indications for scleral patch and amniotic membrane use, over the period, respectively. A significant trend has also been found towards eyelid reconstruction using scleral patches and amniotic membrane for anterior segment inflammation management. This evolving scenario in tissue use for ocular surgery has to be taken into consideration, especially regarding eye banks facing current and futures changes in tissue preservation, storage and indications.

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Introduction

Ocular pathologies and its surgical treatment usually need adjuvant tissues, being sclera and amniotic membrane commonly used among others. Eye banks supply both tissues for ophthalmologic requirements in various types of preservation approaches and for different indications. However, epidemiology of such indications is not always easy and mostly depends on regional eye bank services and bureaucracy aspects of registration.

The sclera is a mostly avascular structure made mainly of connective tissue widely available after eye removal, especially in those cases where the whole globe—instead of a corneoscleral disc—is retrieved. Donor sclera was initially used in 1950s (Hodge et al. 2017) and gained popularity thanks to its minimum risk on infectious agents transmission—including prions (Mentha and WA 2002). Despite the initial use of sclera was supporting iatrogenic surgical complications (Harizman et al. 2005), scleral patches have been currently used in many procedures as adjunctive treatment including glaucoma surgery, anterior segment reconstruction (Prasher 2014), scleral thinning and ulcer (Wen et al. 2018; Sainz de la Maza et al. 1989), cornea perforation (Prasher 2014), orbital implants wrapping (Gawdat and Ahmed 2014), repair of eroded scleral buckles (Wilson and Parker 1975), management of ocular tumors (Barman et al. 2012), spacer material in complicated cases of strabismus surgery (Thorisdottir et al. 2019), and eyelid reconstruction among others (Olver et al. 1998).

The amniotic membrane (AM) is the inner layer of the foetal membranes (Malhotra and Jain 2014; Jirsova and Jones 2017). Its use in ophthalmology and other specialities is nowadays increasing because the AM has been shown to have an outstanding number of properties such as anti-inflammatory, anti-fibrotic, anti-angiogenic, and anti-microbial activity. In addition, it also shows transparency, lack of immunogenicity, and behaves as a substrate for growth, migration and adhesion of epithelial corneal and conjunctival cells (Malhotra and Jain 2014; Jirsova and Jones 2017). AM can be implanted as graft or inlay technique (AM usually placed epithelium up), patch or overlay technique—as a biological bandage contact lens—(epithelium facing down), and layered or fill in technique (Malhotra and Jain 2014; Rahman et al. 2009). Despite AM has been used in ophthalmology since 1940, its expansion just started after the early nineties with a large list of indications in ophthalmology currently (Malhotra and Jain 2014; Kim and Tseng 1995): ocular surface reconstruction with or without limbal stem cell deficiency – persistent epithelial defects, non-healing corneal ulcers, corneal perforations, descematoceles, bullous keratopathy, pterygium, conjunctival reconstruction, sclera melts and perforations, glaucoma surgeries management, and as a carrier for ex vivo expansion for corneal epithelial cells, among others (Malhotra and Jain 2014; Jirsova and Jones 2017).

Different storage methods have been described for both tissues. Cold storage, glycerol, gamma radiation, alcohol, and freeze drying were often used for scleral segments. Also, several authors have used "fresh" sclera while in our tissue bank the sclera provided was preserved in ethanol 70% (Dubey et al. 2017; Tsoukanas et al. 2016). AM can be stored in different forms such as cryopreserved, dried, lyophilizated, and even as a "fresh" material (Malhotra and Jain 2014; Jirsova and Jones 2017).

With such a myriad of different ocular indications and uses for donor sclera and AM, we set up an epidemiologic study to report those in a medium sized region of Spain, Catalonia (7.500.000 inhabitants) during a 6 years period. We aim to describe such characteristics and look for changing trends in sclera and AM eye use.

Materials and methods

This study is a retrospective review of scleral tissue and AM ocular use, recorded from the Catalan Transplant Organization (OCATT; Catalonia, Spain), between January of 2011 and December of 2018. The included data corresponded to scleral and AM retrieved and implanted with clinical application in ophthalmology. Institutional Ethics Committee Board approval was obtained for donor data revision (approval number HCB/2015/0879, Hospital Clinic de Barcelona, amended on 14th November of 2018).

Research methods and analysis plan adhered to the Tenets of the Declaration of Helsinki. Data related to ocular tissue, its traceability, and potential adverse events were treated in accordance with the appropriate European Union directives (2004/23/EC, 2006/17/EC, and 2006/86/EC).

Patient data were encoded for management in accordance with the Spanish legislation on personal data protection (RD05/2018).

Tissue storage setting was 70% ethanol solution preservation, at room temperature, for sclera (1/4 or 1/2 patches) and Glycerol-RPMI medium (vol:vol) cryopreservation, at − 80 °C, for AM membrane (2.5 × 2.5 cm segments) placed on a nitrocellulose paper as carrier. For both, a follow-up form was sent to the implanting surgeon asking for adverse events and surgery indication among other logistic and clinic items.

Reported data outcomes included, as per every year, number of cases and main eye disease indication for scleral or AM implantation. Descriptive results are presented as absolute frequencies and percentages for categorical variables. Linear trends (Mantel-Haenszel statistic) and deviation from linearity were obtained. All tests were performed with a two-sided type I error of 5% with the statistical package STATA v.15.1 (StataCorp, College Station, Texas, USA).

Results

Over a 6-year period (2013–2018), 874 scleral patches were implanted. Despite the high recommendation and enforcing to send it back, the response rate par year was heterogeneous, ranging from 40 to 83.4%. Information about surgical indication was obtained from 556 (63.6%) procedures. Indications for scleral patch implantation are listed in Table 1. The main indication for scleral implant was glaucoma surgery (77.5%), followed by eyelid reconstruction (5.2%) and corneal or scleral ulcers (5%). Further indications included a hotchpotch of rare indications such as retina surgery or suture extrusion.

Trends for scleral patch use were analysed and showed sclera for eyelid reconstruction to present with an increasing trend ($p = 0.0032$) different from linearity ($p = 0.0003$). Scleral patch as a treatment for corneal or scleral ulcers showed a decreasing trend ($p = 0.0042$), with a non-linear performance ($p = 0.0109$). No statistically significant trend of scleral use was found in glaucoma surgery ($p = 0.9689$), orbit surgery ($p = 0.5589$) or ocular globe reconstruction ($p = 0.2032$). Evolving trends in scleral use over years are shown in Fig. 1.

AM was indicated 1665 times during the study period. Information about surgical indication was available in 937 (56.3%) procedures, which are listed in Table 2. The top indication for AM implant was corneal ulcer (26.9%), followed by conjunctival reconstruction (23.8%), and corneal epithelial defect (22.7%). Other indications included few cases of chemical injury, symptomatic bullous keratopathy, descemetocele, limbal stem cell deficiency, and pterygium.

AM clinical application trends were also statistically analysed. AM for inflammation management showed an increasing trend ($p = 0.0198$) which did not differ from linearity ($p = 0.1603$). No statistically significant increasing trends for AM use were found in epithelial defects ($p = 0.5923$), corneal ulcers ($p = 0.3517$) or conjunctival reconstructions ($p = 0.2795$). Evolving trends in amniotic membrane use over years are shown in Fig. 2.

Discussion

The most frequent indication for scleral implant in our study was glaucoma surgery, followed by eyelid reconstruction and ulcers. Regarding AM, main indication was corneal ulcer, followed by conjunctival reconstruction. During the study period, a statistically significantly increasing trend of scleral use for eyelid reconstruction and AM for inflammation management was found.

One hundred and seventy-eight scleral patches were implanted in 2018 in Catalonia, whereas the same year, 33 of 58 eye banks members of European Eye Bank Association (EEBA) reported 2909 scleral patches implanted in Europe (European Eye Bank Association 2020). About 6.1% of all scleras implanted by EEBA members were performed in Catalonia. Regarding AM clinical application, 372 segments were implanted in 2018 in Catalonia (2459

Table 1 Clinical indications for sclera implantation

	n (%)						
	2013	2014	2015	2016	2017	2018	Totals
Glaucoma surgery	**48** (63.2)	**84** (75)	**90** (91.9)	**109** (86.5)	**49** (80.4)	**51** (61.5)	**431** (77.5)
Eyelid reconstruction	**3** (3.9)	**6** (5.4)	**2** (2)	**1** (0.8)	**3** (4.9)	**14** (16.9)	**29** (5.2)
Ulcer or scleral thinning	**8** (10.5)	**12** (10.8)	**2** (2)	**0** (0)	**2** (3.3)	**4** (4.8)	**28** (5)
Ocular globe reconstruction	**1** (1.3)	**2** (1.7)	**3** (3.1)	**10** (7.9)	**3** (4.9)	**2** (2.4)	**21** (3.8)
Orbital implant	**6** (7.9)	**2** (1.7)	**0** (0)	**2** (1.6)	**3** (4.9)	**3** (3.6)	**16** (2.9)
Others	**10** (13.2)	**6** (5.4)	**1** (1)	**4** (3.2)	**1** (1.6)	**9** (10.8)	**31** (5.6)
Total with informed indication	**76** (100)	**112** (100)	**98** (100)	**126** (100)	**61** (100)	**83** (100)	**556** (100)
Total of sclera implanted and proportion of indications informed (%)	**119** (63.9)	**155** (72.3)	**119** (82.4)	**151** (83.4)	**152** (40)	**178** (47)	**874** (63.6)

Bold values in the last row indicate total absolute numbers from both sclera and amniotic membrane implanted in Catalonia over the study period

Bold values in all other rows indicate absolute numbers of INFORMED INDICATIONS from both sclera and amniotic membrane

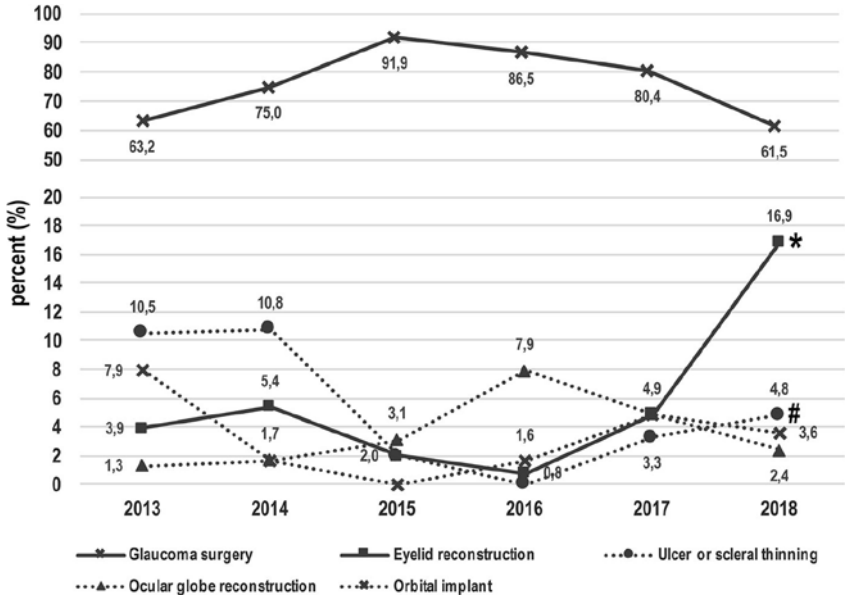

Fig. 1 Evolving trends in sclera clinical application. There was an increased trend for eyelid reconstruction ($p = 0.0032$; *), while decreased as a treatment for corneal or scleral ulcers ($p = 0.0042$; #). No statistically significant trend was found for other scleral patches clinical indications

in Spain) (Trasplantes 2018), whereas the same year 38 of 58 eye banks members of EEBA reported 6974 amniotic membranes implanted in Europe (Association 2020). That means, AM implanted in Catalonia in 2018 corresponded to 21.2% and 5.3% of all AM implanted in Spain and in Europe respectively.

Main indication for scleral patch use is still glaucoma surgery—in conjunction with trabeculectomy revision in treating over-filtering surgeries and tube implants—accounting for 77.5% of all indications during the 6-year period and in line with previous reports (Hodge et al. 2017; Lind et al. 2017). Despite

Table 2 Clinical indications for amniotic membrane implantation

	n (%)						
	2013	2014	2015	2016	2017	2018	Totals
Corneal ulcer	**36** (27.9)	**21** (18.1)	**55** (33.3)	**83** (30.2)	**36** (30.3)	**21** (15.7)	**252** (26.9)
Conjunctival reconstruction	**21** (16.3)	**35** (30.2)	**35** (21.2)	**75** (27.3)	**19** (15.9)	**38** (28.6)	**223** (23.8)
Epithelial defect	**27** (20.9)	**37** (31.9)	**27** (16.4)	**52** (18.9)	**38** (31.9)	**32** (24.1)	**213** (22.7)
Inflammation	**6** (4.7)	**4** (3.4)	**5** (3)	**26** (9.4)	**12** (10.1)	**10** (7.5)	**63** (6.7)
Others	**39** (30.2)	**19** (16.4)	**43** (26.1)	**39** (14.2)	**14** (11.8)	**32** (24.1)	**186** (19.9)
Total with informed indication	**129** (100)	**116** (100)	**165** (100)	**275** (100)	**119** (100)	**133** (100)	**937** (100)
Total of AM implanted and proportion of indications informed (%)	**238** (54.2)	**158** (73.4)	**264** (62.5)	**332** (82.8)	**301** (39.5)	**372** (35.7)	**1665** (56.3)

Bold values in the last row indicate total absolute numbers from both sclera and amniotic membrane implanted in Catalonia over the study period

Bold values in all other rows indicate absolute numbers of INFORMED INDICATIONS from both sclera and amniotic membrane

AM amniotic membrane

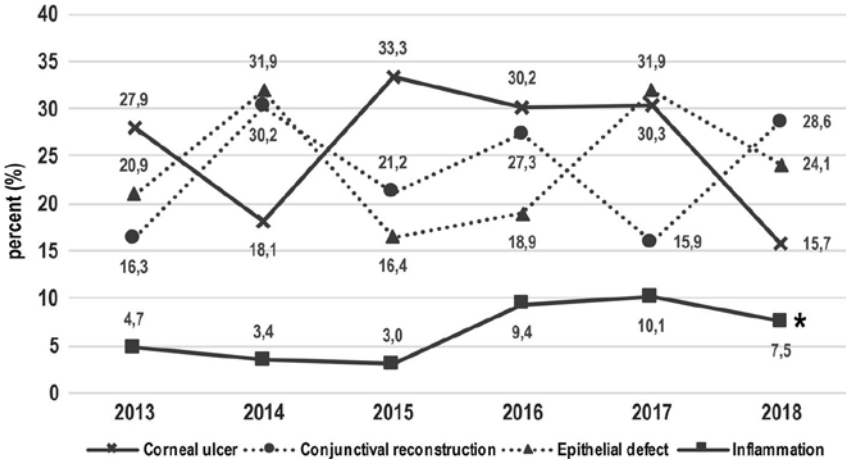

Fig. 2 Evolving trends in amniotic membrane (AM) clinical application. There was an increased trend for inflammation management ($p = 0.0198$; *), while no statistically significant trend was found for other AM segments clinical indications

other patch graft materials—including AM, pericardium, fascia lata, dura mater, buccal mucous membrane, and even corneal tissue—have been successfully used in glaucoma drainage implant surgeries, sclera has shown to have a similar erosion rate (5–7%) (Lind et al. 2017). Scleral segments are cost-efficient and were associated with a low risk of pathogen transmission in addition to its wide availability, strength, flexibility and ease of storage (Hodge et al. 2017; Dubey et al. 2017; Tsoukanas et al. 2016). Eye banks can provide preserved donor cornea as an alternative for scleral patch. Remarkably is the recent increase in the use of corneal tissue for glaucoma shunt patching noted by the Eye Bank Association of America could explain the 9% reduction in sclera use from 2017 to 2018 (https://restoresight.org/who-we-are/statistics/, last entrance 10/01/2020). Proponents argue for it allowing a better examination of glaucoma drainage devices, better cosmesis and low tube exposure rates (Hodge et al. 2017; Spierer et al. 2016; Lambert and Chamberlain 2017). On the other hand, despite the continued requirement of natural donor tissue, in the meantime (Hodge et al. 2017), techniques and bioengineered material to avoid the

need for patch grafts for glaucoma drainage implants are being developed with promising results (Lind et al. 2017; Kugu et al. 2015).

Eyelid reconstruction showed an increasing trend surpassing a linear slope during the study period. Scleral use for eyelid reconstruction is a well-known spacer graft especially for thyroid disease, despite it is not clearly superior to others (hard palate mucosa, tarsal conjunctiva, porous polyethylene, cartilage, dermis or acellular tissue) (Olver et al. 1998; Feldman et al. 1992; Park et al. 2017). Therefore. there are apparently no reasons for this outstanding increase, which is mainly focused on the last years of the study period.

"Ulcer" indication included both scleral thinning and corneal perforation. Interestingly, scleral patch as a treatment for corneal or scleral ulcers has shown a decreasing trend. This could be related with various facts such as current knowledge on graft necrosis in immune scleritis could be avoided with timely initiation of immunosuppression, especially with biologic drugs (Wen et al. 2018; Sainz de la Maza et al. 1989). On the other hand, our eye bank provides more than 1000 corneas per year and some of them, with low endothelial cell quality, are used for tectonic purposes, without any restriction of tissue. Because of this availability, cornea surgeons could be more prone to use cornea than sclera for corneal perforations and therefore the found decreasing trend could be explained.

Scleral patch use for implant exposure prevention has kept stable over the study period with few cases per year. Although other materials have also been used, their higher postoperative complications and potential risk of disease transmission could lessen its extent (Hodge et al. 2017; Lind et al. 2017; Arat et al. 2003).

Main indication for AM in our series was corneal ulcer (26.9%). Conjunctival reconstruction—especially as adjuvant to pterygium surgery—was found in second place for indication. Despite conjunctival autografting for pterygium has been shown to have less recurrence risk, AM is still widely used in our area because of its availability (up to 30 grafts can be prepared with one placenta) among other reasons (Malhotra and Jain 2014; Clearfield et al. 2017; Sabater-Cruz et al. 2020). In 2018, a total of 2459 AM fragments were obtained from 92 donors and corresponded to 1758 AM implants in Spain (Organización Nacional de Trasplantes 2018).

Even though being based on solid and official data on tissue transplantation, this study has still some limitations to disclose. First, as it is based on the OCATT's annual reports, data is kept subject to surgeons' responses accuracy. On average, surgeon responses rate is higher in sclera (63.6%) than amniotic membrane (56.3%). Reasons for different response rates among tissues and years are being studied to improve surgeons' responses rate, which is an important limitation of our study.

Both sclera and AM have proven to be very useful and versatile tissues for ophthalmologists, whose eye indications are rapidly expanding as there is a better understanding of their properties. Despite alternatives for both tissues exist and are in continuous study, they still have prominent indications such as cornea tissue saving in the case of sclera, especially for eye banks to be aware of in case of corneal tissue shortage. A judicious use and appropriate patient selection remain crucial and important points for achieving optimal outcomes when managing eye donor tissue.

Author contributions R.P.C.-M. and N.S.-C. designed the work, L.P.-P., A.G.-V. and J.T. acquired the data, M.F.-R. analysed the data, all authors interpreted the data, N.S.-C. drafted the work and all authors substantively revised it. All authors reviewed and approved the submitted version of the manuscript.

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Data availability The authors declare their data transparency and will send any material on demand

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest or competing interests.

Ethics approval This study was evaluated and approved by the Hospital Clinic de Barcelona Ethics Committee Board, approval number HCB/2015/0879, amended on 14th November of 2018.

Consent for publication The authors consent the publication of this work.

References

Arat YO, Shetlar DJ, Milton B (2003) Bovine pericardium versus homologous sclera as a wrapping for hydroxyapatite orbital implants. Ophthalmic Plast Reconstr Surg

19:189–193. https://doi.org/10.1097/01.iop.0000062850.71572.7d
Barman M, Finger PT, Milman T (2012) Scleral patch grafts in the management of uveal and ocular surface tumors. Ophthalmology 119:2631–2636. https://doi.org/10.1016/j.ophtha.2012.06.024
Clearfield E, Hawkins BS, Kuo IC (2017) Conjunctival Autograft versus amniotic membrane transplantation for treatment of pterygium: findings from a cochrane systematic review. Am J Ophthalmol 182:8–17. https://doi.org/10.1016/j.ajo.2017.07.004
Dubey S, Tajurkar K, Kapur N, Acharya M (2017) Heterologous, human donor sclera as the patch graft in glaucoma drainage device surgery. J Glaucoma 26:e128. https://doi.org/10.1097/IJG.0000000000000479
European Eye Bank Association (2020) Annual directory, 28th edn. Venice, Italy
Feldman K, Putterman A, Farber M (1992) Surgical treatment of thyroid-related lower eyelid retraction: a modified approach. Ophthalmic Plast Reconstr Surg 8:278–286. https://doi.org/10.1097/00002341-199212000-00007
Gawdat TI, Ahmed RA (2014) Scleral shield: primary results of a new surgical technique in augmenting porous orbital implant protection. Eur J Ophthalmol 24:948–952. https://doi.org/10.5301/ejo.5000469
Harizman N, Ben-Cnaan R, Goldenfeld M et al (2005) Donor scleral patch for treating hypotony due to leaking and/or overfiltering blebs. J Glaucoma 14:492–496. https://doi.org/10.1097/01.ijg.0000185618.98915.d2
Hodge C, Sutton G, Devasahayam R et al (2017) The use of donor scleral patch in ophthalmic surgery. Cell Tissue Bank 18:119–128. https://doi.org/10.1007/s10561-016-9603-4
Jirsova K, Jones GLA (2017) Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review. Cell Tissue Bank 18:193–204. https://doi.org/10.1007/s10561-017-9618-5
Kim JC, Tseng SC (1995) The effects on inhibition of corneal neovascularization after human amniotic membrane transplantation in severely damaged rabbit corneas. Korean J Ophthalmol 9:32–46
Kugu S, Erdogan G, Sevim MS, Ozerturk Y (2015) Efficacy of long scleral tunnel technique in preventing ahmed glaucoma valve tube exposure through conjunctiva. Semin Ophthalmol 30:1–5. https://doi.org/10.3109/08820538.2013.807851
Lambert N, Chamberlain W (2017) The structure and evolution of eye banking: a review on eye banks' historical, present, and future contribution to corneal transplantation. J Biorepos Sci Appl Med Volume 5:23–40. https://doi.org/10.2147/bsam.s114197
Lind JT, Shute TS, Sheybani A (2017) Patch graft materials for glaucoma tube implants. Curr Opin Ophthalmol 28:194–198. https://doi.org/10.1097/ICU.0000000000000347
Malhotra C, Jain AK (2014) Human amniotic membrane transplantation: different modalities of its use in ophthalmology. World J Transplant 4:111. https://doi.org/10.5500/wjt.v4.i2.111
Mentha J, WA F (2002) The sclera, the prion, and the ophthalmologist. Br J Opthtalmol 86:587–592
Olver JM, Rose GE, Khaw PT, Collin JRO (1998) Correction of lower eyelid retraction in thyroid eye disease: a randomised controlled trial of retractor tenotomy with adjuvant antimetabolite versus scleral graft. Br J Ophthalmol 82:174–180. https://doi.org/10.1136/bjo.82.2.174
Organización Nacional de Trasplantes (2018) Memoria de actividad de donación y trasplante de tejidos, España 2018. ONT, Madrid
Park E, Lewis K, Alghoul MS (2017) Comparison of efficacy and complications among various spacer grafts in the treatment of lower eyelid retraction: a systematic review. Aesthet Surg J 37:743–754. https://doi.org/10.1093/asj/sjx003
Prasher P (2014) Use of an autologous lamellar scleral graft to repair a corneal perforation. Int Ophthalmol 34:957–960. https://doi.org/10.1007/s10792-013-9883-7
Rahman I, Said DG, Maharajan VS, Dua HS (2009) Amniotic membrane in ophthalmology: indications and limitations. Eye 23:1954–1961. https://doi.org/10.1038/eye.2008.410
Sabater-Cruz N, Dotti-Boada M, Ríos J et al (2020) Postoperative treatment compliance rate and complications with two different protocols after pterygium excision and conjunctival autografting. Eur J Ophthalmol. https://doi.org/10.1177/1120672120917335
Sainz de la Maza M, Tauber J, Foster CS (1989) Scleral grafting for necrotizing scleritis. Ophthalm 96:306–310
Spierer O, Waisbourd M, Golan Y et al (2016) Partial thickness corneal tissue as a patch graft material for prevention of glaucoma drainage device exposure. BMC Ophthalmol 16:5–10. https://doi.org/10.1186/s12886-016-0196-2
Thorisdottir RL, Blohmé J, Malmsjö M (2019) Clinical evidence supporting the use of donor sclera as spacer material in complicated cases of strabismus surgery—retrospective evaluation of surgical results in 117 patients with thyroid-associated ophthalmopathy or congenital strabismus. Acta Ophthalmol 97:74–79. https://doi.org/10.1111/aos.13845
Tsoukanas D, Xanthopoulou P, Charonis AC et al (2016) Heterologous, fresh, human donor sclera as patch graft material in glaucoma drainage device surgery. J Glaucoma 25:558–564. https://doi.org/10.1097/IJG.0000000000000294
Wen JC, Lam J, Banitt MR (2018) Scleral patch graft with a suture reinforcement technique in surgical management of Necrotizing Scleritis With Ectasia. Cornea 37:933–935. https://doi.org/10.1097/ICO.0000000000001598
Wilson R, Parker J (1975) Scleral patch for exposed silicone buckles. Ophthalmic Surg 6:83–85

Artículo 3

Objetivo:
Evaluar el uso de los cultivos microbiológicos de anillos esclerocorneales y medios de preservación corneal como control perioperatorio del tejido donante en el mundo real. Con ello, se destaca analizar el valor añadido y la frecuencia de la utilización del control microbiológico en un entorno real y sus factores asociados a la contaminación del tejido corneal.

Título:
Eye bank and theatre factors for positive microbiological culture of corneoscleral rim and cornea storage medium in the real-world

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RESUMEN

Propósito: evaluar la tasa de realización de cultivos microbiológicos y la positividad de los cultivos de anillos esclerocorneales y medio de preservación, así como los posibles factores de riesgo para la contaminación con datos de vida real.

Métodos: se revisaron los datos de donantes de córneas implantadas de manera consecutiva en el centro de referencia entre enero de 2013 y enero de 2018. Se analizó estadísticamente las características de la córnea (datos demográficos del donante, densidad celular del endotelio, tipo de conservación corneal, días de almacenaje, tejido precortado o no precortado) e información sobre el cultivo microbiológico (anillo esclerocorneal y/o medio de conservación y resultado).

Resultados: durante el periodo de estudio, se implantaron 1369 córneas (737 donantes). Se realizó cultivo microbiológico en el 76,8% (N=1052) con un resultado positivo en el 3,2% de los casos, por bacterias principalmente (84,4%). Las córneas preservadas en hipotermia representaron el 61,8% de todos los resultados positivos ($p<0,001$). Otros factores de riesgo analizados no alcanzaron una asociación estadística con la positividad de los cultivos. Ninguno de los 34 casos con cultivos positivos causó infección ocular en el receptor en al menos seis meses de seguimiento.

Conclusiones: la tasa de realización de los cultivos microbiológicos en la práctica clínica es alta a pesar de no ser obligatorio. Las córneas en preservación organotípica muestran estadísticamente una menor positividad en los cultivos microbiológicos de anillo esclerocorneal y medio de preservación que las hipotérmicas, resultando en otra ventaja de este tipo de preservación corneal. A pesar de la creencia de que las córneas precortadas presentan menos positividad, esta asociación no se halló en este estudio.

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The ROYAL COLLEGE of OPHTHALMOLOGISTS

ARTICLE

Eye bank and theatre factors for positive microbiological culture of corneoscleral rim and cornea storage medium in the real-world

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Abstract

Objectives To evaluate microbiological culture rate and positivity of corneoscleral rim and cornea storage media as well as possible risk factors for contamination with real-world data.

Methods Data of consecutive cornea donors implanted in the reference centre from January 2013 to January 2018 were reviewed. Information about cornea characteristics (donor demographic data, endothelial cell density, type of cornea conservation, days of storage, and precut vs full-thickness tissue), and microbiological culture information (corneoscleral rim *vs* storage sample, positive result) were statistically analysed.

Results During the study period, 1369 corneas (737 donors) were implanted. Cultures were performed in 76.8% ($n = 1052$) of them and were positive in 3.2% of cases, mainly bacteria (84.4%). Corneas preserved in hypothermia represented 61.8% of all positive microbiology results ($p < 0.001$). Other analysed risk factors did not reach statistically significant association with microbiological positivity. None of the 34 cases with positive microbiological cultures reported ocular infection for the recipients in at least 6 months' follow-up.

Conclusions Microbiological tests rate in real-world practice are high despite not being compulsory. Organotypic cultured corneas showed a statistically less positivity in corneoscleral and storage medium than hypothermic ones, resulting in another advantage of this kind of cornea storage. Although precut corneas are thought to present less microbiological positivity, a statistically significant association was not found in the present study.

Introduction

Donated organs and tissue presenting microbiological positivity are usually considered not suitable for transplant, discarded for clinical purposes and never reaching a potential recipient. In Europe, about 2.5% of donated corneas are not suitable for clinical use due to contamination [1]. This may represent a crucial problem for the national healthcare system, since there are countries worldwide which do not achieve sufficient corneal donation to meet the annual demand [2].

Several studies have evaluated donor causes for corneal microbiological contamination [3, 4]. Routinely, samples of corneoscleral rim and/or storage medium of the cornea implanted are sent for microbiological study. Positivity rate of microbiological cultures of corneoscleral rims described in the literature can reach 16.8% [5–8]; however, infectious complications are uncommon after keratoplasty, with reported rates between 0.08% and 0.77% [5–7, 9–12]. Quality control, diagnosis, and, especially, prevention of endophthalmitis and keratitis in the receptor are outstanding advantages and therefore microbiological cultures are strongly motivated [13, 14].

Many reports have tried to determine whether there is a relationship between positive microbiological cultures of donor corneas and postoperative infection in recipients [5, 7, 15, 16] Others recognize donor-to-host transmission

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of bacteria and fungi as serious adverse reaction that can lead to endophthalmitis, keratitis and poor visual outcome [5, 10, 13, 17]. Some authors have claimed that donor-to-host microbial transmission may be as much as 12- to 22-fold times greater in the presence of a positive rim culture [11, 12].

This study aimed to investigate in first place the rate of microbiological positivity tests performed in a real-life setting, in order to then search potential causes for such finding.

Materials and methods

Study design

This was a retrospective study carried out at a single eye bank in Barcelona, Spain (*Barcelona Tissue Bank*; BST). Data was included for donors that had at least one cornea implanted in a high-volume implanting centre in Barcelona (Instituto de Microcirugía Ocular; IMO) over a 5-year period (from January 2013 to January 2018). Institutional Ethics Committee Board approval was obtained for clinical history revision (approval number HCB/2015/0879, Hospital Clinic de Barcelona, amended on 14th November of 2018). Research methods and analysis plan adhered to the tenets of the Declaration of Helsinki.

Patient data were encoded for management in accordance with the Spanish legislation on personal data protection (RD05/2018). Informed consent for surgical procedures was obtained in the implanting centres. Authorization, and informed consent for biological sample analysis were treated in accordance with local law. Data related to ocular tissue, and its traceability were treated in accordance with the appropriate European Union directives (2004/23/EC, 2006/17/EC, and 2006/86/EC).

Procedures

Corneoscleral buttons were obtained by retrieval teams at the donor procurement units' hospital. By routinely standard operating protocol, 5% povidone-iodine solution was applied for 5 min, abundantly washed with 0.9% physiological solution, and then the corneoscleral discs were retrieved under sterile surgical conditions and placed in Optisol GS medium (Bausch & Lomb Surgical Inc., San Dimas, California) for storage at 4 °C. The mean time between death and preservation was 12 ± 8 h (range, 8–24 h). Donated corneoscleral discs were later processed, evaluated, and preserved at 4 °C (cornea in hypothermia) or 31 °C into organotypic culture conditions (cultured cornea) in CorneaMax® (Eurobio, Les Ulis, France) at eye bank facilities. At surgeon's request, corneoscleral material was either sent without further manipulation (surgeon-cut button) or sent as precut tissue for Descemet Membrane Endothelial Keratoplasty (DMEK) or Descemet Stripping Automated Endothelial Keratoplasty (DSAEK).

After transplantation, the trephined remnant of donated corneoscleral rims and/or the original corneal storage medium—Optisol GS for hypothermic cornea; deswelling medium CorneaJet® (Eurobio, Les Ulis, France) for cultured cornea—were sent for conventional microbiological testing. The remnant corneoscleral rims, storage medium or both were cultured depending on surgeon/implanting centre preferences.

Data collection

The following information was collected: donor demographic data (age, sex, cause of death), endothelial cell density, date of cornea retrieval and eye bank entry, type of corneal conservation, date of cornea delivery to the implanting centre, date of surgery, surgical technique and microbiological culture information (type of sample cultured, microbiological result). In those cases, in which the second cornea of the same donor had been implanted in a different centre than the reference centre of this study, tracing was applied to collect information about mate corneas. Surgeons from other implanting centres were interviewed to provide information about the mate cornea in those cases.

Statistical analysis

Descriptive results are presented as median and their 95%CI or absolute frequencies and percentages for quantitative and qualitative variables respectively. All results were tabulated for presence of culture (yes or no), outcome of this culture and type of cornea implanted. Mann–Whitney U Test or Fisher's Exact test were used for statistical analyses for quantitative or qualitative variables respectively. All statistical tests were performed with a two-sided type I error of 5%, with the statistical software SPSS v.25.0 (IBM, Armonk, New York, USA).

Results

From 1st January 2013 to 31st January 2018, 737 consecutive donors had at least one cornea implanted in the reference centre: In some cases, both corneas were implanted in the reference centre, in others the mate cornea was implanted in another centre or was discarded by the eye bank due to quality criteria. Then, a total of 1369 corneas (737 donors) were implanted in the period of study. Destination of donor corneas is summarized in Table 1.

Table 1 Destination of 737 consecutive donor corneas with at least one of them implanted in the reference centre.

Donors	*n*
One cornea implanted in the reference centre	737
Mate cornea	
Implanted in the same centre	145
Implanted in another centre[a]	487
Did not accomplished quality criteria and discarded by the eye bank	105
Total of corneas implanted	1369

[a]Corneas were implanted in 78 different centres elsewhere in Spain by 97 surgeons.

Details about donor characteristics (demographics and cause of death), donor corneal characteristics and criteria quality (endothelial cell density, laterality, days in the eye bank, time from delivery to surgery, type of corneal conservation—hypothermia *vs* organotypic), and technical approach used for tissue delivered are resumed in Table 2.

Microbiological analysis was performed in 76.8% ($n = 1052$) corneal remaining material. Samples sent for microbiological study were corneoscleral rim (89.8%, $n = 945$), cornea storage medium (2.6%, $n = 27$) or both (7.6%, $n = 80$). Test results were available in 97.4% ($n = 1025$) of cases. The real-world flow chart is represented in Fig. 1.

Microbiological culture was positive in 3.2% ($n = 34$), in which 85.3% ($n = 29$) of microorganisms found corresponded to bacteria and the remaining 14.7% ($n = 5$) corresponded to fungal or mixed flora (*Enterococcus faecalis* plus *Candida albicans*). The most prevalent microorganism found was *Staphylococcus epidermidis* ($n = 17$), followed by *Enterococcus faecalis* ($n = 3$), and *Escherichia coli* ($n = 3$). In the reference centre, 78.9% ($n = 830$) of all microbiological cultures were performed compared to an 18% in all other centres ($p = 0.001$), and 85.3% ($n = 29$) of positive microbiological cultures corresponded to corneas implanted in the reference centre ($p = 0.279$). Microorganisms isolated and its corresponding cornea characteristics are detailed in Table 3.

The 34 cases of positivity corresponded to 31 donors, in other words, only 3 donors had both corneas with positive cultures: both corneas of them were positive for the same microorganism (*Staphylococcus epidermidis* in two donors, and mixed *Enterococcus faecalis* plus *Candida albicans* in the other one). Among the other 28 positive microbiological cultured donor corneas, microbiology was indeed negative in mate corneas for 13 cases. In 15 out of the 28 remaining donors we have not information about culture of the mate cornea because in 5 cases mate cornea did not reach quality standards for clinical application and were discarded by the eye bank, and, on the other hand, due to microbiological tests not being performed in 10 cases. None of the 34 cases

Table 2 Demographics and characteristics of donors and donated corneas.

Donor cause of death: *N* (%) ($n = 737$)	
Brain damage	196 (26.6)
Cancer	155 (21)
Respiratory	146 (19.8)
Trauma	42 (5.7)
Non-septic shock	32 (4.4)
Septic shock/Active infection	20 (2.7)
Cardiovascular	9 (1.2)
Other	137 (18.6)
Donor age: mean and range (years)	62 (range 0–89)
Donor sex: *N* (%) female	366 (35.8%)
Cornea laterality: *N* (%) right	540 (52.7%)
Mean ECD: mean and range	2568.5 (range 2000–5102)
Method of corneal preservation: *N* (%)	
Hypothermia	240 (23.4%)
Organotypic (cultured)	785 (76.6%)
Days of storage: Median (95% CI)	
All corneas	26.0 (26.0; 27.0)
Corneas in hypothermia	5.0 (5.0; 6.0)
Corneas in culture	28.0 (28.0; 29.0)
Time from delivery to surgery: *N* (%)	
Same day	599 (58.4%)
One or more days before	426 (41.6%)
Technique of preparation by the eye bank: *N* (%)	
Full-thickness tissue[a]	941 (92.3%)
DSAEK	27 (2.7%)
DMEK	51 (5.0%)

ECD endothelial cell density, *DSAEK* Descemet Stripping Automated Endothelial Keratoplasty precut tissue, *DMEK* Descemet Membrane Endothelial Keratoplasty precut tissue.

[a]Full-thickness tissue was used for either penetrating keratoplasty, anterior or posterior lamellar techniques prepared in theatre.

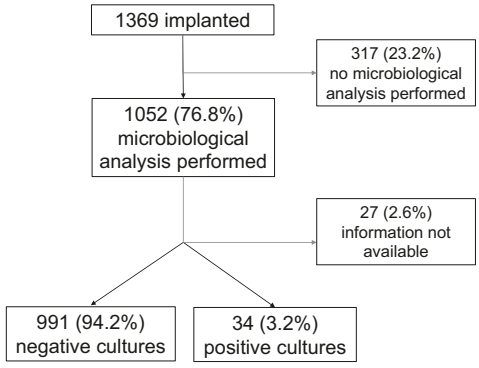

Fig. 1 Flowchart of the study design (real-world data). Microbiological tests performed over the total of corneas implanted and their results.

Table 3 Microbiological results of positive cultures and cornea tissue characteristics.

Cornea n°	preservation	Precut tissue	days delivery to implantation	sample	microorganism	Mate cornea
1	C	No	1	CSR	*Staphylococcus epidermidis*	CSR cultured: negative
2	H	No	0	CSR	*Staphylococcus epidermidis*	No microbiology
3	H	No	0	CSR	*Staphylococcus epidermidis*	CSR cultured: negative
4	C	No	0	CSR	*Escherichia coli*	CSR cultured: negative
5	H	DSAEK	1	CSR	*Staphylococcus aureus*	No microbiology
6	H	No	0	CSR	*Staphylococcus epidermidis*	No microbiology
7	H	DSAEK	1	CSR	*Corynebacterium sp*	CSR cultured: negative
8	H	No	1	CSR	*Candida glabrata*	discarded
9	C	No	1	CSR	*Candida parapsilosis*	discarded
10	C	No	0	CSR	*Burkholderia cepacia*	No microbiology
11	H	No	1	CSR	*Staphylococcus epidermidis*	mate cornea: number 12
12	H	No	1	CSR	*Staphylococcus epidermidis*	mate cornea: number 11
13	H	No	1	CSR	*Staphylococcus epidermidis*	No microbiology
14	H	No	0	CSR	*Staphylococcus epidermidis*	No microbiology
15	H	No	1	CSR	*Candida albicans, Enterococcus faecalis*	mate cornea: number 16
16	H	No	1	CSR	*Candida albicans, Enterococcus faecalis*	mate cornea: number 15
17	C	No	0	CSR	*Escherichia coli*	CSR cultured: negative
18	H	No	1	CSR	*Staphylococcus epidermidis*	No microbiology
19	H	No	1	CSR	*Staphylococcus epidermidis*	Discarded
20	C	No	0	CSR	*Staphylococcus epidermidis*	No microbiology
21	H	No	0	SM+CSR	*Corynebacterium sp*	CSR cultured: negative
22	C	No	0	CSR	*Staphylococcus epidermidis*	CSR cultured: negative
23	C	No	0	CSR	*Bacillus spp*	No microbiology
24	C	No	0	CSR	*Enterococcus faecalis*	SM cultured and negative
25	H	No	0	CSR	*Enterococcus faecalis*	discarded
26	C	No	0	CSR	*Staphylococcus epidermidis*	CSR cultured: negative
27	H	No	0	CSR	*Enterococcus faecalis*	CSR cultured: negative
28	C	No	0	CSR	*Haemophilus influenzae*	CSR cultured: negative
29	H	No	0	CSR	*Staphylococcus epidermidis*	discarded
30	C	No	1	CSR	*Staphylococcus epidermidis*	No microbiology
31	C	No	0	SM+CSR	*Escherichia coli*	SM+CSR cultured: negative
32	H	No	1	CSR	*Aspergillus niger*	CSR cultured: negative
33	H	No	0	SM+CSR	*Staphylococcus epidermidis*	mate cornea: number 34
34	H	No	0	SM+CSR	*Staphylococcus epidermidis*	mate cornea: number 33

H: hypothermia; C: cultured cornea; 0: same day; 1: one or more days before.

CSR corneoscleral rim, *SM* storage medium, *DSAEK* Descemet Stripping Automated Endothelial Keratoplasty precut tissue.

with positive microbiological cultures reported ocular infection for the recipients in at least 6 months' follow-up.

Regarding hypothermic corneas, 8.8% ($n = 21$) had positive microbiological culture whereas only 1.6% ($n = 13$) of organotypic cultured corneas. In other words, corneas preserved in hypothermia represented 61.8% of all positive microbiology results ($p < 0.001$). *Staphylococcus epidermidis* and *Enterococcus faecalis*—alone or isolated with *Candida albicans*—were also more prevalent in hypothermic corneas, corresponding to 57.1 and 19% of all specimens isolated respectively. Corneas in hypothermia ($n = 346$) remained on average 5 days in the eye bank (CI 95% 5; 6) and cultured corneas ($n = 1023$) remained on average 28 days (CI 95% 28.0; 29.0). Median time of corneas with negative microbiological culture ($n = 991$) were 26 days (CI 95% 26; 27) (range 0; 150 days) and with positive

microbiological culture ($n = 34$) were 7 days (CI 95% 7; 26) (range 0; 37 days) ($p = 0.001$).

Septicaemia showed more microbiological positivity than other causes of death ($p = 0.044$). Other factors that could eventually be related to positive microbiological cultures were also studied: precut vs surgeon-cut tissue ($p = 1.000$), one vs both corneas implanted ($p = 0.203$), both corneas sent at the same time vs one after the other ($p = 0.856$), days from eye bank shipment to surgery ($p = 0.748$), endothelial cell density ($p = 0.939$), or sample sent for microbiology (corneoscleral rim, cornea storage medium or both) ($p = 0.251$). Overall, none was found significant for a statistical association with microbiological positivity.

Discussion

Microbiological culture of corneoscleral rim and/or cornea storage medium is widely used as quality control of donors when the cornea is implanted. This study provides real-world information on actual day data about microbiological culture results of corneoscleral rims and storage medium of corneas provided by a single public official eye bank. As a whole, it reports less microbiological culture positivity in organotypic cultured corneas than those conserved in hypothermia. In our study, 78.9% of all donor microbiological control cultures carried out corresponded to corneas implanted in the reference centre ($p = 0.001$). That could suggest that high volume implanting centres could be more prone to perform microbiological cultures.

Causes for positivity related to donor cornea characteristics were searched for, showing more microbiological positive results of those corneas stored in hypothermia than those cultured ($p < 0.001$). Several groups have studied risk factors for donor cornea contamination detected in the eye bank [3, 4, 18, 19]. It seems than cultured corneas have lower risk to have a positive culture because of the eye bank protocol that imply more accurate microbiological controls during the period of culture. For example, in our ocular tissue bank cultured cornea is released after three sequentially negative microbiological analysis by standard protocol [20].

Most of the provided corneas were full thickness tissue (92.4%). Among precut tissue ($n = 78$; 7.6%)—for DMEK or DSAEK—only 2 cases of positivity were found, in this case, in precut tissue for DSAEK. Only a fraction (65.2%) of delivered full-thickness corneas was used for penetrating keratoplasty as some of them (34.8%) were indeed prepared by the surgeon to perform a selective endothelial procedure. This additional manipulation in the theatre to prepare a whole cornea for lamellar surgery could eventually contaminate it although no statistical differences in microbiological testing results between precut corneas and whole corneas than could be cut in theatre were found ($p = 1.000$). Since in 2013 our eye bank started to deliver precut corneas for DSAEK and in 2017 for DMEK, some surgeons and implanting centres opted for purchasing precut tissue while others continued to prepare the corneas for posterior keratoplasties in site. These last corneas were cut with a microkeratome or peeled in theatre without laminar flow in most of the cases, having an extra chance for contamination. Taking into account that the most prevalent microorganism found in our study is *Staphylococcus epidermidis*, which is considered a microorganism of normal mucous membrane flora, we could not discard an eventual theatre contamination. On the other hand, precut tissue has shown less positive bacterial donor rim cultures than surgeon-cut, as previously reported [21, 22]. It is not possible to know the trends in keratoplasty techniques from this study data, as only in some cases (surgeon preferences or implanting centre protocol, for example) DMEK and DSAEK were undertaken using precut tissue [23], while in the reference centre, most of the full-thickness corneas purchased (34.8%) were used for posterior lamellar techniques (data not shown).

Other causes that could be related to microbiological positivity were also studied. For example, the material for sample cultured (corneoscleral rim, cornea storage medium or both) could be associated to the positivity rate. In this study both corneoscleral rim and storage medium were simultaneously cultured in only 80 cases (7.6%), an insufficient number to perform statistical associations. Corneoscleral rim cultures and storage media have been previously compared in literature, observing that corneoscleral remaining tissue showed more positivity [24]. In addition, different methods of microbiological culture or incubation time, can lead to different positivity results due its different sensibility [8, 25]. We could not stablish and compare types of microbiological culture methods due to the heterogeneity of protocols and approaches of the centres involved. Sharma et al. demonstrated that different microbiology protocols directly influence the rates of positive rim cultures [8]. The donor cause of death has also proven to be a major factor for corneal suitability because death related to infections are more prone to cause positive cultures in the eye bank and being discarded consequently [26]. Despite our study showed than septicaemia had a statistically significant microbiological positivity ($p = 0.044$), only one major death cause was reported by donor. Other causes of death—as cancer, respiratory—could cause sepsis as well and be ignored for being a secondary death cause.

Some limitations of this study are related to the real-world origin of the data, sample size, and its related biases. In addition, we observed that 23.2% of the keratoplasties

carried out had no microbiological perioperatively control of the donated tissue. Since this is not required by protocol, it could still be considered a high microbiological control rate compared with other areas [22]. Despite some authors question the prognostic role of corneoscleral rims [15], recent studies are more prone to perform them, based on an efficient rationale [14, 24].

Being able to know the exact moment of donor corneal contamination would be of crucial interest. However, the microbiological control rate, number of corneas with its mate cornea discarded in this study, and the study design itself, leave this question unanswered. For any not clearly defined reason, cultured corneas tend to have less positivity rate than hypothermic ones. Eye banks current trends are to supply organ-cultured corneas instead of hypothermic tissue —BST started to supply organotypic corneas in January 2010 and stands for the 65% of the overall storage method for the last 3 years. It seems they have less risk of have a positive microbiological result: hypothermic storage ranged from 4 to 37% [6, 27]. By contrast, organ culture literature reports values up to only 16% [2, 28, 29]. However, some authors have found major positivity in organotypic corneas compared to hypothermic ones [27]. Our data showed that only 40.6% of all positive microbiological results have been on cultured corneas, corresponding to 76.7% of all supplied tissue. Moreover, cultured corneas have other added advantages over the hypothermic ones, as for example the longer storage time that has improved the logistics of the supply procedure as well as decreased the waiting list in some regions [27].

In summary, this study reports rates of positive microbiological culture of corneoscleral rims and cornea storage medium in real-world practice. It shows a statistically significant more positivity in those corneas conserved in hypothermia compared to those in organotypic cultures. In addition to other commented advantages of cultured cornea method, as greater tissue availability despite improved costs, the future of cornea tissue conservation in eye banks could be expected to consolidate higher rates of organ-cultured corneas.

Summary

What was known before

- several factors are related to keratoplasty postoperative infection ·microbiological cultures are used as predictors for keratoplasty infectious complications ·the relationship between positive microbiological cultures and keratoplasty infection is weak.

What this study adds

- microbiological culture rates in real-world are studied ·causes for microbiological culture positivity are studied ·organotypic cultured corneas are less prone to have positive corneoscleral rims or cornea storage media

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. European Eye Bank Association. Annual Directory. 28th Edition. Venice, Italy; 2020.
2. Builles N, Perraud M, Reverdy M, Burillon C, Crova P, Brun F, et al. Reducing contamination when removing and storing corneas: a multidisciplinary, transversal, and environmental approach. Cornea. 2006;25:185–92.
3. Gruenert AK, Rosenbaum K, Geerlling G, Fuchsluger TA. The influence of donor factors on corneal organ culture contamination. Acta Ophthalmol. 2017;95:733–40.
4. Röck D, Wude J, Bartz-Schmidt KU, Yoeruek E, Thaler S, Röck T. Factors influencing the contamination rate of human organ-cultured corneas. Acta Ophthalmol. 2017;95:e706–12.
5. Rehany U, Balut G, Lefler E, Rumelt S. The Prevalence and Risk Factors for Donor Corneal Button. Cornea. 2004;23:649–54.
6. Kloess PM, Stulting RD, Waring GO, Wilson LA. Bacterial and fungal endophthalmitis after penetrating keratoplasty. Am J Ophthalmol. 1993;115:309–16.
7. Keyhani K, Seedor JA, Shah MK, Terraciano AJ, Ritterband DC. The incidence of fungal keratitis and endophthalmitis following penetrating keratoplasty. Cornea. 2005;24:288–91.
8. Sharma RA, Park JSY, Wang Y, Zhang T, Sharpen L, Dixon W, et al. Association between positive corneal rim cultures and microbiology screening protocols in Ontario. Can J Ophthalmol. 2018;53:272–7.
9. Chen JY, Jones MN, Srinivasan S, Neal TJ, Armitage WJ, Kaye SB. Endophthalmitis After Penetrating Keratoplasty. Ophthalmology. 2015;122:25–30.
10. Hassan SS, Wilhelmus KR. Eye-banking risk factors for fungal endophthalmitis compared with bacterial endophthalmitis after corneal transplantation. Am J Ophthalmol. 2005;139:685–90.
11. Leveille AS, McMullan FD, Cavanagh HD. Endophthalmitis following penetrating keratoplasty. Ophthalmology. 1983;90:38–9.
12. Antonios S, Cameron J, Badr I, Habash N, Cotter J. Contamination of donor cornea: postpenetrating keratoplasty endophthalmitis. Cornea. 1991;10:217–20.
13. Matsumoto M, Suzuma K, Miyamura N, Imamura N, Kitaoka T. Conjunctival swabs and corneoscleral rim cultures from corneal

transplantation donors as possible early indicators for post-transplant endopthalmitis. Jpn J Ophthalmol. 2011;55:321–6.
14. Kiatos E, Armstrong JJ, Hutnik CML, Tsioros SM, Malvankar-Mehta MS, Hodge WG. The value of corneoscleral rim cultures in keratoplasty: a systematic review and cost-effectiveness analysis. Clin Outcomes Res. 2017;9:459–74.
15. Wilhelmus KR, Hassan SS. The prognostic role of donor corneoscleral rim cultures in corneal transplantation. Ophthalmology. 2007;114:440–5.
16. Everts RJ, Fowler WC, Chang DH, Reller LB. Corneoscleral rim cultures: lack of utility and implications for clinical decision-making and infection prevention in the care of patients undergoing corneal transplantation. Cornea. 2001;20:586–9.
17. Hajjar Sesé A, Lindegaard J, Julian HO, Højgaard-olsen K, Møller NF, Heegaard S. A presentation of culture-positive corneal donors and the effect on clinical outcomes. Graefe's Arch Clin Exp Ophthalmol. 2019;257:135–41.
18. Linke SJ, Fricke OH, Eddy M, Bednarz J, Druchkiv V, Kaulfers P, et al. Risk factors for donor cornea contamination: retrospective analysis of 4546 procured corneas in a single eye bank. Cornea. 2013;32:141–8.
19. Linke SJ, Eddy M, Fricke OH, Wulff B, Schro A, Hassenstein A, et al. Thirty years of cornea cultivation: long-term experience in a single eye bank. Acta Ophthalmol. 2013;91:571–8.
20. Zanetti E, Bruni A, Mucignat G, Camposampiero D, Frigo AC, Ponzin D. Bacterial contamination of human organ-cultured corneas. Cornea. 2005;24:603–7.
21. Rauen MP, Goins KM, Sutphin JE, Kitzmann AS, Schmidt GA, Wagoner MD. Impact of eye bank lamellar tissue cutting for endothelial keratoplasty on bacterial and fungal corneoscleral donor rim cultures after corneal transplantation. Cornea. 2012;31:376–9.
22. Mian SI, Aldave AJ, Tu EY, Ayres BD, Jeng BH, Macsai MS, et al. Infections in the cornea preservation time study. Cornea. 2018;37:1102–9.
23. Palma-Carvajal F, Morales P, Salazar-Villegas A, Figueroa-Vercellino JP, Spencer F, Peraza-Nieves J, et al. Trends in corneal transplantation in a single center in Barcelona, Spain. Transitioning to DMEK. J Fr Ophtalmol. 2020;43:1–6. https://doi.org/10.1016/j.jfo.2019.06.026.
24. Tsui E, Luong PM, Fogel J, Fogel ES, Zegans ME. Microbial analysis of donor corneoscleral rims and storage media. Ocul Immunol Inflamm. 2019;27:817–20.
25. Nan-ni C, Pei-Lun W, Hung-Chi C, Tsung-Yu H, Li-Ju L. Prevalence of microbial contamination in donor corneas. Taiwan J Ophthalmol. 2019;9:179–84.
26. Röck T, Hofmann J, Thaler S, Bramkamp M, Bartz-Schimdt K, Yoeruek E, et al. Factors that influence the suitability of human organ-cultured corneas. Graefe's Arch Clin Exp Ophthalmol. 2016;254:135–41.
27. Devasahayam R, Georges P, Hodge C, Treloggen J, Cooper S, Petsoglou C, et al. Implementation of organ culture storage of donor corneas: a 3 year study of its impact on the corneal transplant wait list at the Lions New South Wales Eye Bank. Cell Tissue Bank. 2016;17:377–85.
28. Cunningham WJ, Moffatt SL, Brookes NH, Twohill HC, Pendergrast DGC, Stewart JM, et al. The New Zealand National Eye Bank Study: trends in the acquisition and storage of corneal tissue over the decade 2000 to 2009. Cornea. 2012;31:538–45.
29. Fontana L, Errani PG, Zerbinati A, Musacchi Y, Di Pede B, Tassinari G. Frequency of positive donor rim cultures after penetrating keratoplasty using hypothermic and organ-cultured donor corneas. Cornea. 2007;26:552–6.

Artículo 4

Objetivo:
Evaluar los resultados clínicos de los biosustitutos autólogos, tomando como biosustituto representativo la conjuntiva y analizando factores de riesgo, como la tasa de adherencia al tratamiento postoperatorio, asociados al resultado clínico de la cirugía del pterigión.

Título:
Postoperative treatment compliance rate and complications with two different protocols after pterygium excision and conjunctival autografting.

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RESUMEN

Propósito: evaluar la tasa de adherencia al tratamiento postoperatorio tras cirugía de pterigión con dos protocolos de tratamiento diferentes.

Métodos: se revisaron los datos clínicos de pacientes con indicación de exéresis de pterigión con autoinjerto conjuntival en un solo centro (y por una sola cirujana) en Barcelona entre marzo de 2014 y diciembre de 2017. El protocolo postoperatorio inicial (protocolo 1) consistía en 4 meses de tratamiento con corticoides en pauta descendente. El protocolo 2 consistió en el tratamiento con corticoides tópicos en pauta descendente durante 5 semanas. La tasa de adherencia, complicaciones y resultados clínicos fueron evaluados realizándose comparaciones estadísticas.

Resultados: se realizaron 120 cirugías en 99 pacientes. Se aplicó el protocolo 1 en 63 casos y los siguientes 57 siguieron el protocolo 2. La adherencia al protocolo 1 (57,6%) fue menor que al protocolo 2 (84,9%) (p=0,002). Se encontraron complicaciones intraoperatorias – desgarro del injerto, adelgazamiento corneal, perforación corneal, sangrado – en diez casos del protocolo 1 y tres casos del protocolo 2 (p=0,08). Se encontraron complicaciones postoperatorias – dislocación del injerto, hematoma del injerto, hipertensión ocular y recidiva – en 31 casos del protocolo 1 (46,2%) y en ocho casos del protocolo 2 (14%) (p=0,001). Seis semanas después de la cirugía, se detectó hipertensión ocular en ocho casos del protocolo 1 (13,6%) y dos del protocolo 2 (3,8%) (p=0,099). La tasa de recurrencia durante el primer año fue mayor en el protocolo 1 (26,3%) que en el protocolo 2 (7,6%) (p=0,011). No se registraron casos de disminución de la agudeza visual o de infección.

Conclusiones: el protocolo 2 ha mostrado no solo tener mayor tasa de adherencia que el protocolo 1 sino que también presenta menos complicaciones postoperatorias, por lo que se presenta como un tratamiento postoperatorio para la cirugía del pterigión seguro y efectivo.

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Postoperative treatment compliance rate and complications with two different protocols after pterygium excision and conjunctival autografting

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Abstract

Aim: To evaluate compliance rate to pterygium postoperative treatment with two different protocols.

Methods: Review of clinical data of patients submitted to pterygium excision and conjunctival autografting in a single centre (and a single surgeon) in Barcelona between March 2014 and December 2017. Initial postoperative protocol (protocol 1) consisted of 4 months of topical steroids in a tapering fashion. Protocol 2 consisted of topical steroids tapered over 5 weeks. Compliance rate, complications and clinical outcomes were evaluated, and statistical comparisons were made.

Results: 120 surgeries were performed in 99 patients. Protocol 1 was applied in 63 cases and the next 57 followed protocol 2. Compliance with protocol 1 (57.6%) was lower than with protocol 2 (84.9%) ($p = 0.002$). Intraoperative complications (graft tear, corneal thinning, corneal perforation and bleeding) were found in 10 cases of protocol 1 and three cases of protocol 2, $p = 0.08$. Postoperative complications (graft dislocation, graft haematoma, ocular hypertension and recurrence) were found in 31 cases of protocol 1 (46.2%) and eight cases of protocol 2 (14%), $p = 0.001$. Six weeks after surgery, ocular hypertension was detected in eight cases corresponding to protocol 1 (13.6%) and two cases of protocol 2 (3.8%), $p = 0.099$. Recurrence rate during first year was higher in protocol 1 (26.3%) compared to protocol 2 (7.6%), $p = 0.011$. No cases of visual acuity worsening or infection were registered.

Conclusion: Protocol 2 has shown to have higher compliance rate than protocol 1 and less postoperative complications, proving to be a safe and effective postoperative treatment after pterygium surgery.

Keywords

Pterygium, cornea/external disease, pharmacology, conjunctival degenerations, diseases of the ocular surface, ocular surface surgery

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Introduction

Pterygium is characterized by the presence of a fibrovascular tissue which slowly grows over the limbic corneal surface and whose prevalence can reach up to 22%, mainly in countries close to the equator.[1–3]

Although ocular lubrication is required in the presence of symptoms (irritation, red and dry eye), surgery is indeed necessary when the visual axis is covered or the ocular movement restricted. Another indication for pterygium surgery is cosmetic disturbance. The main complication of pterygium surgery is recurrence, which can be up to 89% according to the different surgical techniques.[4] Pterygium excision with conjunctival autografting is currently the gold standard technique, which provides the lowest recurrence rate, between 3.3% and 16.7%.[4–7] Low compliance with postoperative treatment has been associated with a higher risk of recurrence.[8]

The aim of this study was to evaluate the compliance rate and outcomes with postoperative treatment in patients who have undergone surgery to treat pterygium. With just one surgical approach performed by a single surgeon, two different postoperative protocols were prescribed and clinical outcomes were compared retrospectively between groups.

Methods

This was a retrospective analysis study carried out at a single centre in Barcelona, Spain (*Hospital Universitari Sagrat Cor*). The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics institutional review board approval was obtained (2018/88-OFT-HUSC).

Data of consecutive cases of pterygium surgery over a 3-year period (between March 2014 and December 2017) were included. Sex, nationality, visual acuity, intraocular pressure (IOP), pterygium morphology (atrophic, intermedium or fleshy),[9,10] extension (millimetres) of corneal invasion, postoperative regime protocol and complications were reviewed.

The surgical technique employed in all cases was excision with conjunctival autografting. Under peribulbar anaesthesia, the pterygium head was pulled and subconjunctival tissue dissected. In high-risk cases of recurrence, 0.02% mitomycin C (MMC; 0.2 mg/mL) was applied over the bare sclera intraoperatively for 2 min followed by profuse rinsing with saline (100 mL).[11] The following cases were defined as high-risk pterygia: (a) new excision on a previous pterygium surgery that recurred, (b) recurrence in the contralateral eye and (c) fleshy pterygium with large corneal invasion. After measuring the bare scleral area, a graft of 1×1 mm larger in size was harvested from the upper bulbar conjunctiva from the same eye and glued over the naked sclera using fibrin sealant (Tisseel®; Baxter, Vienna, Austria). The free graft was placed over the cornea, epithelium facing down and limbus-limbus oriented. Next, the 'liquid' component of Tisseel® (human thrombin) was applied to the bare sclera, and the 'dense' component of Tisseel® (soluble human fibrinogen) on the graft. The graft was turned over and placed on the naked sclera allowing more time to set the graft properly. For those patients with suspected or diagnosed glaucoma and those with previous pterygium recurrence, the graft was obtained from the inferior bulbar conjunctiva. Dexamethasone and chloramphenicol ointment (0.5/10 mg/g) was applied at the end of surgery, and eye occlusion was maintained for a day.

Postoperative treatment was carried out under two protocols. Protocol 1 consisted of dexamethasone and chloramphenicol ointment (0.5/10 mg/g) 4 times a day during 1 week, followed by topical 0.1% dexamethasone (10 mg/mL) 4 times a day tapered for 4 months (first month was applied 4 times a day, 3 times a day during the second month, twice a day during third month and once a day during last month). Protocol 2 had 5 weeks duration: after 1 week with dexamethasone and chloramphenicol ointment 4 times a day, topical dexamethasone drops 4 times a day were tapered weekly (4 times a day for a week, then 3 times a day for a week, twice a day for a week and once a day during last week). Follow-up was on postoperative day 1, at 4–6 weeks, at 6 months and at 1 year after surgery. Postoperative compliance was checked at each visit, asking directly to the patient, until month sixth after surgery. Bad compliance was registered as underdose when the patient admitted a lower frequency of treatment than prescribed or overdose when the patient applied the topical treatment with a higher frequency than prescribed. Complications were checked at each visit and registered. In those patients with any type of complication or recurrence suspicion, follow-up was adapted and changed.

Recurrences were managed according to the time of appearance: weekly subconjunctival injections of 0.1-mL 5-fluorouracil (5-FU; 25 mg/mL) were used in cases presenting early recurrence (less than 3 months after surgery) until progression stopped. If recurrence was detected more than 3 months after surgery, observation or new excision (using a graft from the inferior bulbar conjunctiva) was used, according to patient preferences.

Descriptive results were presented as mean with 95% confidence intervals (95% CIs) or absolute frequencies and percentages for quantitative or qualitative variables respectively. In the case of the absence of a Gaussian distribution, median and 95% CI were used. All results were tabulated for whole population and by postoperative protocol. Inferential analyses were performed by visual outcome, compliance and complications (intra and postoperative). The t-test for independent groups or Fisher's exact test were used for statistical analyses for quantitative or qualitative variables, respectively; in case of ordinal variables or non-Gaussian distributions Mann–Whitney U test was applied. Differences in changes in IOP at 6 weeks post-surgery between protocols were calculated by analysis of covariance (ANCOVA) model

Table 1. Demographics of both groups of postoperative treatment protocols.

			All	Protocol 1	Protocol 2	p-value
		N	120	63 (52.5%)	57 (47.5%)	
Age		Mean (95% CI)	46.4 (44.6, 48.3)	47.5 (45.3, 49.6)	45.3 (42.2, 48.4)	0.245
BCVA preop		Mean (95% CI)	0.07 (0.04, 0.10)	0.07 (0.04, 0.10)	0.07 (0.01, 0.12)	0.940
IOP preop		Mean (95% CI)	14.3 (13.7, 14.9)	15.2 (14.2, 16.1)	13.3 (12.6, 14)	0.002
Pterygium type	A	N	8 (6.7%)	2 (3.2%)	6 (10.5%)	
	I	N	77 (64.2%)	45 (71.4%)	32 (56.1%)	0.832
	C	N	35 (29.2%)	16 (25.4%)	19 (33.3%)	
Location	Nasal	N	113 (94.2%)	59 (93.6%)	54 (94.7%)	0.878
	Double	N	4 (3.3%)	2 (3.2%)	2 (3.5%)	
	Temporal	N	3 (2.5%)	2 (3.2%)	1 (1.8%)	
Primary pterygium		N	114 (95%)	58 (92.1%)	56 (98.2%)	0.210
MMC		N	5 (4.2%)	2 (3.2%)	3 (5.3%)	0.667

BCVA: best-corrected visual acuity; IOP: intraocular pressure; MMC: mitomycin C.

adjusting the effect of protocol by baseline value of IOP for each patient. All statistical tests were performed with a two-sided type I error of 5%, with the statistical software SPSS v.25.0 (IBM, Armonk, New York, USA).

Results

A total of 120 surgical interventions (57.5% on left eyes) on 99 patients were carried out by a single surgeon (N.S.). A postoperative treatment protocol of 4 months (Protocol 1) was employed in the first 63 consecutive cases, and the 5 weeks protocol (Protocol 2) was used in the subsequent 57 cases. No demographic or clinical data were statistically different between groups, except preoperative IOP (15.2 mmHg in protocol 1 vs 13.3 mmHg in protocol 2, p = 0.002), with no clinical significance: the mean age of the patients was 46.4 years (range 23; 86 years) and 55.5% were women. Patients were originally from Central and South America (82.5%), Asia (9.2%), Europe (5%) and North Africa (3.3%). The mean horizontal corneal invasion was 1.72 mm (range 1; 3.5 mm), and the predominant morphology was 'intermediate' (64.2%). The LogMAR mean preoperative best-corrected visual acuity (BCVA) was 0.07 (95% CI = 0.04, 0.1). The mean preoperative IOP was 14.3 (95% CI = 13.7, 14.9) with statistically significant differences between groups (p = 0.002): mean basal IOP group 1 was 15.2 (95% CI = 14.2, 16.1) and for group 2 was 13.3 (95% CI = 12.6, 14.0).

Systemic comorbidity was present in 13.3% of patients: diabetes mellitus (n = 5), high blood pressure (n = 5), migraine, hyper and hypothyroidism, and anxiety. Ocular comorbidity was present in 5% of patients: cataract (n = 3), being one case associated with macular degeneration, amblyopia (n = 2) and one had previously undergone laser refractive surgery. No cases of preoperative glaucoma were detected in either group. Descriptive data are summarized in Table 1.

Major surgical indication was primary nasal pterygium (94.2%); other indications were one primary temporal pterygium confirmed by histology, six double pterygia (two of them with previous nasal pterygium excised), one double pinguecula; and six pterygia recurrences – or secondary pterygia – (one of them on a previous pinguecula surgery). The median follow-up time was 14.1 months (95% CI = 10.4, 24.4). Mean graft area used in both groups was similar, p = 0.429. However, the range was wider in group 1 (8.0–132.0 mm^2) than in group 2 (28.0–54.0 mm^2).

Inferior bulbar conjunctiva was used in eight cases. MMC was applied intraoperatively in five cases of high-risk recurrence (three recurrent pterygia and two primary pterygia with contralateral recurrence). Three patients with recurrence undergoing surgery refused to have MMC.

Eight patients failed to come to follow-up visits (four of them corresponded to protocol 2). Good compliance with postoperative treatment globally in our study was achieved in 79 cases (70.5%). Compliance with postoperative treatment protocol 2 (84.9%) was higher than with protocol 1 (57.6%), p = 0.002. A poor compliance profile differed between protocols, p = 0.015: in protocol 1, underdose was the reason for poor compliance in 19 cases (76%), and in protocol 2 overdose was the main cause of poor compliance to postoperative treatment (75%).

Intraoperative and postoperative complications are summarized in Table 2. There was a higher number of postoperative complications with statistical significance in protocol 1 compared to protocol 2, p = 0.001.

Recurrence was detected in 19 cases (17.3%) one year after surgery, which increased to 21 cases at long-term follow-up. There were more recurrences during the first year in protocol 1 than in protocol 2 (26.3% vs 7.5%; p = 0.011). No recurrences were detected on secondary pterygia cases treated with MMC intraoperatively, or on grafts with tears. The median time to diagnose and treat a recurrence was 78 days; the median time in protocol 1 was

Table 2. Intraoperative and postoperative complications.

	Protocol 1 (n = 63)	Protocol 2 (n = 57)	p-value
Intraoperative			
Tear in graft	7 (11.3%)	1 (1.7%)	
Corneal thinning	2 (3.2%)	none	
Corneal perforation	none	1 (1.7%)	
Bleeding	1 (1.6%)	1 (1.7%)	
Total intraoperative	10	3	0.080
Postoperative			
Partial graft dislocation	7 (11.3%)	2 (3.4%)	
Graft haematoma	1 (1.6%)	none	
Recurrence after a year	15 (26.3%)	4 (7.5%)	
Total postoperative	31	8	0.001

Totals were for number of patients with a least one complication (intra or postoperative).

87 days (95% CI = 56, 202) and 37 days (95% CI = 36, 64) in protocol 2, with no statistically significant difference between protocols, p = 0.096.

Eight cases were managed conservatively (six cases in protocol 1), one case (protocol 2) had further surgery with a new inferior conjunctival autografting and MMC for 2 min, and the other 12 cases (eight cases in protocol 1) were treated with one subconjunctival injection of 5-FU (six cases), two injections (four cases) or three injections (two cases). Progression of recurrence halted in all 12 cases and even reversed in one case (Figure 1). There were no complications due to 5-FU injections. No statistically significant difference was found in recurrence management between protocols, p = 0.455.

No cases of visual worsening or infection were registered. The mean IOP kept higher in protocol 1 at 4–6 weeks follow-up visit (p = 0.008). However, this change in IOP (estimated difference) from preoperative visits to 4–6 weeks follow-up had no statistically significant difference between protocols 1 and 2 (p = 0.089). Ten cases (8.3%) of ocular hypertension (eight cases in protocol 1) were detected at the second follow-up visit, 4–6 weeks after surgery, p = 0.099. All the cases were treated with topical prostaglandin analogue (tafluprost 15 mg/mL once at night) until postoperative treatment ended; none of them evolved to glaucoma.

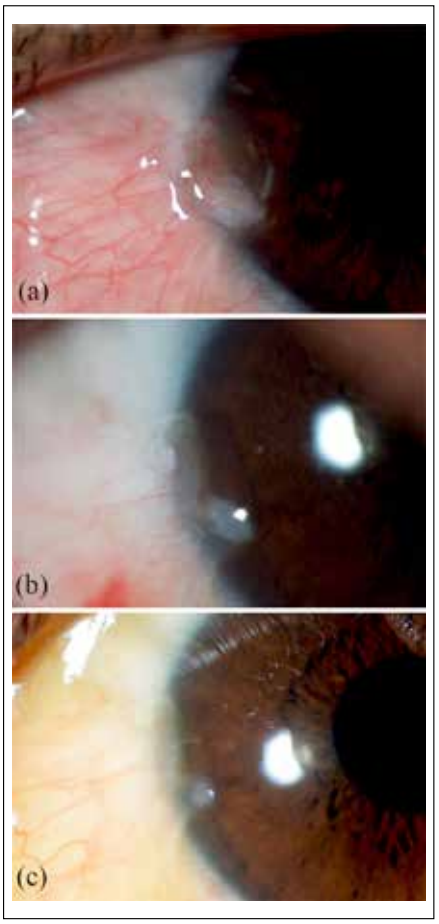

Figure 1. (a) A nasal pterygium recurrence diagnosed 2 months after excision. (b) Three weeks after subconjunctival 5-FU injection, a partial regression is observed. (c) Three months after a single subconjunctival 5-FU injection, the recurrence is halted and the pterygium appears atrophic.

Discussion

Recurrence risk, main complication of pterygium surgery, has been related to patient age, environmental factors, surgical technique, adjuvant treatments, sutures instead of tissue glue, postoperative treatment and fleshy morphology.[4,9,10,12,13]

Surgical technique proved to be a major factor of recurrence. Simple excision (with bare sclera or simple closure) has higher rates of recurrence (up to 89%)[4] as well as amniotic membrane transplantation (up to 42.3%).[6] Currently, excision with conjunctival autografting is considered the technique with the lowest recurrence rate,

between 3.3% and 16.7%.[4–7] Our overall 17.3% of recurrence rate using excision with the conjunctival autografting technique and gluing is comparable to that reported in the literature.

Previous reports observed a significantly higher risk of recurrence in patients with poor postoperative treatment compliance.[8] First cases of the present study followed a 4-months protocol similar to that described in the literature.[14–16] After observation of poor compliance with this protocol 1, a review of literature came up with a shorter protocol regime,[17–19] protocol 2. Applying this protocol to the next group of patients increased the compliance rate compared to a longer postoperative treatment in a statistically significant way. Moreover, protocol 1, used in the first 63 cases showed a statistically higher recurrence rate than protocol 2. Our results showed postoperative treatment compliance has to be taken into account as a risk factor for pterygium recurrence, among others (e.g., surgeon learning curve). A shorter duration regimen (5 weeks vs 4 months) does not increase the recurrence rate, probably due to its better compliance. Interestingly, the pattern of poor compliance to postoperative treatment was significantly different between protocols. In protocol 1, it seemed patients tend to get tired of a long treatment and abandon it, being apparently more prone to recurrences as previously reported.[8] Protocol 2, a protocol treatment of 5 weeks, seems to kept them too 'motivated' and they tend to 'forget' to taper the treatment. However, compliance to treatment protocols may be affected by many other factors such as poor communication, conflicting goals, lack of knowledge, poor skills, regime complexity, disease duration, physiological distress, financial restriction, and lack of cohesion or social support.[20]

Ocular hypertension rate at 4–6 weeks was lower with protocol 2, although this difference had no statistical significance ($p=0.089$). Other factors could explain higher ocular hypertension in patients receiving protocol 1: preoperative IOP was significantly higher in group 1 ($p=0.002$) and remained higher at weeks 4–6 ($p=0.008$). Steroid-induced ocular hypertension has been reported in literature ranging from 2.97% to up to 40%.[21–23] Our overall outcome of 8.3% is in line with findings in the literature. Treatment of steroid-induced ocular hypertension is indeed stopping the use of topical steroids. However, because an early cessation can be related to a higher rate of recurrences,[8] cases of ocular hypertension in our patients were treated with topical prostaglandin until postoperative treatment ended.

Other postoperative complications (graft hematoma, partial graft dislocation) showed to be statistically less frequent in protocol 2. However, this difference does not seem to have any real relation to the postoperative treatment duration but may rather be explained by increased surgical experience.

Postoperative treatment strategies must minimize side effects (ocular hypertension) and recurrence rates.[4,8,10–13] This study showed proper treatment compliance is associated with lower side effects and less recurrences, so strategies should be designed to engage the patient after the pterygium surgery. We suggest accurate explanation about the postoperative management prior to surgery and a follow-up visit during the second week to check proper treatment compliance.

This study has some limitations to disclose. First, it has a non-randomized, retrospective design. Since the surgical technique, age and patient origin were essentially the same between groups, the recurrence rate could be explained not only by the compliance rate, but also by the surgeon's learning curve (i.e. having more experienced and harvesting a more reproducible grafting area when operating patients from protocol 2) introducing a bias in the study and weakening it. Moreover, in the absence of a standardized questionnaire to check the patients' compliance, probably patients of protocol 2 had a wider explanation about their postoperative protocol increasing their compliance. Standardized questionnaires should be introduced in the clinical practice in order to improve the compliance checking.

Management of recurrence traditionally consists of observation or new surgery. On the other hand, several studies have proposed subconjunctival injections of 5-FU as a management option.[16,24,25] In our series, 12 out of 19 recurrences were detected in early stages and progression stopped in all of the cases and even partially reversed in one case with subconjunctival injections of 5-FU. These findings are comparable to those of other authors.[25]

In conclusion, this study showed a significantly elevated rate of recurrences and complications in cases with inadequate compliance with postoperative treatment, and proved that a shorter treatment course may provide similar surgical success than long-term tapering protocols.

Informed consent

Patients whose images appear in this article consented to publish them.

Declaration of conflicting interests

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References

1. Cardenas-Cantu E, Zavala J, Valenzuela J, et al. Molecular basis of pterygium development. *Semin Ophthalmol* 2016; 31(6): 567–583.

2. Hovanesian JA, Starr CE, Vroman DT, et al. Surgical techniques and adjuvants for the management of primary and recurrent pterygia. *J Cataract Refract Surg* 2017; 43(3): 405–419.
3. Liu T, Liu Y, Xie L, et al. Progress in the pathogenesis of pterygium. *Curr Eye Res* 2013; 38(12): 1191–1197.
4. Alpay A, Ugurbas SH and Erdogan B. Comparing techniques for pterygium surgery. *Clin Ophthalmol* 2009; 3: 69–74.
5. Ang LP, Chua JL and Tan DT. Current concepts and techniques in pterygium treatment. *Curr Opin Ophthalmol* 2007; 18(4): 308–313.
6. Clearfield E, Hawkins BS and Kuo IC. Conjunctival autograft versus amniotic membrane transplantation for treatment of pterygium: findings from a Cochrane systematic review. *Am J Ophthalmol* 2017; 182: 8–17.
7. Kocamis O and Bilgec M. Evaluation of the recurrence rate for pterygium treated with conjunctival autograft. *Graefes Arch Clin Exp Ophthalmol* 2014; 252(5): 817–820.
8. Yaisawang S and Piyapattanakorn P. Role of post-operative topical corticosteroids in recurrence rate after pterygium excision with conjunctival autograft. *J Med Assoc Thai* 2003; 86(Suppl. 2): S215–S223.
9. Safi H, Kheirkhah A, Mahbod M, et al. Correlations between histopathologic changes and clinical features in pterygia. *J Ophthalmic Vis Res* 2016; 11(2): 153–158.
10. Tan DT, Chee SP, Dear KB, et al. Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. *Arch Ophthalmol* 1997; 115(10): 1235–1240.
11. Kaufman SC, Jacobs DS, Lee WB, et al. Options and adjuvants in surgery for pterygium. *Ophthalmology* 2013; 120(1): 201–208.
12. Karalezli A, Kucukerdonmez C, Akova YA, et al. Fibrin glue versus sutures for conjunctival autografting in pterygium surgery: a prospective comparative study. *Br J Ophthalmol* 2008; 92(9): 1206–1210.
13. Pan HW, Zhong JX and Jing CX. Comparison of fibrin glue versus suture for conjunctival autografting in pterygium surgery: a meta-analysis. *Ophthalmology* 2011; 118(6): 1049–1054.
14. Akbari M, Soltani-Moghadam R, Elmi R, et al. Comparison of free conjunctival autograft versus amniotic membrane transplantation for pterygium surgery. *J Curr Ophthalmol* 2017; 29(4): 282–286.
15. Nakamura T, Inatomi T, Sekiyama E, et al. Novel clinical application of membrane to treat patients with pterygium. *Acta Ophthalmol Scand* 2006; 84: 401–405.
16. Pikkel J, Porges Y and Ophir A. Halting pterygium recurrence by postoperative 5-fluorouracil. *Cornea* 2001; 20(2): 168–171.
17. Ha SW, Park JH, Shin IH, et al. Clinical analysis of risk factors contributing to recurrence of pterygium after excision and graft surgery. *Int J Ophthalmol* 2014; 8: 522–527.
18. Toker E and Eraslan M. Recurrence after primary pterygium excision: amniotic membrane transplantation with fibrin glue versus conjunctival autograft with fibrin glue. *Curr Eye Res* 2016; 41(1): 1–8.
19. Küçükerdönmez C, Akova YA and Altinörs DD. Comparison of conjunctival autograft with amniotic membrane transplantation for pterygium surgery. *Cornea* 2007; 26(4): 407–413.
20. Fielding D and Duff A. Compliance with treatment protocols: interventions for children with chronic illness. *Arch Dis Child* 1999; 80: 196–200.
21. Dibas A and Yorio T. Glucocorticoid therapy and ocular hypertension. *Eur J Pharmacol* 2016; 787: 57–71.
22. Busool Y, Mimouni M, Vainer I, et al. Risk factors predicting steroid-induced ocular hypertension after photorefractive keratectomy. *J Cataract Refract Surg* 2017; 43(3): 389–393.
23. Yülek F, Gerçeker S, Akçay E, et al. Corneal biomechanics in steroid induced ocular hypertension. *Contact Lens Anterior Eye* 2015; 38: 181–184.
24. Prabhasawat P, Tesavibul N, Leelapatranura K, et al. Efficacy of subconjunctival 5-fluorouracil and triamcinolone injection in impending recurrent pterygium. *Ophthalmology* 2006; 113(7): 1102–1109.
25. Said DG, Faraj LA, Elalfy MS, et al. Intra-lesional 5 fluorouracil for the management of recurrent pterygium. *Eye* 2013; 27(10): 1123–1129.

Manuscrito 1

Objetivo:
Evaluar los resultados clínicos de los recientes formatos de la membrana amniótica disponibles en Cataluña – liofilizada y colirio de extracto de membrana amniótica – desde un punto de vista de validación y estudio en la práctica clínica.

Título:
Lyophilized amniotic membrane graft for primary pterygium surgery: preliminary results

Autores:
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Revista, número de revisión
Cell and Tissue Banking, R1

RESUMEN

El objetivo de este estudio fue investigar la tolerabilidad, seguridad y eficacia de la membrana amniótica liofilizada (LAM) para uso ocular. De manera prospectiva, en una cohorte de 4 pacientes con pterigión primario nasal a los que se les realizó exéresis e implante de LAM, se evaluaron resultados clínicos y complicaciones. La manipulación quirúrgica de la LAM fue también analizada. La LAM era rígida y fácil de manipular, no se produjeron desgarros en la membrana durante la cirugía ni la sutura. El confort ocular fue comprobado y similar entre los pacientes con el tejido suturado o pegado. Tras doce meses no se encontraron problemas respecto a tolerabilidad o reacciones adversas. Se detectó un bajo resultado estético, en forma de recidiva, en tres pacientes. Nuestro estudio muestra que la LAM puede ser una alternativa efectiva a la membrana amniótica criopreservada como parche para la cirugía de exéresis del pterigión. Su principal ventaja es el almacenaje a temperatura ambiente, que puede hacerla disponible de inmediato. Se requieren estudios adicionales de los resultados clínicos de la cirugía del pterigión con membrana amniótica criopreservada comparada con LAM que puedan confirmar los beneficios de la última.

Cell and Tissue Banking

Lyophilized amniotic membrane graft for primary pterygium surgery: preliminary results

--Manuscript Draft--

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Abstract:	The aim of this study was to investigate the tolerability, safety and efficacy of lyophilized amniotic membrane (LAM) presentation for ocular use. A prospective case-series cohort of four patients with primary nasal pterygium which undergone excision and LAM implantation were evaluated for complications and clinical outcomes. Surgical manipulation of LAM was also assessed. LAM was stiff and easy to manipulate as well as no tearing occurred during surgery or suturing. Ocular comfort was checked and similar among those patients with LAM glued or sutured. After 12 months, there were no issues about tolerability or adverse events. Lower cosmetic outcomes (recurrence) was stated in 3 patients. Our study showed that LAM could be an effective alternative to cryopreserved amniotic membrane for graft after pterygium excision surgery. Its main advantage, storage at room temperature, can make it of immediate availability. Further studies comparing clinical outcomes of pterygium surgery with cryopreserved amniotic membrane versus LAM would confirm the benefits of the last.
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Lyophilized amniotic membrane graft for primary pterygium surgery: preliminary results

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Authors' contributions: Ricardo P Casaroli-Marano and Noelia Sabater-Cruz designed the work, Eva Martinez-Conesa and Noelia Sabater-Cruz acquired the data, all authors analysed and interpreted the data, Noelia Sabater-Cruz and Anna Vilarrodona drafted the work and all authors substantively revised it. All authors reviewed and approved the submitted version of the manuscript.

ABSTRACT

The aim of this study was to investigate the tolerability, safety and efficacy of lyophilized amniotic membrane (LAM) presentation for ocular use. A prospective case-series cohort of four patients with primary nasal pterygium which undergone excision and LAM implantation were evaluated for complications and clinical outcomes. Surgical manipulation of LAM was also assessed. LAM was stiff and easy to manipulate as well as no tearing occurred during surgery or suturing. Ocular comfort was checked and similar among those patients with LAM glued or sutured. After 12 months, there were no issues about tolerability or adverse events. Lower cosmetic outcomes (recurrence) was stated in 3 patients. Our study showed that LAM could be an effective alternative to cryopreserved amniotic membrane for graft after pterygium excision surgery. Its main advantage, storage at room temperature, can make it of immediate availability. Further studies comparing clinical outcomes of pterygium surgery with cryopreserved amniotic membrane versus LAM would confirm the benefits of the last.

Key words: Lyophilized amniotic membrane, cryopreserved amniotic membrane, eye bank; tissue bank; pterygium, recurrence

INTRODUCTION

Amniotic membrane (AM) is widely used in ophthalmology for conjunctival or ocular surface reconstruction: persistent epithelial defects, non-healing corneal ulcers, corneal perforations and descemetoceles, bullous keratopathy, limbal stem cell deficiency, corneoscleral melts and perforations, ocular surface squamous neoplasia, glaucoma surgery, and pterygium surgery (Jirsova and Jones 2017).

Pterygium can lead to ocular irritation so its management is initially symptomatic approach using ocular lubrication, despite surgical excision is eventually needed. Even though different techniques are appropriate for pterygium excision, cosmetic outcomes – recurrence rates - can vary depending on the technique. Despite pterygium excision with conjunctival autografting is currently considered the gold standard technique (Wang et al. 2017; Küçükerdönmez et al. 2007; Sabater-Cruz et al. 2020), AM grafts are an effective alternative to conjunctival autografts because of its anti-inflammatory properties and in those cases than conjunctiva must be preserved (Toker and Eraslan 2016). AM can be implanted fresh or be stored under various conditions (Jirsova and Jones 2017). Lyophilized amniotic membrane (LAM) is an innovative and optimized presentation, characterized by low water concentration (5-10%) (Jirsova and Jones 2017), which allows storage at room temperature and an easy-to-use availability for surgical application.

We carried out a preliminary study as proof of concept in order to investigate tolerability, safety and efficacy of LAM presentation as an alternative to cryopreserved AM (CAM) graft for ocular surgery - pterygium excision.

MATERIALS AND METHODS

This prospective case-series study was carried out at a single centre in Barcelona, Spain (*Hospital Universitari Sagrat* Cor). The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics Institutional Review Board approval was obtained (HCB/2018/1199).

We included patients who presented nasal primary pterygium and had surgery indication. Gender, age, best-corrected visual acuity (BCVA), intraocular pressure (IOP), pterygium morphology - atrophic, intermedium and fleshy - (Safi et al. 2016; Tan et al. 1997), extension (millimetres) of corneal invasion, and complications were recorded.

LAM was prepared in the Barcelona Tissue Bank (*Banc de Sang i Teixits*, Barcelona, Spain; https://www.bancsang.net/en_index/) under good manufacturing practices and according to previous approach (European Committee on Organ Transplantation and Keitel 2019).

Under peribulbar anaesthesia, pterygium head was pulled and subconjunctival tissue dissected. After measuring the bare scleral area, LAM (1 x 1 mm) was glued using fibrin sealant (Tisseel®, Baxter, Vienna, Austria) or sutured (8-0 braided absorbable suture) over the naked sclera. Eye occlusion was kept for a day, sutures were removed after two weeks, and a five-weeks postoperative treatment was used as described (Sabater-Cruz et al. 2020). Follow-up visits were performed at postoperative day 1, 14, month 3, 6, and 12. Ocular discomfort was graded (none/mild, moderate, and severe) preoperatively and at postoperative day 1, month 6, and month 12. Cosmetic outcomes were graded (1-4) as previous reported (Küçükerdönmez et al. 2007; Prabhasawat et al. 1997). A grade 4 indicated bad cosmetic outcome - pterygium recurrence.

RESULTS

Four patients (3 males) aged between 26 and 67 years, with primary nasal pterygium, were operated in January 2019. Surgical technique consisted in the pterygium excision and LAM implantation. LAM was previously rehydrated and washed several times in order to remove the lyoprotective solution and improve its manipulation during surgery (Figure 1). Routinely microbiological testing was performed at the time of surgery and were negative.

No infection, inflammation or granuloma was observed during the 12-months period. One patient presented high intraocular pressure at the second postoperative follow-up visit (day 14) which was treated with topical tafluprost 15mg/mL once at night until postoperative treatment ended.

Patient demographics, surgery characteristics, postoperative complications and outcomes are summarized in Table 1. Figure 2 shows LAM appearance shortly after implantation and ocular surface semblance after a year.

DISCUSSION

The objective of this study was to check LAM ocular tolerability, its easiness of manipulation during surgery, efficacy, and security. LAM was stiffer than the cryopreserved one and consequently easier to manipulate, no holes or rolling were observed during surgical manipulation. It could be glued or sutured without tearing it as in our case series. No complications were observed during follow-up period. Regarding to the patient with ocular hypertension, it could be explained by the postoperative treatment with topical steroids more than because of the AM presentation itself.

Tolerability and ocular comfort were examined: all patients pointed out a moderate or mild ocular discomfort 2 weeks after surgery and mild or no discomfort after a year. This discomfort is in agreement with the literature and is probably associated to the sutures because all sutured patients referred moderate discomfort two weeks after surgery while the fibrin glue ones reported none or mild discomfort. Moreover, 6 and 12 months after surgery, none of them had any - or only mild – discomfort; in line with previous reports (Wang et al. 2017). Regarding cosmetic outcomes, they were less encouraging because three of our patients with LAM (all males) had pterygium recurrence at 12 months follow-up without a clear association to age or gender. Pterygium recurrence rate after CAM implant has been described to be up to 42.3% at 6 months (Clearfield et al. 2017). Other complications related to AM and fibrin glue have been reported (graft dislocation, dellen, infection, bleeding, scleromalacia, and pyogenic granuloma) (Nguyen et al. 2019), but none of them were observed in our case series.

Main limitation of this study is the small number of cases and the absence of a control group to be compared with. In this study there are not enough cases to establish the effectiveness of the LAM versus other AM presentation types or even versus conjunctival autografting in terms of recurrence prevention.

LAM can be an effective option to CAM for the pterygium surgery. Its main advantage is the immediate availability and its storage at room temperature. Further studies comparing clinical outcomes with cryopreserved versus lyophilized AM in pterygium surgery would confirm the benefits of the second.

REFERENCES

Clearfield E, Hawkins BS, Kuo IC (2017) Conjunctival Autograft Versus Amniotic Membrane Transplantation for Treatment of Pterygium: Findings From a Cochrane Systematic Review. Am J Ophthalmol 182:8–17. https://doi.org/https://doi.org/10.1016/j.ajo.2017.07.004

European Committee on Organ Transplantation, Keitel S (2019) Guide to the quality and safety of tissues and cells for human application - EDQM 4th Edition, 4th edn. Strasbourg

Jirsova K, Jones GLA (2017) Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting — a review. Cell Tissue Bank 18:193–204. https://doi.org/10.1007/s10561-017-9618-5

Küçükerdönmez C, Akova YA, Altinörs DD (2007) Comparison of Conjunctival Autograft With Amniotic Membrane Transplantation for Pterygium Surgery. Cornea 26:407–413

Nguyen L, Wang Z, Molina J, et al (2019) Complications of fibrin glue in pterygium surgery with amniotic membrane transplant. Eye Sci 27:19–14. https://doi.org/10.3969/j.issn.1000-4432.2012.01.004.Complications

Prabhasawat P, Barton K, Burkett G, Tseng SC (1997) Comparison of Conjunctival Autografts, Amniotic Membrane Grafts , and Primary Closure for Pterygium Excision. Ophthalmology 104:974–985. https://doi.org/10.1016/S0161-6420(97)30197-3

Sabater-Cruz N, Dotti-Boada M, Ríos J, et al (2020) Postoperative treatment compliance rate and complications with two different protocols after pterygium excision and conjunctival autografting. Eur J Ophthalmol Online ahe: https://doi.org/10.1177/1120672120917335

Safi H, Kheirkhah A, Mahbod M, et al (2016) Correlations Between Histopathologic Changes and Clinical Features in Pterygia. J Ophthalmic Vis Res 11:153–158. https://doi.org/10.4103/2008-322x.183917

Tan DT, Chee SP, Dear KB, Lim AS (1997) Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. Arch Ophthalmol 115:1235–1240

Toker E, Eraslan M (2016) Recurrence After Primary Pterygium Excision: Amniotic Membrane Transplantation with Fibrin Glue Versus Conjunctival Autograft with Fibrin Glue. Curr Eye Res 41:1–8. https://doi.org/10.3109/02713683.2014.999947

Wang X, Zhang Y, Zhou L, et al (2017) Comparison of fibrin glue and Vicryl sutures in conjunctival autografting for pterygium surgery. Mol Vis 23:275–285

Figure and table legends

Table 1: Patient demographics, surgery characteristics, postoperative complications and outcomes.

Figure 1: Rehydration of lyophilized amniotic membrane (LAM) before the use. First, the amniotic membrane is washed several times (a); LAM is peeled from the carrier after rehydration, just before to use (b).

Figure 2: Postoperative image of patient number 1 one day after surgery (a) and 12 months after surgery (b).

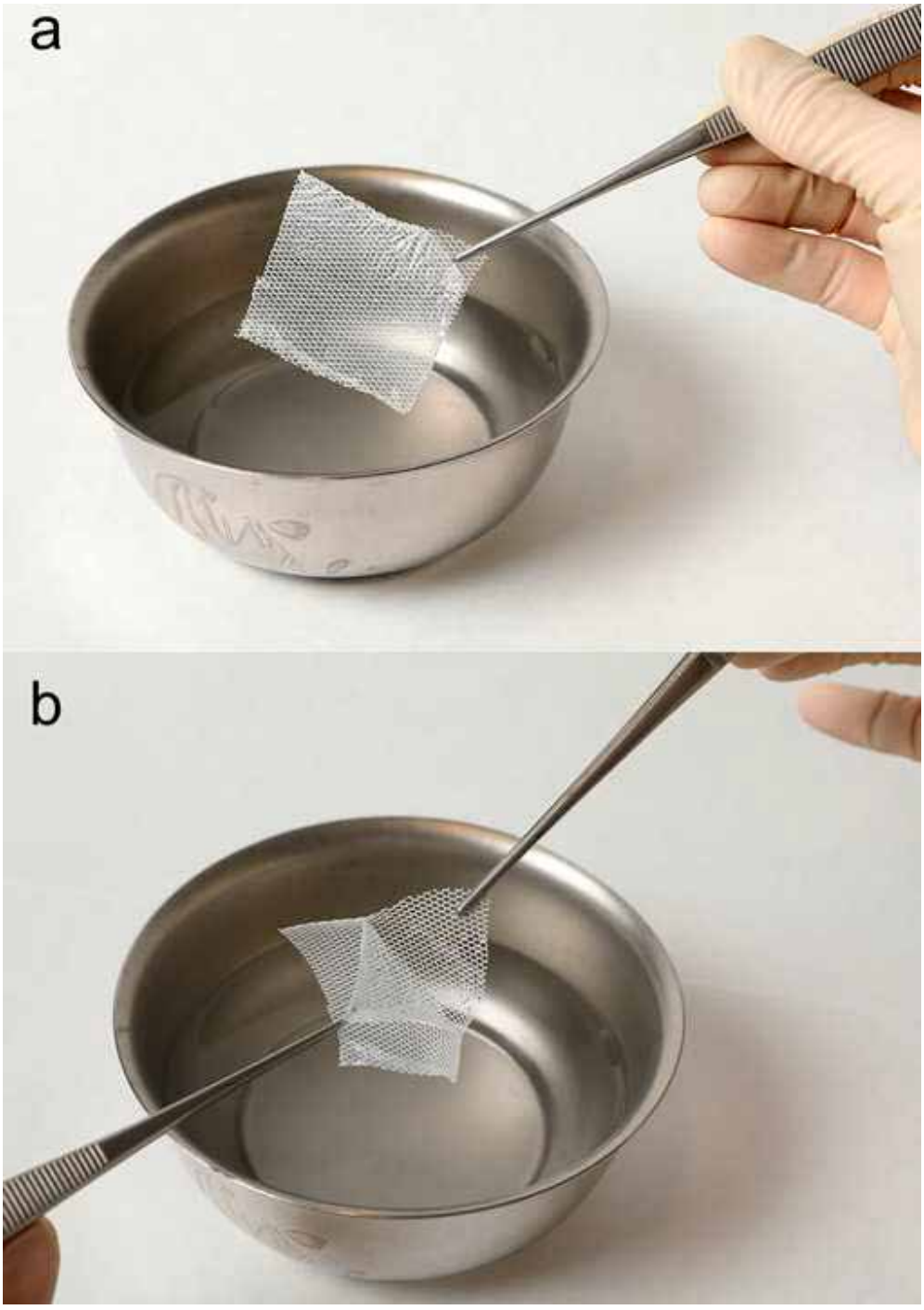

FIGURA 1

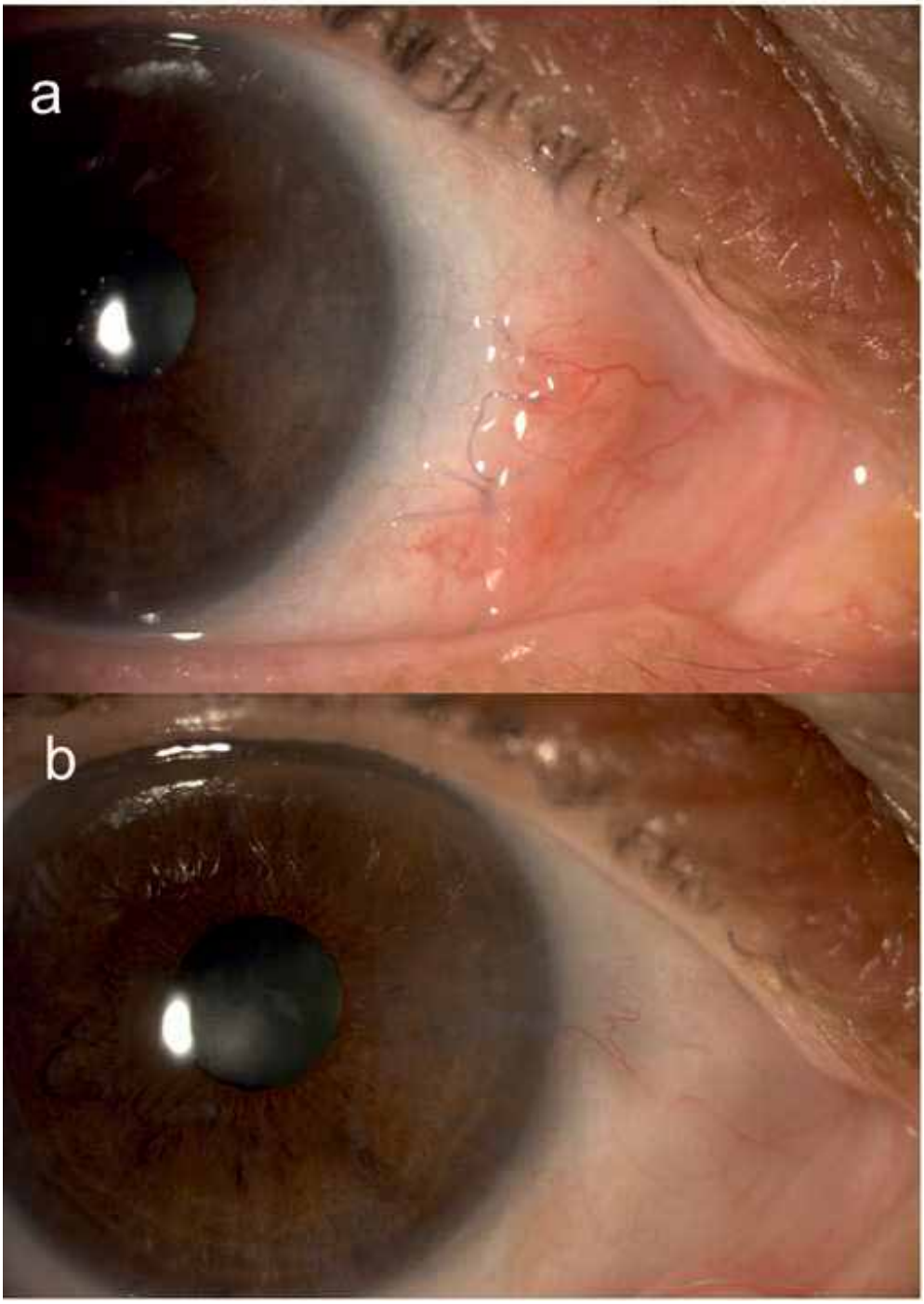

FIGURA 2

Table 1: Patient demographics, surgery characteristics, postoperative complications and outcomes.

patient	age	sex	eye	Corneal invasion; mm	Pterygium morphology	BCVA preop	BCVA month 12	IOP preop (mmHg)	IOP month 12 (mmHg)	Attachment method	Ocular discomfort			COG 12 months	postop complications
											preop	postop day 14	postop month 12		
1	67	F	R	2	FI	20/25	20/20	14	16	Sutures	none/mild	moderate	none/mild	1	none
2	26	M	R	1	I	25/20	25/20	15	16	Tisseel®	none/mild	none/mild	none/mild	4	OHT
3	31	M	R	1	FI	20/20	20/20	12	14	Sutures	none/mild	moderate	none/mild	4	none
4	42	M	R	3.5	FI	20/20	20/20	14	17	Tisseel®	none/mild	none/mild	none/mild	4	none

M: male; F: female; R: right; BCVA: best corrected visual acuity; OHT: ocular hypertension; FI: Fleshy; I: intermediate; Preop: preoperative, postop: postoperative, COG: cosmetic outcome grading.

Manuscrito 2

Objetivo:
Evaluar los resultados clínicos de los recientes formatos de la membrana amniótica disponibles en Cataluña – liofilizada y colirio de extracto de membrana amniótica – desde un punto de vista de validación y estudio en la práctica clínica.

Título:
Amniotic membrane extract eye drops: a new approach to severe ocular surface pathologies

Autores:
Pérez ML, Barreales Soto S, **Sabater-Cruz N**, Martinez-Conesa EM, Vilarrodona A, Casaroli-Marano RP, on behalf of the AMEED Research Group.

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Cell and Tissue Banking, R1

RESUMEN

Se ha desarrollado un protocolo para el procesamiento de la membrana amniótica como un extracto para ser rehidratado y administrado tópicamente como colirio ocular (colirio de extracto de membrana amniótica, *Amniotic Membrane Extract Eye Drops*, AMEED) para el tratamiento de patologías graves de la superficie ocular. Pacientes con patologías de la superficie ocular en los cuales el tratamiento convencional no es efectivo, podrían beneficiarse de las propiedades regenerativas y antiinflamatorias de la membrana amniótica, a través del AMEED y evitando procesos quirúrgicos.

Propósito: evaluar la seguridad y eficacia de la administración tópica de AMEED para patologías de la superficie ocular graves

Métodos: se evaluó de forma prospectiva la clínica ocular antes y después de la aplicación regular de AMEED durante al menos cuatro semanas en pacientes con enfermedades de la superficie ocular refractarios al tratamiento convencional. La eficacia y seguridad fueron evaluadas en base a los síntomas, medidas objetivables y notificaciones de reacciones adversas.

Resultados: se incluyeron treinta y seis ojos de veinticinco pacientes. A pesar de que la puntuación de la función de calidad visual, mediante el cuestionario VQF25, no fue estadísticamente significativa tras el tratamiento (p=0,4657), hubo una clara tendencia hacia la mejoría en los síntomas oculares y la presentación clínica de la patología. Los defectos epiteliales, desepitelizaciones y úlceras corneales mejoraron.

Conclusión: La aplicación tópica de AMEED ha mostrado ser segura, bien tolerada y efectiva para reducir los síntomas de la patología ocular grave. Estudios adicionales son necesarios para confirmar las mejores indicaciones del uso de AMEED.

Cell and Tissue Banking

Amniotic membrane extract eye drops: a new approach to severe ocular surface pathologies

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Abstract:	Background: a protocol for processing amniotic membrane as an extract to be re-hydrated and administered topically as eye drops (amniotic membrane extract eye drops, AMEED) has been developed. Safety and efficacy of AMEED was assessed in patients with severe ocular surface pathologies. Methods: prospective clinical follow-up of ocular surface symptoms before and after regular application of the AMEED for at least 4 weeks on patients with severe ocular surface disorders that were refractory to conventional treatment. Efficacy and tolerability were assessed based on patient-reported symptoms, objective measurements, and reports of adverse events. Results : thirty-six eyes from 25 patients were included. Although the visual quality function score, by means of a VQF25 questionnaire, was not statistically different after the treatment (p=0.4657), there was a clear trend towards the improvement in ocular symptoms and clinical presentation of the pathology. Corneal ulcer, de-epithelization and epithelial defect improved. Conclusions : topically applied AMEED proved to be safe, well tolerated and effective in reducing the symptoms and clinical signs of severe ocular disease. Further studies are needed to confirm the best indications for AMEED use.
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1.- Title page

Title: **Amniotic membrane extract eye drops: a new approach to severe ocular surface pathologies.**

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- Ethics approval: the ethics institutional review board (IRB) approval was obtained (CEIm Hospital Clinic de Barcelona; HCB/2015/0985).

- Consent to participate: all patients in the study were fully informed and gave their signed informed consent. In addition, all tissue samples come from donors who signed informed consent.
- Consent for publication: clinical data from patients was recorded after informed consent.
- Availability of data and material: due to the nature of this research, participants of this study did not agree for their data to be shared publicly, so supporting data is not available.
- Code availability: Not applicable.

- Authors' contributions:
  - AV: design of the study, validation of the protocol leading to the obtaining of the AMEED, responsible for biovigilance.
  - RCM: design of the study, supervising of the study; writer and reviewer manuscript.
  - MLP: set up and validation of the protocol for obtaining the AMEED; data collection and analysis, writer and reviewer manuscript.
  - SB: data collection, statistical analysis and interpretation.
  - NSC: patient recruitment and follow-up, critical review of the manuscript.
  - EMC: supervising eye drop production, critical review of the manuscript.

Acknowledgements

Does not apply

Abstract

Background: a protocol for processing amniotic membrane as an extract to be re-hydrated and administered topically as eye drops (amniotic membrane extract eye drops, AMEED) has been developed. Safety and efficacy of AMEED was assessed in patients with severe ocular surface pathologies.

Methods: prospective clinical follow-up of ocular surface symptoms before and after regular application of the AMEED for at least 4 weeks on patients with severe ocular surface disorders that were refractory to conventional treatment. Efficacy and tolerability were assessed based on patient-reported symptoms, objective measurements, and reports of adverse events.

Results: thirty-six eyes from 25 patients were included. Although the visual quality function score, by means of a VQF25 questionnaire, was not statistically different after the treatment (p=0.4657), there was a clear trend towards the improvement in ocular symptoms and clinical presentation of the pathology. Corneal ulcer, de-epithelization and epithelial defect improved.

Conclusions: topically applied AMEED proved to be safe, well tolerated and effective in reducing the symptoms and clinical signs of severe ocular disease. Further studies are needed to confirm the best indications for AMEED use.

Keywords**:** amnion, ophthalmic solutions, tissue bank, dry eye disease.

2.- Text

Introduction

Ocular surface disorders affecting the cornea, conjunctiva or related structures occur in a wide range of clinical conditions such as traumatisms, infection, inflammation, autoimmune and genetic diseases, or caused by tear deficiency, among others.

Tear biosubstitutes – such as several blood derivatives-- or amniotic membrane (AM) and limbal stem cell presentations have been proposed for the treatment of ocular surface disorders like dry eye disease (DED)(Rauz and Saw, 2010). The AM - the innermost layer of the placenta, composed of an epithelium, a basement membrane underneath and a rich stroma – contributes to promote re-epithelization, as well as to reduce inflammation and neovascularization(Kim, Shin and Kim, 2012) by means of properties attributed to cytokines, growth factors and other plasmatic proteins (Hopkinson *et al.*, 2006; Mamede *et al.*, 2012). Moreover, the AM gives structural support and has anti-microbial properties(Jirsova and Jones, 2017). Since tissue banks have developed safe and effective protocols for the procurement, processing and preservation of the AM, its clinical indications are increasing. Fresh, frozen, cryopreserved, and lyophilized patches can be prepared for AM transplantation; hence, new approaches arise looking for a new presentation that allows its use as eye drops. Compared to autologous serum (AS) or artificial tears, AM derivatives achieve promising results in wound healing on animal models of ocular chemical or mechanical injury(Shahriari *et al.*, 2008; Choi, Choi and Joo, 2011; Guo *et al.*, 2011). Preliminary results in patients with acute chemical burns (with or without limbic involvement) and the topical use of AM extracts show an ocular surface healing improvement(Liang *et al.*, 2009; Sheha *et al.*, 2010). In addition, corneal surface recovery has been observed in cases of persistent epithelial defects following the topical application of AM homogenates(Bonci, Bonci and Lia, 2005).

The present work analyses the safety and efficacy of AM extract eye drops (AMEED) for the treatment of severe ocular surface alterations.

Patients and Methods

This was a multicentric, interventional, prospective, comparative case-series study, designed to evaluate the safety and efficacy of AMEED in the treatment of patients with severe ocular surface pathology. The study was performed taking into consideration the requirements of the Guidelines for Good Clinical Practice in Clinical Trials and in accordance with the Declaration of Helsinki. The ethics institutional review board (IRB) approval was obtained (CEIm Hospital Clinic de Barcelona; HCB/2015/0985). All patients were fully informed and gave their signed informed consent.

AMEED preparation

Placentas were collected from elective caesarean delivery donors after informed consent; donors were serologically screened, samples for microbiological testing were taken during the collection and placentas were taken to the tissue bank where a specialised team processed the tissue following the requirements of the European Directives for Tissues and Cells. After rinsing the placenta to remove blood clots, a detachment of the AM by blunt dissection was performed. Then, the AM was rinsed and decontaminated in an antibiotic/anti-mycotic solution containing amphotericin B (Xalabarder Pharmacy, Barcelona, Spain), penicillin (ERN Laboratories, Barcelona, Spain) and streptomycin (Normon Laboratories, Madrid, Spain) in RPMI 1640 Medium (Corning, Manassas, USA). The following day, the AM was rinsed in saline solution to remove antibiotics and was frozen by immersion in liquid nitrogen. The frozen AM was ground (IKA, A11) and centrifuged at room temperature. The supernatant was put into aliquots of 1 mL fractions (vials), lyophilized (CoolVacuum, Barcelona, Spain) and stored at room temperature until use. Each vial with the powdered AMEED was packaged individually in a Tyvek® pouch (DuPont de Nemours Inc., Wilmington, USA) with the eye drop bottle. The final packaging included all material needed for the re-hydration of the

powder, which was a syringe, a needle, and a 10-mL bottle of sterile water. Before use, each vial of AMEED was reconstituted in 4 mL of sterilised water.

Study design and participants

Participants with severe ocular surface pathology and without a successful response to conventional treatment were included. Ocular surface was assessed at baseline and after treatment with topical application of AMEED. Patients were screened at five clinical centres in Barcelona. Inclusion criteria (Table S1; supplementary data) were patients aged 18 years and older, with a severe ocular surface pathology that was refractory to conventional treatment, including lubricant eye drops, autologous serum, and/or surgical AM transplantation. Exclusion criteria included current treatment with autologous serum derivatives and ocular infection.

AMEED were applied every two hours during the day, the container being discarded after 5 days. Each patient was asked to use the extract for at least 4 weeks, excepting remission of the pathology or adverse event and for no longer than 8 weeks. Patients were evaluated at baseline, day 7, 14, 30 and 60 by their ophthalmologist who fulfilled the information requested in the follow-up form about visual acuity and symptomatology.

Study assessments

The data collected were demographics, medical history including previous and current ocular treatment, diagnosis, clinical presentation, comorbidities, duration of treatment with AMEED and concomitant medications. Clinical signs (slit lamp staining, defect size, punctate epithelial erosions), ancillary tests such as the Schirmer test and tear break-up time (TBUT), subjective improvement (itching, foreign body sensation, tearing, pain, dryness, and general relief), the Visual Quality Function-25 (VQF-25) questionnaire, and adverse events were recorded as well. Seven parameters helped assess the ocular surface pathology: epithelial defect, de-epithelization, corneal ulcer, corneal Dellen, corneal opacity, conjunctivalization, neovascularization, and symblepharon. Epithelial defect was defined as a failure of re-epithelization, assumed as persistent when lasting

more than two weeks(Vaidyanathan *et al.*, 2019); de-epithelization was defined as a partially removed cornea epithelium. Finally, corneal ulcer was considered in the event of an open sore in the cornea. The monitoring method of the clinical outcomes and assessment were documented in a case report notebook (CRN) for each patient and visit. Clinical parameters (signs and symptoms) were evaluated according to standard criteria(Bron *et al.*, 2007).

Statistical analysis

The statistical test used to analyse the data about symptomatology before and after the instillation of AMEED was the McNemar test for paired data samples. This test assessed the difference between the percentage of patients with a presence of any symptom at the beginning of the study and the percentage of patients showing the same symptom at the end of the treatment. A positive result meant that the number of patients with that symptom at the end was lower than at the beginning. The statistical test used to analyse the quantitative parameters was a T-test for paired data. The change in the median number of symptoms was assessed. Finally, for the analysis of the quality of visual function, a coefficient was given for the initial and final evaluation; to assess the difference between VQF-25 values at the beginning and at the end, the statistical test used was a T-test for paired data. All statistical tests were performed with a two-sided type I error of 5% with the statistical package STATA v.15.1 (StataCorp, College Station, Texas, USA).

Results

Participants

Twenty-five patients from five recruiting centres met the eligibility criteria and completed the CRN for the analysis. A total number of 36 eyes of 25 patients were analysed. Sixty-one percent of patients recruited fulfilled at least four out of the seven inclusion criteria.

The mean age of the cohort was 65.96 ± 19.1 (range 28-92), 64% of the patients (n=16) were women; the females' mean age was 65.81 ± 19.0 (range 28-92), without differences with males (p=0.4806). The main therapeutic indications for AMEED were DED (n=13; 52%), severe lacrimal dysfunction (n=10, 40%), and persistent epithelial defect (n=6; 24%). Table 1 summarises the diagnosis, clinical characteristics and previous treatments of the patient cohort.

Efficacy assessments

Severity in ocular symptoms. The characteristics of each eye were registered with the presence/absence of foreign body sensation, itching, stinging, lacrimation, ocular pain, hyperemia and chemosis. Results showed a general positive tendency when AMEED was instilled in the eye. Improvements in the patient-reported symptoms were observed at each post-baseline visit, including the 4-week evaluation and the 8-week assessment. Foreign body sensation, itching and stinging had a statistically significant improvement ($p<0.05$) (Figure 1A). Considering the total number of ocular symptoms per patient – with 3.61 symptoms on average per patient -, statistically significant improvement was observed from day 0 to day 30 – 2.5 symptoms per patient on average, (p=0.0001) - and to day 60 – 2.44 symptoms per patient on average (p=0.0001).

Evaluation of the ocular surface. The presence or absence of ocular surface signs - epithelial defect, de-epithelization, corneal ulcer, corneal Dellen, corneal opacity, conjunctivalization, neovascularization, and symblepharon - was registered. It was a trend towards a clinically improvement between the initial and final ocular sign evaluation, although no statistical differences could be reported (Figure 1B and Figure 2).

TBUT showed a statistically significant improvement on day 60 (3.7 sec. *vs* 5.7 sec.; p=0.0034). Although the Schirmer test also showed an improvement, it did not reach statistical significance on day 30 (6.4 mm *vs* 7.7 mm; p=0.8269) or day 60 (6.4 mm *vs* 7.1 mm; p=0.8938).

The analysis of the visual function through the VQF-25 questionnaire showed an ocular self-perception with low scores both on day 0 and after the AMEED instillation with no statistical significance (p=0.4657).

Safety assessments

Based on the review of patients' medical records, one patient has decided to discontinue AMEED use because of some itching sensation. No adverse events were reported.

Discussion

In this study, we investigated the safety and efficacy of AMEED in a cohort of patients with a severe ocular surface pathology that was refractory to conventional treatment. The AM extract was prepared following the current regulatory framework for Tissues and Cells(Directive 2004/23/EC of the European Parliament and of the Council, 2004; Commission, The and Communities, 2006b, 2006a). Although the inclusion criteria of patients in the study were stringent, revealing a complex ocular status, 25 patients (36 eyes) from five centres were instilled with AMEED for at least four weeks and completed the CRN. The final global ocular symptomatology, signs and tests improved significantly. Although experimental studies on cell cultures and animals confirm the potential of preparations based on AM(Murri *et al.*, 2018), few clinical studies are performed with human AM derivatives. Previous studies(Liang *et al.*, 2009; Sheha *et al.*, 2010) concluded the efficacy of an AM extract in eyes with ocular chemical burns, and in the healing of corneal epithelial defect after acute chemical burn. Recently, a relief of symptoms as well as corneal epithelization in patients with ocular cicatricial diseases, treated with a combined solution of AM and umbilical cord tissues, has been reported(Cheng *et al.*, 2016). Furthermore, in a clinical trial conducted with a cohort of different ocular surface disease patients, a significant improvement of symptoms and epithelization after use of human AM homogenized frozen suspension was reported(Bonci, Bonci and Lia, 2005). In addition, the AM extract presented efficacy in

cases of neurotrophic keratopathy(Kordi} *et al.*, 2013) and more recently, a cryopreserved AM cytokine extract demonstrated beneficial results in DED(Yeu *et al.*, 2019). Our study, with a cohort of 25 patients composed mainly (92%) of patients with chronic DED that is refractory to conventional treatment, reports results of patients improving their foreign body sensation, itching and stinging symptomatology with a powdered extract of human AM prepared in a tissue establishment. Moreover, all patients with corneal ulcer (8%) showed complete epithelization after topical use of AMEED.

AM derivatives offer the advantages of their properties avoiding surgery for their implantation. However, there is no standardized way to obtain a unique product and different preparations like homogenate, frozen or lyophilized extracts, serum and fluids can be found in the literature(Murri *et al.*, 2018). This study presents the AMEED, a powdered extract prepared in a clean room environment, which is reconstituted by the patient before use and lasts for 5 days once reconstituted. The rationale underlying the proposed advantage of an AM extract over conventional treatment was the presence of growth factors in AM, mimicking the composition of natural tears, and its maintenance in AMEED (data not shown). Apart from collagen and proteoglycans, key molecules that have been identified in the amniotic tissue, including growth factors and cytokines with regenerative and anti-inflammatory properties(Mamede *et al.*, 2012). Manipulation conditions can affect the release of angiogenic and growth factors(Wolbank *et al.*, 2009) showing than pulverization achieve better factor release(Mahbod *et al.*, 2014) and lyophilisation process can cause greater loss of protein than cryopreservation(Rodríguez-Ares *et al.*, 2009). Considering these reports, an appropriate and adapted processing methodology let us achieve better results after the homogenization compared to freeze-drying as the molecules are released from the cell(Russo, Bonci and Bonci, 2012). Two main advantages come up with AMEED presentation over other preparations: one of them is its availability, as it is allogeneic.

The other is the long-term storage at room temperature, with no need for special logistical conditions.

Our results could be attributed to the biochemical properties present in AMEED (Imanishi *et al.*, 2000). which were proven similar in some growth factors and other molecules observed in autologous serum (AS)(Noble *et al.*, 2004; Jirsova *et al.*, 2014; Anitua *et al.*, 2015). To our knowledge, AS eye drops are not always available because patients must fulfil some requirements or may present drawbacks, like a positive serology test or an inability to have blood drawn(Geerling, MacLennan and Hartwig, 2004). The use of the AM extract opens up new horizons to an alternative treatment, as it is safe and effective in complex ocular surface pathology treatment.

Despite the satisfactory results, the limitations of the present study include the low correlation between symptoms and standard dry eye tests, differences reported previously by others(Semeraro *et al.*, 2016). In addition, the self-reported questionnaire raised problems in terms of the comprehension of the questions, the language and the impossibility to fulfil for a blinded patient as well as for an illiterate patient. The scores obtained on day 0 showed a poor visual function, but, although the symptomatology improved, the self-ocular-perception did not change over time. Maybe other questionnaires such as the faces scale or the ocular surface disease index (OSDI) score questionnaire would have been easier for patients to fulfil and made it easier to grade the pathology more clearly(Schiffman *et al.*, 2000). On the other hand, there are no reports on the effect and risks of prolonged use, for more than a year, of derivatives containing growth factors in the human ocular surface(Beylerian *et al.*, 2018), so further studies should be carried out in this way.

Finally, based on the absence of adverse events in the medical records evaluated in this study and the improvements observed in patient-reported symptoms, AMEED appears to be well tolerated by patients with DED and corneal ulcer. Although the precise effect of the AMEED in the treatment of DED is unknown, a combined effect is likely due to

biologically active agents that have been identified as present in the AM. Two main effects have been identified in this study: a regenerative effect in patients with corneal ulcer and anti-inflammatory effect in DED patients, leading to a symptomatic relief. A wide range of pathological conditions could benefit from AM properties through AMEED topical use, even among those patients for whom amniotic membrane transplantation did not succeed in cases of limbal stem cell deficiency(Paolin *et al.*, 2016). Moreover, in the light of the results of this assessment, patients suffering from other ocular surface pathologies may benefit from the AMEED as the first clinical option.

Conclusion

AMEED is safe and show a beneficial effect on epithelial healing, as well as proving to be a promising alternative for symptom relief in DED patients.

ANNEX 1. AMEED Research Group

Researchers are members of the AMEED Research Group and considered to be the co-authors of the present study.

- Miriam Ferrán Fuertes and Diana Mora Ramírez, Hospital General de L'Hospitalet (L'Hospitalet de Llobregat, Spain) (patient recruitment and follow-up);
- Oscar Gris Castellón and Miriam Barbany Rodríguez, Instituto de Microcirugía Ocular (Barcelona, Spain) (patient recruitment and follow-up);
- Carlos Luis Moser Wurth and Mónica Lecumberri López, Hospital de Sant Joan Despí Moisès Broggi (Sant Joan Despí, Spain) (patient recruitment and follow-up);
- Sara Martín Nalda and Laia Bisbe López, Hospital Universitario Valle Hebrón (Barcelona, Spain) (patient recruitment and follow-up);
- Maite Sáinz de la Maza, Noelia Sabater-Cruz and Josep Torras Sanvicens, Hospital Clínic i Provincial de Barcelona (Barcelona, Spain) (patient recruitment and follow-up).

3.- References

Anitua, E. et al. (2015) 'Autologous serum and plasma rich in growth factors in ophthalmology: Preclinical and clinical studies', Acta Ophthalmologica, 93(8), pp. e605–e614. doi: 10.1111/aos.12710.

Beylerian, M. et al. (2018) 'Collyres de sérum autologue : traitement à long-terme dans le syndrome sec oculaire', Journal Francais d'Ophtalmologie. Elsevier Masson SAS, 41(3), pp. 246–254. doi: 10.1016/j.jfo.2017.11.008.

Bonci, Po, Bonci, Paola and Lia, A. (2005) 'Suspension made with amniotic membrane: Clinical trial', European Journal of Ophthalmology, 15(4), pp. 441–445. doi: 10.1177/112067210501500403.

Bron, A. J. et al. (2007) 'Methodologies to diagnose and monitor dry eye disease: Report of the diagnostic methodology subcommittee of the international Dry Eye Workshop (2007)', Ocular Surface, 5(2), pp. 108–152. doi: 10.1016/s1542-0124(12)70083-6.

Cheng, A. M. S. et al. (2016) 'Morselized amniotic membrane tissue for refractory corneal epithelial defects in cicatricial ocular surface diseases', Translational Vision Science and Technology, 5(3). doi: 10.1167/tvst.5.3.9.

Choi, J. A., Choi, J.-S. and Joo, C.-K. (2011) 'Effects of amniotic membrane suspension in the rat alkali burn model.', Molecular vision, 17(February), pp. 404–412.

Commission, T. H. E., The, O. F. and Communities, E. (2006a) 'L 294/32', pp. 32–50.

Commission, T. H. E., The, O. F. and Communities, E. (2006b) 'L 38/40', (d), pp. 40–52.

Directive 2004/23/EC of the European Parliament and of the Council (2004) 'EU Direcitve on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells', Official Journal of the European Union, L102, pp. 48–58.

Geerling, G., MacLennan, S. and Hartwig, D. (2004) 'Autologous serum eye drops for ocular surface disorders', British Journal of Ophthalmology, 88(11), pp. 1467–1474. doi: 10.1136/bjo.2004.044347.

Guo, Q. et al. (2011) 'A comparison of the effectiveness between amniotic membrane homogenate and transplanted amniotic membrane in healing corneal damage in a rabbit model', Acta Ophthalmologica, 89(4), pp. 315–319. doi: 10.1111/j.1755-3768.2010.02097.x.

Hopkinson, A. et al. (2006) 'Proteomic analysis of amniotic membrane prepared for human transplantation: Characterization of proteins and clinical implications', Journal of Proteome Research, 5(9), pp. 2226–2235. doi: 10.1021/pr050425q.

Imanishi, J. et al. (2000) 'Growth factors: Importance in wound healing and maintenance of transparency of the cornea', Progress in Retinal and Eye Research, 19(1), pp. 113–129. doi: 10.1016/S1350-9462(99)00007-5.

Jirsova, K. et al. (2014) 'The application of autologous serum eye drops in severe dry eye patients; subjective and objective parameters before and after treatment', Current Eye Research, 39(1), pp. 21–30. doi: 10.3109/02713683.2013.824987.

Jirsova, K. and Jones, G. L. A. (2017) 'Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review', Cell and Tissue Banking. Springer Netherlands, 18(2), pp. 193–204. doi: 10.1007/s10561-017-9618-5.

Kim, K. M., Shin, Y. T. and Kim, H. K. (2012) 'Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis', Japanese Journal of Ophthalmology, 56(6), pp. 544–550. doi: 10.1007/s10384-012-0175-y.

Kordi}, R. et al. (2013) 'Application of the Amniotic Membrane Extract (AMX) for the Persistent Epithelial Defect (PED) of the Cornea', Coll. Antropol, 37(1), pp. 161–

164.
Liang, L. et al. (2009) 'Amniotic membrane extraction solution for ocular chemical burns', Clinical and Experimental Ophthalmology, 37, pp. 855–863. doi: 10.1111/j.1442-9071.2009.02159.x.
Mahbod, M. et al. (2014) 'Amniotic Membrane Extract Preparation: What is the Best Method?', Journal of Ophthalmic and Vision Research, 9(3), pp. 314–319. doi: 10.4103/2008-322X.143367.
Mamede, A. C. et al. (2012) 'Amniotic membrane : from structure and functions to clinical applications', Cell Tissue Research, 349(2), pp. 447–458. doi: 10.1007/s00441-012-1424-6.
Murri, M. S. et al. (2018) 'Amniotic membrane extract and eye drops: A review of literature and clinical application', Clinical Ophthalmology, 12, pp. 1105–1112. doi: 10.2147/OPTH.S165553.
Noble, B. A. et al. (2004) 'Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease', British Journal of Ophthalmology, 88(5), pp. 647–652. doi: 10.1136/bjo.2003.026211.
Paolin, A. et al. (2016) 'Amniotic membranes in ophthalmology : long term data on transplantation outcomes', Cell and Tissue Banking. Springer Netherlands, 17(1), pp. 51–58. doi: 10.1007/s10561-015-9520-y.
Rauz, S. and Saw, V. P. (2010) 'Serum eye drops, amniotic membrane and limbal epithelial stem cells-tools in the treatment of ocular surface disease', Cell and Tissue Banking, 11, pp. 13–27. doi: 10.1007/s10561-009-9128-1.
Rodríguez-Ares, M. T. et al. (2009) 'Effects of lyophilization on human amniotic membrane', Acta Ophthalmologica, 87(4), pp. 396–403. doi: 10.1111/j.1755-3768.2008.01261.x.
Russo, A., Bonci, Paola and Bonci, Paolo (2012) 'The effects of different preservation processes on the total protein and growth factor content in a new biological product developed from human amniotic membrane', Cell and Tissue Bank, 13, p. 353. doi: 10.1007/s10561-011-9261-5.
Schiffman, R. M. et al. (2000) 'Reliability and validity of the ocular surface disease index', Archives of Ophthalmology, 118(5), pp. 615–621. doi: 10.1001/archopht.118.5.615.
Semeraro, F. et al. (2016) 'Effect of autologous serum eye drops in patients with Sjögren syndrome-related dry eye: Clinical and In Vivo confocal microscopy evaluation of the ocular surface', In Vivo, 30(6), pp. 931–938. doi: 10.21873/invivo.11016.
Shahriari, H. A. et al. (2008) 'Comparison of the effect of amniotic membrane suspension and autologous serum on alkaline corneal epithelial wound healing in the rabbit model', Cornea, 27(10), pp. 1148–1150. doi: 10.1097/ICO.0b013e318173138a\r00003226-200812000-00010 [pii].
Sheha, H. et al. (2010) 'Amniotic membrane extract for acute ocular chemical burns', Techniques in Ophthalmology, 8(4), pp. 146–150. doi: 10.1097/ITO.0b013e318203feba.
Vaidyanathan, U. et al. (2019) 'Persistent Corneal Epithelial Defects: A Review Article.', Medical hypothesis, discovery & innovation ophthalmology journal, 8(3), pp. 163–176.
Wolbank, S. et al. (2009) 'Impact of human amniotic membrane preparation on release of angiogenic factors', Journal of Tissue Engineering and Regenerative Medicine, 3, pp. 651–654. doi: 10.1002/term.207.
Yeu, E. et al. (2019) 'Safety and efficacy of amniotic cytokine extract in the treatment of dry eye disease', Clinical Ophthalmology, 13, pp. 887–894. doi: 10.2147/OPTH.S203510.

4.- Figures and Tables legends

Fig. 1. Symptoms and ocular surface characteristics at baseline and after AMEED. (A) Ocular symptoms evolution expressed in terms of percentage of eyes affected from baseline to day 60. A significant improvement was observed in three main parameters related to DED: foreign body sensation, itching and stinging. (B) Corneal alterations expressed in terms of percentage of eyes affected over time from baseline to day 60. There was an improvement trend in the main parameters related to corneal surface: epithelial defect, de-epithelization and ulcer. *, $p<0.05$.

Fig. 2. AMEED for neurotrophic ulcer. An improvement of corneal healing and epithelization was observed (patient 17). An efficacy assessment was performed through the measurement of the corneal defect during treatment.

TABLE LEGENDS

Table 1. Clinical cohort characteristics at the baseline

SUPPLEMENTARY DATA

Table S1. Entry criteria for cohort.

5.- Figures and Tables

Figure 1.

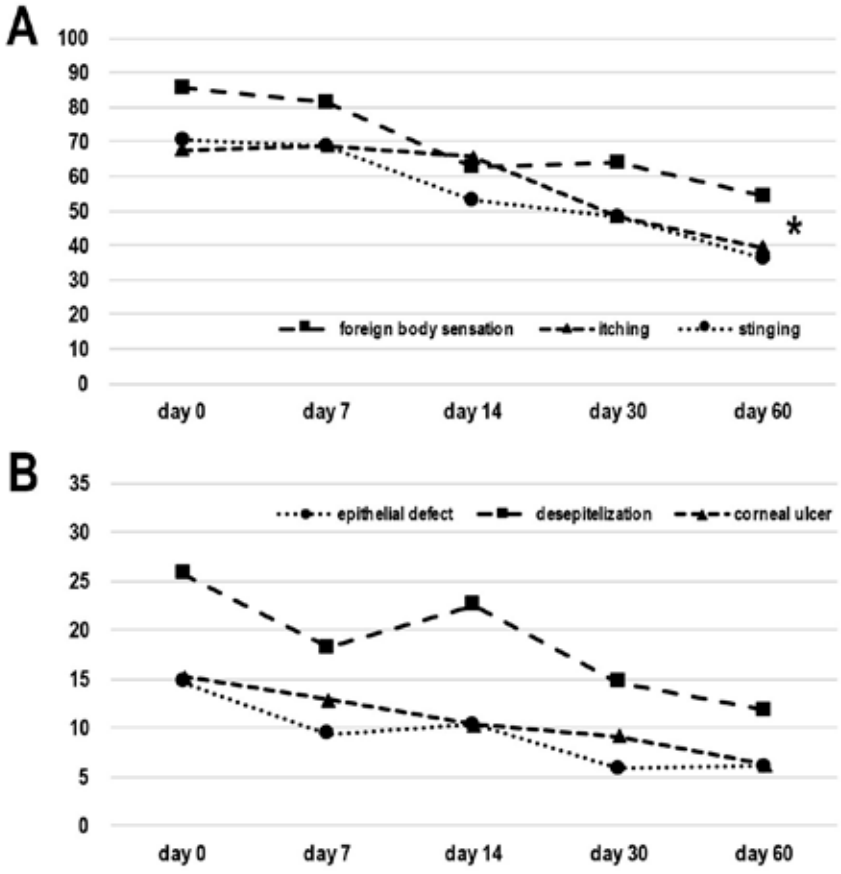

Figure 2.

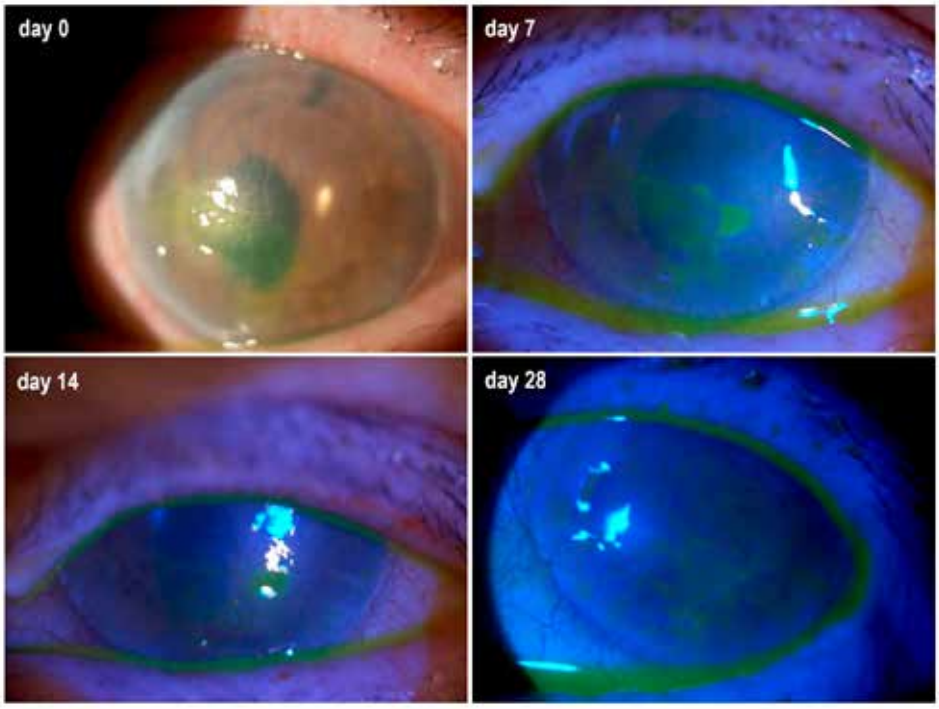

Table 1. Clinical cohort characteristics at the baseline

Patient	Sex	Age	Eye	Diagnosis	Conventional treatment at baseline
1	F	59	BE	DED, Sjogren´s syndrome	AT, AS, cyclosporine
2	F	72	BE	LSCD, Sjogren´s syndrome	AT, AS
3	M	85	LE	LSCD, DED	AT, AS
4	M	55	BE	DED	not reported
5	M	53	BE	LSCD, DED	AT, AS, steroids, tetracycline
6	F	55	BE	Severe DED	AT
7	F	47	LE	Neurotrophic keratitis	AT, AS
8	F	78	BE	Severe DED	AT, AS
9	F	86	BE	DED, Sjogren´s syndrome	AT, cyclosporine, steroids
10	F	79	LE	DED, filamentary keratitis	AT, AS
11	F	85	BE	PED	AT
12	F	63	LE	DED, Lyell´s syndrome	AS
13	F	80	LE	PED	AT
14	F	32	BE	PED, Sjogren´s syndrome	AT, cyclosporine
15	F	67	RE	Filamentary keratitis, Sjogren´s syndrome	AT, AS
16	M	51	LE	DED, filamentary keratitis	AT, cyclosporine, steroids
17	F	28	LE	Neurotrophic ulcer	AS, AMT
18	M	91	LE	PED	AT, AS
19	M	32	LE	PED	therapeutic corneal lenses, AS
20	M	76	LE	PED	therapeutic corneal lenses, AS
21	M	90	LE	DED	AT
22	F	53	LE	DED, Sjogren´s syndrome	AS
23	F	92	LE	Neurotrophic ulcer	AT, AMT
24	F	77	BE	LSCD, persistent keratitis	AT
25	M	63	BE	DED	AT, AS

F, female; M, male; LE, left eye; RE, right eye; BE, both eyes; AS, autologous serum; AT, artificial tears; AMT,
DED, dry eye disease; LSCD, limbal stem cell defficiency; PED, persitent epitehlial defect

6.- Supporting information

Table S1. Entry criteria for patients.

Inclusion criteria	Exclusion criteria
Severe DED primary or secondary to systemic and autoimmune diseases	Successful response to conventional treatment with tear substitutes, AS or PRP
LSCD secondary to external trauma (chemical acid or alkali burns, thermal burns)	Ocular surface alterations secondary to a previous or current infectious process
Neurotrophic ulcers	Non-compliance to the study protocol
No response to tear substitutes with or without adjuvants	Patients under 18 years-old
No response or impossibility to AS or PRP use	

DED, dry eye disease; LSCD, limbal stem cell deficiency; AS, autologous serum; PRP, platelet rich plasma

Artículo 5

Objetivo:
Evaluar los resultados clínicos de los recientes formatos de la membrana amniótica disponibles en Cataluña – liofilizada y colirio de extracto de membrana amniótica – desde un punto de vista de validación y estudio en la práctica clínica.

Título:
Amniotic membrane extract eye drops for ocular surface diseases: use and clinical outcome in real-world practice

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Cuartil y área de conocimiento:
Q4 *Ophthalmology* (JCR)
Q2 *Ophthalmology* (Scimago)

RESUMEN

Propósito: estudiar las indicaciones y resultados clínicos, en un entorno de vida real, del uso de colirio de extracto de membrana amniótica (*Amniotic Membrane Extract Eye Drops*, AMEED) para el tratamiento de patologías de la superficie ocular.

Métodos: se llevó a cabo un estudio retrospectivo de pacientes tratados con AMEED tópico entre enero de 2018 y enero de 2020. Los pacientes fueron clasificados en dos grupos en función del tipo de patología de la superficie ocular que tuvieran enfermedad de ojo seco (*dry eye disease*; grupo DED) o retraso en el cierre epitelial (*wound healing delay*; grupo WHD). Se analizaron datos demográficos, comorbilidades, duración del tratamiento y resultados clínicos.

Resultados: se incluyó un total de cincuenta ojos de treinta y seis pacientes con o sin tratamiento previo. Los pacientes del grupo DED presentaron más comorbilidades sistémicas (83% vs 22%; p<0,001) y estuvieron más tiempo en tratamiento con AMEED (10 vs 7,2 meses de media) que los del grupo WHD (p=0,0104). En cuatro pacientes, se comunicó un tratamiento por largo tiempo (más de 24 meses). Se halló una mejoría sintomática global en ambos grupos (DED 88,9% vs WHD 100%; p=0,486). En el grupo WHD se notificó preferentemente alivio general (78%) y en el grupo DED mejoría del dolor (44%) (p=0,011). En relación a los pacientes con tratamiento previo con suero autólogo, no se encontraron diferencias estadísticas en términos de mejoría subjetiva u objetiva. Se consiguió una mejoría general en el 94,4% de los casos y no se notificaron reacciones adversas.

Conclusión: el AMEED es un tratamiento prometedor para mejorar las patologías de la superficie ocular, tales como ojo seco, defecto epitelial persistente y úlceras corneales. A pesar de que el AMEED puede ser efectivo en el tratamiento del ojo seco severo, de las úlceras corneales y de los defectos epiteliales persistentes, las conclusiones son limitadas por la ausencia de ensayos clínicos controlados.

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ORIGINAL PAPER

Amniotic membrane extract eye drops for ocular surface diseases: use and clinical outcome in real-world practice

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Abstract

Purpose To study the indications and clinical outcomes, in a real-word setting, of amniotic membrane extract eye drops (AMEED) use for ocular surface disease (OSD).

Methods A retrospective study of patients treated with topical AMEED between January 2018 and January 2020 was conducted. Patients were classified in two groups according to specific OSD—dry eye disease (DED) and wound healing delay (WHD) groups. Demographics, comorbidities, treatment duration and clinical outcomes were analysed.

Results A total of 50 eyes of 36 patients with or without previous treatments were included. Patients in the DED group presented more systemic comorbidities (83 vs 22%; $p < 0.001$) and spent more mean time under AMEED treatment (10 vs 7.2 months average) than the WHD group ($p = 0.0104$). In four patients, long-term treatment (more than 24 months) was reported. Global similar symptomatic improvement

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was reported for both groups (DED 88.9% vs WHD 100%; $p = 0.486$), with the WHD group especially consisting in general relief (78%) and the DED group reporting more pain improvement (44%) ($p = 0.011$). Regarding patients with autologous serum as a previous treatment, no statistical differences were found in subjective or objective improvement. An overall success was achieved in 94.4% of the cases and no adverse events were found.

Conclusion AMEED administration is a promising mean to treat OSD such as dry eye, persistent epithelial defect and corneal ulcers. Although AMEED may be effective in the treatment of severe DED and persistent epithelial defect or corneal ulcers, conclusions are limited owing to the absence of controlled clinical trials.

Keywords Amniotic membrane extract eye drops (AMEED) · Persistent epithelial defect · Ocular surface disease · Dry eye disease · Tissue bank

Introduction

Tear supplementation with ocular lubricants—often termed artificial tears (AT)—is traditionally considered a mainstay of dry eye disease and other ocular surface diseases therapy and thus there are numerous topical formulations available [1]. Nonetheless, biological tear substitutes—blood derived drops and amniotic membrane extract—are increasingly been used [2, 3].

The amniotic membrane (AM)—the innermost layer of the foetal membranes—is known for its lack of immunogenicity behaving as a substrate for growth, migration and adhesion of epithelial corneal and conjunctival cells [4–6]. Since its introduction for ocular use in the 1940s, AM has gained popularity as an ocular surface disease treatment due its anti-inflammatory, anti-angiogenic, immunomodulatory, anti-scarring, and hemocompatibility properties [3–8]. AM presents a high content of foetal hyaluronic acid in its stromal matrix, involved in the signalling mechanisms of some growth factors, as transforming growth factor β (TGF-β), which regulates, in part, the proliferation of fibroblasts on the ocular surface. The differentiation of these into myofibroblasts is therefore inhibited, which reduces the formation of scar tissue through an anti-fibrotic effect. Likewise, the properties of AM have been seen in the inhibition of the expression of some pro-inflammatory interleukins (IL), such as IL-1, IL-2, as well as interferon-gamma (INF-γ) and tumour necrosis factor (TNF). In addition to the anti-inflammatory properties that also retard new-vessels formation, the anti-angiogenic effect of AM has been observed by stimulating the production of thrombospondin-1 and tissue inhibitors of metalloproteinase (TIMP-1–4), among others [4–6].

Several presentations of AM are currently available: cryopreserved, lyophilized or dried, commercially placed in a bandage contact lens, and as amniotic membrane extract eye drops [3, 5, 9]. Amniotic membrane extract eye drops (AMEED) have been used both experimentally [10, 11] and clinically for chemical burns [12], delayed epithelialization [13], neurotrophic corneal ulcers [13], and, as a whole, dry eye disease (DED) [14].

The aim of this study is to evaluate the indications, uses and clinical outcomes of AMEED treatment in different ocular surface diseases in the real-world practice.

Materials and methods

This was a retrospective analysis study carried out in 12 ophthalmological clinical centres in Spain. The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics institutional review board (IRB) approval was obtained (CEIm Hospital Clinic de Barcelona; HCB/2015/0985). All data were recorded in such a manner that subjects could not be identified, directly or through identifiers linked to their records; thus, consent requirement was waived by the IRB.

AMEED preparation started with the collection of placentas from elective caesarean delivery donors after informed consent; donors were serologically screened, samples for microbiological testing were taken during the collection, and placentas were taken to the tissue bank where a specialized team processed the tissue following the requirements of the European Directives for Tissues and Cells (Regulation EC (No). 1394/2007). After rinsing the placenta to remove blood clots, a detachment of the AM by blunt dissection was performed. Then, the AM was rinsed and decontaminated in an antibiotic/anti-mycotic

solution: 10ug/mL amphotericin B (Xalabarder Pharmacy, Barcelona, Spain), 100 U/mL penicillin (ERN Laboratories, Barcelona, Spain), 2 mg/mL streptomycin (Normon Laboratories, Madrid, Spain), in RPMI 1640 Medium (Corning, Manassas, USA). The following day, the AM was rinsed in saline solution to remove antibiotics and was frozen by immersion in liquid nitrogen. The frozen AM was then ground (IKA, A11) and centrifuged at 4500 rpm room temperature. The supernatant was put into aliquots of 1 mL fractions (vials), lyophilized (CoolVacuum, Barcelona, Spain) and stored at room temperature until use. Each vial with the powdered AMEED was packaged individually in a Tyvek® pouch (DuPont de Nemours Inc., Wilmington, USA) with the eye drop bottle. The final packaging included all material needed for the re-hydration of the powder, which was a syringe, a needle, and a 10-mL bottle of sterile water. Before use, each vial of AMEED was reconstituted in 4 mL of sterilized water and discarded after 15 days. Inclusion criteria consisted of subjects aged 18 years and older who had different corneal surface diseases such as moderate-to-severe dry eye disease (DED), grades III–V, defined by Oxford staining score [15], persistent epithelial defect—persisting more than 2 weeks without improvement despite conventional treatment—[16], and corneal ulcer, lasting more than two weeks. Patients were classified into two main pathological groups as per subgroup analysis: DED and wound healing delay (WHD) groups.

Data collected from a follow-up questionnaire sent at the end of the treatment or the end of the study period were demographics, medical history, diagnosis, clinical presentation, comorbidities, duration and frequency of treatment with AMEED, objective improvement (slit lamp staining—Oxford staining score, defect size, punctate epithelial erosions), subjective improvement (itching—yes/no, foreign body sensation—yes/no/improvement-, tearing—yes/no -, pain– yes/no -, dryness—yes/no/improvement -, general relief—yes/no-) and adverse events. Overall success was defined by the treating clinician on the basis of a treatment achievement effect including epithelial defect closure.

AMEED was indicated under medical criteria 3–6 times a day for a minimum of a month. Follow-up visits were planned as per common practice given the observational nature of this real-world study. End treatment results were compared to baseline.

Descriptive results are presented as mean and standard deviation (SD) for quantitative variables and absolute frequencies and percentages for categorical variables. The Shapiro–Wilk test was used to assess the normality of the distributions. T-test for independent groups for quantitative variables (or Mann–Whitney test for nonparametric ones) and Chi-squared tests or Fisher's exact test for categorical data were used. All statistical tests were performed with a two-sided type I error of 5% with the statistical package STATA v.15.1 (StataCorp, College Station, Texas, USA).

Results

A total of 50 eyes from 36 patients—24 (66.7%) female between January 2018 and January 2020 from 12 centres—were included. Indications for AMEED were classified into two groups—DED and WHD, which are detailed in Table 1. Differences in demographics, bilateral presentation, systemic comorbidities, previous treatments, and time on AMEED treatment are detailed in Table 2.

DED group was more frequently present as a bilateral disease (66.7%) and with systemic comorbidities (83%) than the WHD group (11.1% and 22%, respectively). Only two patients (both with persistent epithelial defect) had no previous treatments, while 15 patients (41.6%) were receiving treatment with

Table 1 Ocular surface diseases treated with amniotic membrane extract eye drops (AMEED)

	Patients (*n*)	Eyes (*n*)
DED group		
Severe DED	15	25
Moderate DED	3	5
WHD group		
Neurotrophic ulcer	5	5
Other corneal ulcers	4	4
Non-specified keratitis	4	4
Persistent epithelial defect	3	3
Limbal stem cell deficiency	2	4
Total	36	50

DED dry eye disease, *WHD* wound healing disease, *n* number of patients or eyes

Table 2 Characteristics, systemic comorbidity, previous treatments, and comparative between dry eye disease (DED) and wound healing disease (WHD) groups

	Overall (n = 36)	DED (n = 18)	WHD (n = 18)	p-value
Bilateral disease, %	38.9	66.7	11.1	$p = 0.002$[a]
Age (years), MD ± SD (range)	63.1 ± 19.7 (5–98)	62.9 ± 17.8 (31–98)	63.3 ± 21.9 (5–89)	$p = 0.947$[b]
Systemic comorbidity, n (%)	19, (53)	15, (83)	4, (22)	$p < 0.001$[a]
Sjögren syndrome, n	8	7	1	
Rheumatoid arthritis, n	4	4	0	
Donor-vs-host disease, n	2	2	0	
Stevens-Johnson syndrome, n	1	1	0	
Systemic lupus erythematosus, n	1	0	1	
Chronic immunosuppression, n	2	0	2	
Diabetes mellitus, n	1	1	0	
Previous treatments, n	34	18	16	$p = 0.109$[a]
AS alone, n	2	0	2	
AS + AT, n	13	10	3	
AT alone, n	4	1	3	
AT + topical NSAIDs or steroids, n	6	3	3	
AT + topical cyclosporine A, n	2	2	0	
AM transplantation, n	1	0	1	
Non-specified, n	6	2	4	

DED dry eye disease, *WHD* wound healing disease, *MD* mean, *SD* standard deviation, *AT* artificial tear, *AS* autologous serum, *AM* amniotic membrane, *NSAID* nonsteroidal anti-inflammatory drugs, *n* number of patients or eyes

[a]Chi-squared test (or Fisher's exact test); [b]Independent samples *T*-test

autologous serum combined with artificial tears (n = 13) or alone (n = 2) prior to AMEED. Other previous treatments reported are detailed in Table 2.

Average median time on AMEED treatment was 2.4 months (range 1–28.0), presenting a statistical difference (p = 0.0104) between AMEED treatment time in DED group (median 6.5 months, range 1–28.0) versus WHD group (median 4.2 months, range 1–23.3). Four patients (3 with severe DED and 1 with limbal stem cell deficiency) kept using AMEED after 24 months. Global symptomatic, objective and overall improvement was similar between groups (Table 3). Specific symptoms improvement significantly differ between groups (p = 0.011): WHD group related especially general relief (78%), while in DED group pain relief was the most improved symptom (44%). No adverse reactions were reported.

Discussion

Several blood derivatives and AM formulations (globally called biological tear substitutes) [17] have been proposed for the treatment of various ocular diseases [18]. AMEED has the potential for increased clinical use in corneal surface disorders [3]. This study investigated the clinical outcomes of AMEED in real-life practice. Given the intrinsic difficulties of chronic treatments in the real world for highly assistance-demanding diseases, such as DED and WHD, observational reports are of great interest.

The therapeutic effect of AM (AMEED) could be attributed to multiple mechanisms of action according to current research. It can act by controlling ocular surface inflammation, critical for the pathogenesis and chronicity of DED [9, 19]. On the other hand, AM has been reported to provide the ability to regenerate corneal nerves [20] through the nerve growth factor (NGF) present in this tissue [8, 9, 21], while other authors were unable to find it [22]. In our series, all

Table 3 Symptomatic and objective improvement comparative between dry eye disease (DED) and wound healing disease (WHD) groups

	Overall (%)	DED (%)	WHD (%)	*p*-value
Symptomatic improvement	94.4	88.9	100	$p = 0.486$[a]
Main improved symptom distribution				$p = 0.011$[a]
General relief	58.8	37.5	77.8	
Itching	8.8	12.5	5.6	
Foreign body sensation	2.9	0	5.6	
Tearing	2.9	0	5.6	
Pain	23.5	43.7	5.6	
Dryness	2.9	5.6	0	
Objective improvement	86.1	83.3	88.9	$p = 1.000$[a]
Main objective finding				$p = 0.067$[a]
Epithelial defect persistence	2.8	0	5.6	
Epithelial defect closure	11.1	0	22.2	
Punctate epithelial erosion improvement	75	83.3	66.7	
Punctate epithelial erosion non-improved	5.6	11.1	0	

DED dry eye disease, *WHD* wound healing disease

[a]Chi-squared test (or Fisher's exact test)

patients with WHD had an overall success—a complete closure of the epithelial defect in corneal ulcers and PED—so the AMEED can be potentially used as an alternative to AM transplant avoiding surgical procedures and allowing a continued treatment until healing occurs [3]. Further, we observed that almost half of AM transplant indications in Catalonia (Spain) were corneal ulcer (26.9%) and PED (22.7%) [23]. Therefore, it is foreseeable than AMEED use trends will increase in the future.

Regarding the comorbidities report, half of the patients in this report presented autoimmune diseases. Some lead to pro-immune and pro-inflammatory elements circulating in peripheral blood than can end up in the autologous serum and hence limit its effectiveness [24–26]. Due its foetal origin and anti-inflammatory and immunomodulatory proprieties, AMEED can avoid this caveat. Interestingly, AM expresses CD59—a negative regulator of complement activation—the human leukocyte antigen G (HLA-G) and lack the antigens HLA-A, HLA-B, HLA-C and HLA-DR, making it also immunologically inert [6].

Several studies have also compared efficacy of autologous serum versus artificial tears in DED [27, 28]: autologous serum showed to improve the ocular surface disease index (OSDI) score, the tear break-up time (TBUT) and rose Bengal staining score more than the tear substitutes, with no differences in the Schirmer I test or the fluorescein staining score [27]. On the other hand, an autologous serum for ocular surface diseases review concluded than the evidence on the safety and effectiveness of this treatment was limited by the lack of controlled studies and by the variability in components of the study protocols [29]. To our best knowledge, no studies have compared the efficacy of autologous serum versus AMEED. However, contamination of autologous serum eye drops can be found up to almost all cases [30], despite that related adverse events have been rarely described in the literature [29]. While there are no reports of recognized complications of AMEED treatment, some authors claim a longer observation period and a larger patient population are still mandatory to validate its safety [3]. In this regard, our study showed long-term treatment without no adverse events; others reported foreign body sensation and ocular burning as adverse events [14].In conclusion, according to our findings, AMEED could be considered a promising treatment to enhance the recovery of ocular surface health and reduce signs and symptoms in patients with ocular surface diseases. The presented real-world observational data strengthen AMEED use validity in a myriad of clinical settings. Further studies are warranted in order to determine AMEED long-term effects, consolidate security findings and assess whether repetitive use of AMEED generates a more lasting effect or, by contrast, a decreasing one.

Further core advantages of AMEED over autologous serum are associated with housing temperature storage needs: autologous serum must be kept frozen until its use, while AMEED can be kept at room

temperature until reconstituted; then, its expiry time lasts for 2 weeks. Nevertheless, the different manufacturing processes may still lead to changes in the structural or functional properties of several AMEED active biomolecules [3, 31].

This study has some limitations to disclose. First, as it is based on the follow-up reports, data are kept subject to clinicians' responses accuracy. Due to its real-world observational origin, no standardized questionnaires or scores were used on searched outcomes. Despite that a certain burning or foreign body sensation complaint could be expected according to previous reports [9, 14], no adverse events were found in this investigation. That could be related to the difficulty on reporting these mild-adverse events in the follow-up visit without a structured form. Because this is a real-world observational study, most of the participants had several previous failed treatments and the starting date and duration of the AMEED was decided by the treating clinician. Therefore, despite baseline measurements were compared with the last follow-up visit in all patients, treatment timeline duration could differ between patients, as well as subjacent corneal surface disease and treatment indication, which may had introduced a bias in our study. Finally, this was a retrospective study and had no control arm.

As a whole, the beneficial effects of AMEED should be corroborated with additional studies involving a comparison between AMEED and different tear substitutes [3]. Studies involving a comparison of AMEED with autologous blood-derived products, such as serum, platelet-rich plasma, plasma rich in growth factors and others, which have been studied in relation to ocular surface disorders should be conducted in order to establish its comparative effectiveness and better describe the indications of the different biological eye drops [3].

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Declarations

Conflict of interest The authors declare no conflicts of interest.

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Informed consent Consent requirement was waived by the IRB.

References

1. Jones L, Downie LE, Korb D et al (2017) TFOS DEWS II management and therapy report. Ocul Surf 15:575–628. https://doi.org/10.1016/j.jtos.2017.05.006
2. Soni NG, Jeng BH (2016) Blood-derived topical therapy for ocular surface diseases. Br J Ophthalmol 100:22–27. https://doi.org/10.1136/bjophthalmol-2015-306842
3. Murri MS, Moshirfar M, Birdsong OC et al (2018) Amniotic membrane extract and eye drops: A review of literature and clinical application. Clin Ophthalmol 12:1105–1112. https://doi.org/10.2147/OPTH.S165553
4. Malhotra C, Jain AK (2014) Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. World J Transp 4:111–21. https://doi.org/10.5500/wjt.v4.i2.111
5. Jirsova K, Jones GLA (2017) Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting—a review. Cell Tissue Bank 18:193–204. https://doi.org/10.1007/s10561-017-9618-5
6. Li J, Koike-Soko C, Sugimoto J et al (2015) Human amnion-derived Stem Cells have immunosuppressive properties on NK Cells and Monocytes. Cell Transp 24:2065–2076. https://doi.org/10.3727/096368914X685230
7. Baradaran-Rafii A, Asl NS, Ebrahimi M et al (2018) The role of amniotic membrane extract eye drop (AMEED) in in vivo cultivation of limbal stem cells. Ocul Surf 16:146–153. https://doi.org/10.1016/j.jtos.2017.11.001
8. Tighe S, Mead OG, Lee A, Tseng SC (2020) Basic science review of birth tissue uses in ophthalmology. Taiwan J Ophthalmol 10:3–12. https://doi.org/10.4103/tjo.tjo_4_20
9. McDonald MB, Sheha H, Tighe S et al (2018) Treatment outcomes in the DRy eye amniotic membrane (DREAM)

study. Clin Ophthalmol 12:677–681. https://doi.org/10.2147/OPTH.S162203
10. Tighe S, Moein HR, Chua L et al (2017) Topical cryopreserved amniotic membrane and umbilical cord eye drops promote re-epithelialization in a murine corneal abrasion model. Investig Ophthalmol Vis Sci 58:1586–1593. https://doi.org/10.1167/iovs.16-20834
11. Shahriari HA, Famil Tokhmehchi MR, Hashemi NF (2008) Comparison of the effect of amniotic membrane suspension and autologous serum on alkaline corneal epithelial wound healing in the rabbit model. Cornea 27:1148–1150. https://doi.org/10.1097/ICO.0b013e318173138a
12. Liang L, Li W, Ling S et al (2009) Amniotic membrane extraction solution for ocular chemical burns. Clin Exp Ophthalmol 37:855–863. https://doi.org/10.1111/j.1442-9071.2009.02159.x
13. Bonci P, Bonci P, Lia A (2005) Suspension made with amniotic membrane: clinical trial. Eur J Ophthalmol 15:441–445
14. Yeu E, Goldberg DF, Mah FS et al (2019) Safety and efficacy of amniotic cytokine extract in the treatment of dry eye disease. Clin Ophthalmol 13:887–894. https://doi.org/10.2147/OPTH.S203510
15. Bron AJ, Evans VE, Smith JA (2003) Grading of corneal and conjunctival staining in the context of other dry eye tests. Cornea 22:640–650. https://doi.org/10.1097/00003226-200310000-00008
16. Tsubota K, Goto E, Shimmura S, Shimazaki J (1999) Treatment of persistent corneal epithelial defect by autologous serum application. Ophthalmology 106:1984–1989. https://doi.org/10.1016/S0161-6420(99)90412-8
17. Dua HS, Said DG, Messmer EM et al (2018) Neurotrophic keratopathy. Prog Retin Eye Res 66:107–131. https://doi.org/10.1016/j.preteyeres.2018.04.003
18. Giannaccare G, Carnevali A, Senni C et al (2020) Umbilical Cord blood and serum for the treatment of ocular diseases: a comprehensive review. Ophthalmol Ther 9:235–248. https://doi.org/10.1007/s40123-020-00239-9
19. Wei Y, Asbell PA (2014) The core mechanism of dry eye disease is inflammation. Eye Contact Lens 40:248–256. https://doi.org/10.1097/ICL.0000000000000042
20. Bourgeois M, Loisel F, Obert L et al (2019) Can the amniotic membrane be used to treat peripheral nerve defects? a review of literature. Hand Surg Rehabil 38:223–232. https://doi.org/10.1016/j.hansur.2019.05.006
21. Sacchetti M, Lambiase A (2017) Neurotrophic factors and corneal nerve regeneration. Neural Regen Res 12:1220–1224. https://doi.org/10.4103/1673-5374.213534
22. Bischoff M, Stachon T, Seitz B et al (2017) Growth factor and interleukin concentrations in amniotic membrane-conditioned medium. Curr Eye Res 42:174–180. https://doi.org/10.3109/02713683.2016.1164189
23. Sabater-Cruz N, Figueras-Roca M, González Ventosa A et al (2020) Current clinical application of sclera and amniotic membrane for ocular tissue bio-replacement. Cell Tissue Bank 21:597–603. https://doi.org/10.1007/s10561-020-09848-x
24. Harloff S, Hartwig D, Kasper K et al (2008) Epitheliotrophic capacity of serum eye drops from healthy donors versus serum from immunosuppressed patients with rheumatoid arthritis Epitheliotrophe Kapazität von Serum-Augentropfen gesunder versus immunsupprimierter Patienten mit rheumatoider Arthritis. Klin Monbl Augenheilkd 225:200–206. https://doi.org/10.1055/s-2008-1027199
25. Stenwall PA, Bergström M, Seiron P et al (2015) Improving the anti-inflammatory effect of serum eye drops using allogeneic serum permissive for regulatory T cell induction. Acta Ophthalmol 93:654–657. https://doi.org/10.1111/aos.12801
26. Giannaccare G, Versura P, Buzzi M et al (2017) Blood derived eye drops for the treatment of cornea and ocular surface diseases. Transfus Apher Sci 56:595–604. https://doi.org/10.1016/j.transci.2017.07.023
27. Wang L, Cao K, Wei Z et al (2020) Autologous serum eye drops versus artificial tear drops for dry eye disease: a systematic review and meta-analysis of randomized controlled trials. Ophthalmic Res 63:443–451. https://doi.org/10.1159/000505630
28. Yilmaz U, Küçük E, Koç Ç, Gökler E (2017) Comparison of autologous serum versus preservative free artificial tear in patients with dry eyes due to systemic isotretinoin therapy. Curr Eye Res 42:827–831. https://doi.org/10.1080/02713683.2016.1255758
29. Shtein RM, Shen JF, Kuo AN et al (2020) Autologous serum-based eye drops for treatment of ocular surface disease: a report by the American academy of ophthalmology. Ophthalmology 127:128–133. https://doi.org/10.1016/j.ophtha.2019.08.018
30. Thanathanee O, Phanphruk W, Anutarapongpan O et al (2013) Contamination risk of 100% autologous serum eye drops in management of ocular surface diseases. Cornea 32:1116–1119. https://doi.org/10.1097/ICO.0b013e3182910036
31. Russo A, Bonci P, Bonci P (2012) The effects of different preservation processes on the total protein and growth factor content in a new biological product developed from human amniotic membrane. Cell Tissue Bank 13:353–361. https://doi.org/10.1007/s10561-011-9261-5

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DISCUSIÓN

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Los SoHO constituyen una aproximación terapéutica efectiva en el tratamiento de diferentes patologías de la córnea y superficie ocular. La continua investigación clínica y básica ha dado como resultado recientes cambios en la formulación, preparación, usos, indicaciones, controles de calidad y epidemiología de los diferentes biosustitutos. Así pues, en esta memoria se ha pretendido estudiar los aspectos más representativos en nuestro medio y establecer comparaciones con otras regiones. Se definieron como objetivos de nuestro estudio las características epidemiológicas de las tendencias e indicaciones para la córnea, membrana amniótica y esclera, la evaluación del control de calidad de los biosustitutos mediante el estudio de aproximaciones basadas en el cultivo microbiológico en las queratoplastias, los resultados clínicos de un SoHO autólogo según el protocolo de tratamiento postoperatorio y, finalmente, el estudio de los resultados clínicos y de validación de los nuevos formatos de membrana amniótica – liofilizada y extracto - suministrados. Los resultados derivados de esta investigación se han materializado en las conclusiones que se analizarán y discutirán seguidamente.

En relación a la epidemiología de los biosutitutos, se observa como las técnicas lamelares se están imponiendo sobre las penetrantes. Este cambio viene determinado por ventajas críticas como mejores resultados visuales, menor tasa de rechazo y mayor recuperación postoperatoria a pesar de sus inconvenientes, como una mayor dificultad técnica (Maier et al. 2013; Kymionis et al. 2014; Singh et al. 2019). En el caso de otros SoHO como esclera y membrana amniótica se ha podido estimar un cambio significativo de tendencias en las indicaciones.

La principal indicación del implante de esclera en nuestro estudio fue como coadyuvante en la cirugía de glaucoma, seguida de la reconstrucción palpebral y manejo de úlceras de la superficie ocular. Entre los años 2013 y 2018, se encontró de forma significativa una tendencia creciente en el uso de esclera para reconstrucción palpebral y decreciente para las úlceras. A diferencia de lo que ocurre con las indicaciones de la queratoplastia, son escasas las publicaciones sobre tendencias y epidemiología de las indicaciones del implante de esclera. Éstas describen, al igual que nuestro estudio, la cirugía de glaucoma como principal indicación del implante de esclera (Hodge et al. 2017; Lind et al. 2017). La demanda de esclera para la cirugía del glaucoma es alta

comparada con otros materiales por sus características - bajo riesgo de transmisión de patógenos, flexibilidad y resistencia -, coste-efectividad, disponibilidad y facilidad de almacenamiento (Tsoukanas et al. 2016; Dubey et al. 2017; Hodge et al. 2017; Lind et al. 2017). Sin embargo, el tejido corneal pudiera ser una excepción: el EBAA ha notado una reducción en el uso de esclera en la cirugía de glaucoma a favor del tejido corneal por, aparentemente, la mejor visualización y menor tasa de exposición del tubo del dispositivo de drenaje (https://restoresight.org/who-we-are/statistics/; Spierer et al. 2016; Hodge et al. 2017; Lambert & Chamberlain 2017); este cambio progresivo en las preferencias de los cirujanos de glaucoma podría implicar un cambio de tendencias en los próximos años. Las otras indicaciones del uso de esclera y sus tendencias han sido también estudiados. Cabe destacar que el manejo de úlceras esclerales y/o corneales con parche de esclera ha mostrado una tendencia decreciente que podría estar relacionada, por un lado, con el actual manejo de las escleritis inmunológicas mediante inmunosupresión precoz con fármacos biológicos, que disminuye el riesgo y la evolución a necrosis escleral (Sainz de la Maza et al. 1989; Wen et al. 2018). Y por el otro, con la amplia disponibilidad de córneas donantes con baja densidad de células endoteliales - lo que las invalida para queratoplastia pero que pueden ser utilizadas como parches tectónicos - que podría haber favorecido un cambio en las preferencias de los cirujanos para el manejo de las perforaciones oculares, con la consecuente tendencia descendente a usar esclera.

En referencia a la membrana amniótica, SoHO ampliamente estudiado en esta investigación, su principal indicación fue el tratamiento de la úlcera corneal seguida de reconstrucción conjuntival, defecto epitelial y control de la inflamación ocular. Esta última indicación mostró una tendencia creciente estadísticamente significativa durante el periodo de estudio. La reconstrucción conjuntival – especialmente como coadyuvante en la cirugía de pterigión – fue la segunda indicación para el uso de membrana amniótica. A pesar de que el autoinjerto conjuntival, considerado el *gold standard*, ha demostrado tener menor tasa de recurrencias, la membrana amniótica es muy empleada en nuestra área, entre otras razones por su gran disponibilidad - pudiéndose obtener hasta treinta parches de membrana amniótica de una sola placenta (Malhotra & Jain 2014; Clearfield et al. 2017).

El uso de los SoHO de origen autólogo se ha estudiado tomando como ejemplo el uso de conjuntiva autóloga en el tratamiento quirúrgico del pterigión y valorando su recurrencia como principal complicación. La recurrencia ha sido asociada a diversos factores, especialmente la técnica quirúrgica (Tan et al. 1997; Karalezli et al. 2008; Alpay et al. 2009; Pan et al. 2011; Kaufman et al. 2013; Safi et al. 2016): la exéresis simple tiene las mayores tasas de recurrencia, de hasta el 89% (Alpay et al. 2009), mientras que el implante de membrana amniótica puede asociarse a una tasa de recurrencias de hasta el 42,3%; por este motivo se suele reservar como segunda opción, cuando se descarta el uso de conjuntiva (Clearfield et al. 2017). Finalmente, la exéresis con autoinjerto conjuntival se considera la técnica con la menor tasa de recurrencias, inferiores al 17% (Ang et al. 2007; Alpay et al. 2009; Kocamis & Bilgec 2014; Clearfield et al. 2017). La tasa de recurrencias del 17,3% encontrada en nuestra investigación es comparable con la literatura. La mala adherencia al tratamiento postoperatorio se ha descrito como otro factor de riesgo para la recidiva (Yaisawang & Piyapattanakorn 2003). Nuestro estudio dividió los pacientes en dos grupos según el protocolo de tratamiento postoperatorio indicado y previamente descrito en la literatura (Pikkel et al. 2001; Nakamura et al. 2006; Küçükerdönmez et al. 2007; Ha et al. 2014; Toker & Eraslan 2016; Akbari et al. 2017): un grupo siguió un protocolo de 4 meses (protocolo 1) y el otro un protocolo de 5 semanas (protocolo 2). El protocolo 2 mostró una mayor tasa de adherencia y una menor tasa de recurrencias que el protocolo 1, demostrando que una mala adherencia al tratamiento se asocia significativamente a una mayor tasa de recurrencia del pterigión.

Para el manejo quirúrgico del pterigión, en este proyecto se ha evaluado también el uso de SoHO alogénicos, como la membrana amniótica liofilizada. Se estudió la tolerabilidad ocular, facilidad de manipulación perioperatoria, eficacia y seguridad de esta presentación novedosa, suministrada desde 2019 por el BST. Los resultados mostraron mayor rigidez y, por tanto, mejor manipulación que la criopreservada; no se produjeron agujeros o enrollamientos durante la cirugía, se observó facilidad para suturar o fijarla con pegamento biológico y no se registraron complicaciones durante los doce meses de seguimiento. Se valoró también la tolerabilidad y confort ocular: todos los pacientes refirieron una leve o moderada incomodidad ocular a las dos semanas de la cirugía y mínima o ninguna molestia al año, comparable a publicaciones previas (Wang et al. 2017). En cuanto a los resultados estéticos, estos fueron poco

alentadores pero esperables ya que tres de los pacientes presentaron una recidiva a los doce meses, comparable a lo descrito en la literatura (Clearfield et al. 2017).

Como resultado del gran potencial en I+D de la membrana amniótica, nuestro banco de tejidos regional suministra desde 2018 colirio de extracto de membrana amniótica (*Amniotic Membrane Extract Eye Drops*; AMEED), con indicación para diversas patologías de la córnea y superficie ocular. En este trabajo de investigación se ha llevado a cabo la necesaria evaluación de la seguridad y eficacia de esta presentación. Se ha realizado un primer estudio en una cohorte de pacientes con unos criterios de inclusión estrictos y un segundo estudio sobre los resultados clínicos del AMEED en la práctica clínica habitual. Dadas las dificultades intrínsecas de los tratamientos crónicos en patologías de gran demanda asistencial en la vida real, tales como el ojo seco, las úlceras corneales y los defectos epiteliales persistentes, los estudios observaciones de la práctica clínica resultan de gran interés.

En nuestro primer estudio se evaluaron diferentes signos, síntomas y pruebas complementarias de una cohorte de pacientes con ojo seco y/o úlcera corneal. Los parámetros de sintomatología estudiados mejoraron y se constató una epitelización corneal completa tras el tratamiento con AMEED. En el segundo estudio, de vida real, también se constató epitelización completa en todos los pacientes con retraso del cierre epitelial, lo que muestra que el AMEED podría usarse como alternativa al implante de membrana amniótica, ahorrándose el procedimiento quirúrgico y permitiendo un tratamiento continuado hasta la curación (Murri et al. 2018). Además, como ya se ha comentado, nuestro estudio epidemiológico sobre los cambios de tendencia en las indicaciones de la membrana amniótica en Catalunya ha mostrado que casi la mitad de las indicaciones de implante de este tejido fueron úlcera corneal y defecto epitelial persistente, por lo que es previsible que el uso del AMEED en estas indicaciones tenga una tendencia creciente en el futuro. Se ha identificado un efecto antiinflamatorio en pacientes con ojo seco que producía un alivio sintomático. El efecto terapéutico del AMEED puede ser atribuido a múltiples mecanismos de acción de control de la inflamación de la superficie ocular, cosa que resulta crítica en la patogénesis y cronicidad de la enfermedad del ojo seco (Wei & Asbell 2014; McDonald et al. 2018). Las propiedades biomecánicas del AMEED resultan similares en algunas moléculas y factores de crecimiento al suero autólogo (Imanishi et al. 2000; Noble et al. 2004; Jirsova et

al. 2014; Anitua et al. 2015), pero dado el origen fetal de la membrana amniótica, que expresa CD59 (un regulador negativo de la activación del complemento) y HLA-G y carece de HLA-A, HLA-B, HLA-C y HLA-DR, el AMEED podría evitar los problemas asociados al suero autólogo, como la aparición de elementos circulantes proinflamatorios y proinmunes presentes en la sangre periférica de pacientes con ciertos tipos de enfermedades autoinmunes e inflamatorias (Geerling et al. 2004; Harloff et al. 2008; Li et al. 2015; Stenwall et al. 2015; Giannaccare et al. 2017). Diferentes estudios han comparado la eficacia del suero autólogo y las lágrimas artificiales en el manejo del ojo seco (Yilmaz et al. 2017; Wang et al. 2020), aunque la evidencia sobre la seguridad y efectividad del suero autólogo se ve limitada por la falta de estudios controlados y la variabilidad en el diseño y protocolos de los estudios (Shtein et al. 2020). Hasta la fecha, no hay estudios que hayan comparado la eficacia del suero autólogo con el AMEED. El uso de AMEED abre nuevos horizontes en el tratamiento de la patología compleja de la superficie ocular, pero los efectos beneficiosos del AMEED deben ser corroborados con un mayor número de pacientes y por un periodo de tiempo más prolongado; además son necesarios estudios adicionales que comparen AMEED con diferentes sustitutos de la lágrima, incluyendo SoHO hemoderivados - suero, plasma rico en plaquetas y plasma rico en factores de crecimiento - entre otros.

La indicación más frecuente para la queratoplastia durante el periodo de estudio fue la queratopatía bullosa seguida de la distrofia endotelial de Fuchs, los retrasplantes y las ectasias corneales - incluyendo queratocono. Las indicaciones para el trasplante corneal aún son diferentes de una región a otra a pesar de que las tendencias globales están cambiando y convergiendo: la queratopatía bullosa y la distrofia endotelial de Fuchs son la primera indicación en los países del primer mundo mientras que, en países en vías de desarrollo, las queratitis infecciosas y las opacidades corneales aún son más frecuentes (Chen et al. 2001; Tan et al. 2014; Gupta et al. 2015; Singh et al. 2019). Además, en algunas áreas de países industrializados, la queratopatía bullosa se ha descrito como la segunda indicación, tras la distrofia de Fuchs (Zhang et al. 2013; Tan et al. 2014; Park et al. 2015; Le et al. 2017) o el queratocono (Fasolo et al. 2006; Zare et al. 2012), e incluso como tercera (Röck et al. 2017); diferencias que podrían explicarse más por cuestiones metodológicas, como diferencias en la definición de cada indicación, que por prevalencias.

La técnica más indicada entre 2011 y 2018 fue la queratoplastia penetrante en más del 60% de los casos, a pesar de que se halló un aumento estadísticamente significativo de las técnicas lamelares posteriores, especialmente la DMEK. Este incremento en las técnicas lamelares puede tener una relación directa con el inicio del suministro de tejido precortado - DSAEK en 2013 y DMEK en 2017 - por el banco de tejidos. La DMEK, al ser una técnica más anatómica y con una mejor recuperación visual (Guerra et al. 2011; Patel 2012; Tourtas et al. 2012; Singh, Said & Dua 2018), podría considerarse la primera opción para el tratamiento de enfermedades endoteliales; sin embargo, su adopción está siendo tórpida por su curva de aprendizaje y la dificultad en la preparación del tejido (Terry 2012; Singh et al. 2018). Algunos estudios han destacado la tendencia ascendente en el uso de técnicas lamelares tras la introducción de tejido precortado por parte de los bancos de ojos, dado que los cirujanos tienden más a realizar técnicas lamelares posteriores cuando el riesgo de dañar el tejido corneal donante es menor (Kitzmann et al. 2008; Woodward et al. 2012). Otra ventaja del tejido precortado es la menor tasa de positividad en el cultivo microbiológico y, por tanto, menor riesgo de potenciales complicaciones infecciosas en el receptor (Rauen et al. 2012; Mathes et al. 2019). Sin embargo, la popularización del uso de técnicas lamelares en nuestra comunidad ha empezado tarde en comparación a otras áreas, como en la región de Columbia (Canadá), donde en 2011 las técnicas lamelares supusieron el 57,7%, mientras que en Catalunya fueron el 19% (Tan et al. 2014). Otro ejemplo es Estados Unidos, donde cerca del 50% de las técnicas de queratoplastia son lamelares mientras que, en nuestra región, globalmente no llegan al 10% (Ple-plakon & Shtein 2014; Park et al. 2015). En Europa, concretamente en Italia, el incremento de las técnicas lamelares posteriores se produjo entre los años 2002 y 2008, mientras que en Catalunya esto ha ocurrido después de 2013 (Frigo et al. 2015). Teniendo en cuenta los hallazgos de este estudio epidemiológico, es presumible que la tendencia creciente de la DMEK en nuestro medio seguirá en los próximos años gracias, entre otros, a la disponibilidad del tejido precortado.

Los controles de calidad del tejido resultan esenciales para mantener la seguridad del receptor. Los sistemas de biovigilancia requieren del *feedback* tanto de los equipos extractores, a fin de que se detecten los posibles efectos adversos, como de los cirujanos implantadores de tejido, para la detección de las reacciones adversas. La realización de cultivos microbiológicos por parte de los cirujanos permite trazar el tejido hasta otro

receptor del mismo donante y anticiparse a posibles complicaciones infecciosas ante un resultado positivo. Como parte de esta investigación sobre diferentes aspectos de los SoHO de la córnea y superficie ocular, se ha realizado un estudio que proporciona información de vida real y práctica clínica habitual sobre los cultivos microbiológicos del anillo escleral y medio de preservación. Se ha elegido cuidadosamente el biosustituto a estudiar – la córnea - dado que los controles de calidad microbiológicos tras el implante de tejido corneal han sido ampliamente estudiados y cuestionados en la literatura. Se han buscado causas en el donante que pudieran estar relacionadas con la positividad, hallándose que las córneas preservadas en cultivo organotípico han mostrado significativamente menor tasa de positividad que las preservadas en hipotermia. Este hecho se podría justificar posiblemente por el estricto protocolo de control microbiológico usado en las aproximaciones de preservación para cultivo organotípico (Zanetti et al. 2005; Linke et al. 2013; Gruenert et al. 2017; Röck et al. 2017), que en el caso del BST implica tres análisis microbiológicos secuenciales con resultado negativo, durante el periodo de preservación. Se han estudiado otros posibles factores asociados a la positividad de los cultivos microbiológicos, tales como la muestra analizada a partir del anillo esclerocorneal, el medio de preservación o ambos, la causa de muerte, el momento del envío de la córnea o la densidad endotelial, que no han alcanzado la significación estadística, exceptuando la muerte por septicemia. Debe tomarse con cautela este último resultado, dado que los registros recogen una única causa de muerte y, además, podría ser también una causa secundaria no registrada en algunos casos. El microorganismo más identificado en los cultivos de nuestro estudio fue *Staphylococcus epidermidis*, considerado parte de la flora normal de la mucosa conjuntival y un potencial contaminante ambiental en el quirófano.

El banco de tejidos local apostó por este tipo de preservación corneal y empezó a suministrar córneas en cultivo organotípico en enero de 2010. Actualmente, corresponde al 65% de las córneas suministradas. Nuestros resultados muestran que solo el 40,6% de todos los resultados microbiológicos positivos se han encontrado en córneas organotípicas, mientras que estas correspondieron al 76,7% de todo el tejido empleado. Por tanto, en nuestro medio este tipo de preservación presenta una menor tasa de resultados microbiológicos positivos y reafirma el uso preferencial de este tipo de aproximación para preservar el tejido corneal.

Nuestro estudio tiene algunas limitaciones que destacar. Primero, puesto que se basa en parte en las memorias anuales de la OCATT, los datos están sujetos a la precisión de las respuestas de los formularios. La tasa de respuestas de los cirujanos en otros países puede variar entre el 50 y el 97% (Melles et al. 2000; Park et al. 2015). En nuestro medio, la tasa de respuesta de los cirujanos es de media mayor en esclera (63,6%) que en membrana amniótica (56,3%). Los motivos para estas diferentes tasas de respuesta entre años y tejidos se están estudiando para poder implementar mejoras. Los cirujanos deberían tener presente la importancia de retornar los formularios de seguimiento para la exactitud y fiabilidad de las memorias anuales. La misma limitación acontece en el estudio de los resultados clínicos con AMEED. Al estar basado en los formularios de respuesta de los resultados clínicos, los datos están supeditados a la precisión de la respuesta de los oftalmólogos de referencia.

La ausencia de cuestionarios estandarizados ha sido una limitación recurrente en nuestra investigación. Dado que los resultados clínicos por el uso de AMEED provenían de un diseño observacional en la práctica clínica habitual, no se usaron cuestionarios estandarizados. Además, no se detectó ninguna reacción adversa, por leve que fuera – como sensación de cuerpo extraño, ardor -, cosa que pudiera estar más relacionada con la dificultad en comunicar este tipo de reacciones leves al no contar con un formulario estructurado, que con la ausencia real de dichos síntomas. Incluso en el primer estudio sobre los resultados de AMEED, más estandarizado, los cuestionarios autoevaluativos presentaron algunos problemas de comprensión. Las puntuaciones obtenidas al final del estudio mejoraron respecto la basal, sin embargo, la propia percepción no cambió. Quizás cuestionarios estandarizados hubieran podido clasificar el grado de gravedad de la sintomatología con más claridad.

Además, hay algunas limitaciones específicas de partes de nuestra investigación a comentar. Por ejemplo, el tamaño de la muestra ha limitado la posibilidad de realizar comparaciones en algunos casos, como en el estudio del uso de membrana amniótica liofilizada en la cirugía del pterigión y su tasa de recurrencias respecto a otros tipos de presentación de membrana amniótica o al autoinjerto conjuntival. El origen retrospectivo y de la práctica clínica habitual de los datos podría estar relacionado con la tasa de realización del control de calidad mediante cultivo microbiológico del anillo esclerocorneal, que fue del 76,8%, o la imposibilidad de aleatorizar los dos protoco-

los de tratamiento tras la cirugía del pterigión con autoinjerto conjuntival. En este último, pudiera haberse introducido algunos sesgos por la aplicación secuencial de los protocolos ya que la tasa de recurrencias también podría explicarse, al menos parcialmente, por dos motivos: primero, por la curva de aprendizaje de la cirujana – más experimentada con los pacientes del protocolo 2 y por tanto con una cirugía y tamaño de injerto mucho más reproducibles. Y segundo, por la ausencia de un cuestionario estandarizado sobre el cumplimiento del tratamiento, con lo que los pacientes del protocolo 2 podrían haber recibido una mejor y más amplia explicación del tratamiento, que hubiera mejorado su adherencia.

Por todo ello, concluimos que la caracterización de tendencias en la evolución de los biosustitutos de la córnea y superficie ocular, así como el conocimiento del control de donantes en nuestro medio y la evaluación de nuevas presentaciones de SoHO, permite y permitirá ajustar recursos y sentar las bases de múltiples líneas de estudios clínicos, aportando datos de gran importancia para la práctica asistencial futura en nuestro medio.

6

CONCLUSIONES

CONCLUSIONES

El estudio de la epidemiología, evolución, indicaciones y controles de calidad de diferentes biosustitutos de la córnea y superficie ocular en Catalunya ha mostrado:

1. Las principales indicaciones del trasplante de córnea (queratoplastia) en Catalunya entre los años 2011 y 2018 son la queratopatía bullosa y la distrofia de Fuchs.

2. Las principales indicaciones del implante de tejido escleral en Catalunya entre los años 2011 y 2018 son la cirugía del glaucoma y la reconstrucción palpebral.

3. Las principales indicaciones de la utilización de la membrana amniótica en Catalunya entre los años 2011 y 2018 son las úlceras corneales y la reconstrucción conjuntival.

4. Entre los años 2011 y 2018, las queratoplastias lamelares endoteliales, especialmente la DMEK, han aumentado su indicación de manera significativa, en estrecha relación con la disponibilidad de tejido precortado.

5. Con relación a los controles microbiológicos de las córneas utilizadas para queratoplastia, las técnicas de preservación de tejido mediante las aproximaciones en hipotermia muestran una mayor tasa de contaminación de los tejidos que las aproximaciones en cultivos organotípicos.

6. En el tratamiento quirúrgico del pterigión con autoinjerto conjuntival, la tasa de adherencia de los pacientes al tratamiento postoperatorio, está disminuida de manera estadísticamente significativa en relación al tipo de protocolo postquirúrgico.

7. La membrana amniótica es un biosustituto que ha demostrado tener efectos beneficiosos tanto en la presentación liofilizada - como coadyuvante para la reconstrucción de la superficie ocular en la cirugía del pterigión -, como en forma de colirio de extracto - para el cierre epitelial y en la disminución de la sintomatología asociada al ojo seco.

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REFERENCIAS

REFERENCIAS

Akbari M, Soltani-Moghadam R, Elmi R & Kazemnejad E (2017): Comparison of free conjunctival autograft versus amniotic membrane transplantation for pterygium surgery. J Curr Ophthalmol **29**: 282–286.

Aldave AJ, DeMatteo J, Glasser DB, Elmer TY, Iliakis B, Nordlund ML et al. (2013): Report of the Eye Bank Association of America Medical Advisory Board Subcommittee on fungal infection after corneal transplantation. Cornea **32**: 149–154.

Alio JL, Rodriguez AE & WróbelDudzińska D (2015): Eye platelet-rich plasma in the treatment of ocular surface disorders. Curr Opin Ophthalmol **26**: 325–332.

Alpay A, Uğurbaş SH & Erdoğan B (2009): Comparing techniques for pterygium surgery. Clin Ophthalmol **3**: 69–74.

Ang LPK, Chua JLL & Tan DTH (2007): Current concepts and techniques in pterygium treatment. Curr Opin Ophthalmol **18**: 308–313.

Anitua E, Muruzabal F, Tayebba A, Riestra A, Perez VL, Merayo-Lloves J et al (2015): Autologous serum and plasma rich in growth factors in ophthalmology: Preclinical and clinical studies. Acta Ophthalmol **93**: e605–e614.

Antonios S, Cameron J, Badr I, Habash N & Cotter J (1991): Contamination of donor cornea: postpenetrating keratoplasty endophthalmitis. Cornea **10**: 217–20.

Balal S, Nitiahpapand R, Hassan A, Than J, Patel A, Kumar B et al (2020): Finger-prick autologous blood in the treatment of persistent corneal epithelial defects. Cornea **39**: 594–597.

Balal S, Udoh A, Pappas Y, Cook E, Barton G, Hassan A et al. (2018): The feasibility of finger prick autologous blood (FAB) as a novel treatment for severe dry eye disease (DED): Protocol for a randomised controlled trial. BMJ Open **8**: 1–7.

Barman M, Finger PT & Milman T (2012): Scleral patch grafts in the management of uveal and ocular surface tumors. Ophthalmology **119**: 2631–2636.

Bernabei F, Roda M, Buzzi M, Pellegrini M, Giannaccare G & Versura P (2019): Blood-based treatments for severe dry eye disease: The need of a consensus. J Clin Med **8**: 1478.

Bonci P, Bonci P & Lia A (2005): Suspension made with amniotic membrane: clinical trial. Eur J Ophthalmol **15**: 441–5.

Buzzonetti L, Bohringer D, Liskova P, Lang S & Valente P (2020): Keratoconus in children: A literature review. Cornea **39**: 1592–1598.

Campos E, Versura P, Buzzi M, Fontana L, Giannaccare G, Pellegrini M et al. (2020): Blood derived treatment from two allogeneic sources for severe dry eye associated to keratopathy: A multicentre randomised cross over clinical trial. Br J Ophthalmol **104**: 1142–1147.

Cárdenas-Cantú E, Zavala J, Valenzuela J & Valdez-García JE (2016): Molecular basis of pterygium development. Semin Ophthalmol **31**: 567–583.

Chen JY, Jones MN, Srinivasan S, Neal TJ, Armitage WJ & Kaye SB (2015): Endophthalmitis after penetrating keratoplasty. Ophthalmology **122**: 25–30.

Chen NN, Wu PL, Chen HC, Huang TY & Lai LJ (2019): Prevalence of microbial contamination in donor corneas. Taiwan J Ophthalmol **9**: 179–184.

Chen W, Hu F & Wang I (2001): Changing indications for penetrating keratoplasty in Taiwan from 1987 to 1999. Cornea **20**: 141–144.

Choi JA, Choi JS & Joo CK (2011): Effects of amniotic membrane suspension in the rat alkali burn model. Mol Vis **17**: 404–412.

Clearfield E, Hawkins BS & Kuo IC (2017): Conjunctival autograft versus amniotic membrane transplantation for treatment of pterygium: Findings from a Cochrane systematic review. Am J Ophthalmol **182**: 8–17.

Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK et al. (2017): TFOS DEWS II definition and classification report. Ocul Surf **15**: 276–283.

Das S, Ramappa M, Mohamed A, Chaurasia S, Sharma S & Das T (2020): Acute endophthtalmitis after penetrating and endothelial keratoplasty at a tertiary eye care center over a 13-year period. Indian J Ophthalmol **68**: 2445–50.

Dhillon HK, Bahadur H & Raj A (2019): A comparative study of tarsorrhaphy and amniotic membrane transplantation in the healing of persistent corneal epithelial defects. Indian J Ophthalmol **68**: 29–33.

Dubey S, Tajurkar K, Kapur N & Acharya M (2017): Heterologous, human donor sclera as the patch graft in glaucoma drainage device surgery. J Glaucoma **26**: e128.

EURO GTP II, Good tissue and cell practices (2019): Good Practices for evaluating quality, safety and efficacy of novel tissue and cellular therapies and products. 1st Edition. Barcelona.

European Committee on Organ Transplantation & Keitel S (2019): Guide to the quality and safety of tissues and cells for human application - EDQM 4th Edition. Strasbourg.

European Eye Bank Association (2020): Annual Directory. 28th Edition. Venice.

Everts RJ, Fowler WC, Chang DH & Reller LB (2001): Corneoscleral rim cultures: Lack of utility and implications for clinical decision-making and infection prevention in the care of patients undergoing corneal transplantation. Cornea **20**: 586–589.

Fasolo A, Frigo AC, Böhm E, Genisi C, Rama P, Spadea L et al. (2006): The CORTES Study : Corneal transplant indications and graft survival in an Italian cohort of patients. Cornea **25**: 507–515.

Feu-Basilio S, Sabater-Cruz N, Spencer F, Peraza J, Quintana R, Rocha de Lossada C et al (2020): Cross-linking corneal para el tratamiento del queratocono: resultados clínicos en el Hospital Clínic de Barcelona. Ann d'oftalmologia **28**: 2–6.

Fontana L, Errani PG, Zerbinati A, Musacchi Y, Di Pede B & Tassinari G (2007): Frequency of positive donor rim cultures after penetrating keratoplasty using hypothermic and organ-cultured donor corneas. Cornea **26**: 552–556.

Fox RI, Chan R, Michelson JB, Belmont JB & Michelson PE (1984): Beneficial effect of artificial tears made with autologous serum in patients with keratoconjunctivitis sicca. Arthritis Rheum **27**: 459–461.

Frigo AC, Fasolo A, Capuzzo C, Fornea M, Bellucci R, Busin M et al. (2015): Corneal transplantation activity over 7 years: Changing trends for indications, patient demographics and surgical techniques from the corneal transplant epidemiological study (CORTES). Transplant Proc **47**: 528–535.

Gawdat TI & Ahmed RA (2014): Scleral shield: primary results of a new surgical technique in augmenting porous orbital implant protection. Eur J Ophthalmol **24**: 948–952.

Geerling G, MacLennan S & Hartwig D (2004): Autologous serum eye drops for ocular surface disorders. Br J Ophthalmol **88**: 1467–1474.

Giannaccare G, Carnevali A, Senni C, Logozzo L & Scorcia V (2020): Umbilical cord blood and serum for the treatment of ocular diseases: A comprehensive review. Ophthalmol Ther **9**: 235–248.

Giannaccare G, Versura P, Buzzi M, Primavera L, Pellegrini M & Campos EC (2017): Blood derived eye drops for the treatment of cornea and ocular surface diseases. Transfus Apher Sci **56**: 595–604.

Gonçalves ED, Campos M, Paris F, Gomes JÁP & De Farias CC (2008): Ceratopatia bolhosa: etiopatogênese e tratamento. Arq Bras Oftalmol **71**: 61–64.

Gruenert AK, Rosenbaum K, Geerlling G & Fuchsluger TA (2017): The influence of donor factors on corneal organ culture contamination. Acta Ophthalmol **95**: 733–740.

Güell JL, Morral M, Gris O, Elies D & Manero F (2012): Treatment of symptomatic bullous keratopathy with poor visual prognosis using a modified Gundersen conjunctival flap and amniotic membrane. Ophthalmic Surg Lasers Imaging **43**: 508–512.

Guerra FP, Anshu A, Price MO & Price FW (2011): Endothelial keratoplasty: Fellow eyes comparison of Descemet Stripping Automated Endothelial Keratoplasty and Descemet Membrane Endothelial Keratoplasty. Cornea **30**: 1382–1386.

Guo Q, Hao J, Yang Q, Guan L, Ouyang S & Wang J (2011): A comparison of the effectiveness between amniotic membrane homogenate and transplanted amniotic membrane in healing corneal damage in a rabbit model. Acta Ophthalmol **89**: 315–319.

Gupta N, Vashist P, Tandon R, Gupta SK, Dwivedi S & Mani K (2015): Prevalence of corneal diseases in the rural Indian population: the Corneal Opacity Rural Epidemiological (CORE) study. Br J Opthtalmology **99**: 147–152.

Ha SW, Park JH, Shin IH & Kim HK (2014): Clinical analysis of risk factors contributing to recurrence of pterygium after excision and graft surgery. Int J Ophthalmol **8**: 522–527.

Hajjar Sesé A, Lindegaard J, Julian HO, Højgaard-olsen K, Møller NF & Heegaard S (2019): A presentation of culture-positive corneal donors and the effect on clinical outcomes. Graefe's Arch Clin Exp Ophthalmol **257**: 135–141.

Harizman N, Ben-Cnaan R, Goldenfeld M, Levkovitch-Verbin H & Melamed S (2005): Donor scleral patch for treating hypotony due to leaking and/or overfiltering blebs. J Glaucoma **14**: 492–496.

Harloff S, Hartwig D, Kasper K, Wedel T, Müller M & Geerling G (2008): Epitheliotrophe Kapazität von Serum-Augentropfen gesunder versus immunsupprimierter Patienten mit rheumatoider Arthritis. Klin Monbl Augenheilkd **225**: 200–6.

Hodge C, Sutton G, Devasahayam R, Georges P, Treloggen J, Cooper S et al. (2017): The use of donor scleral patch in ophthalmic surgery. Cell Tissue Bank **18**: 119–128.

Hopkinson A, McIntosh RS, Shanmuganathan V, Tighe PJ & Dua HS (2006): Proteomic analysis of amniotic membrane prepared for human transplantation: Characterization of proteins and clinical implications. J Proteome Res **5**: 2226–2235.

Hovanesian JA, Starr CE, Vroman DT, Mah FS, Gomes JAP, Farid M et al. (2017): Surgical techniques and adjuvants for the management of primary and recurrent pterygia. J Cataract Refract Surg **43**: 405–419.

Imanishi J, Kamiyama K, Iguchi I, Kita M, Sotozono C & Kinoshita S (2000): Growth factors: Importance in wound healing and maintenance of transparency of the cornea. Prog Retin Eye Res **19**: 113–129.

Jin SX, Dackowski E & Chuck RS (2020): Risk factors for postlaser refractive surgery corneal ectasia. Curr Opin Ophthalmol **31**: 288–292.

Jirsova K, Brejchova K, Krabcova I, Filipec M, Al Fakih A, Palos M et al. (2014): The application of autologous serum eye drops in severe dry eye patients; subjective and objective parameters before and after treatment. Curr Eye Res **39**: 21–30.

Jirsova K & Jones GLA (2017): Amniotic membrane in ophthalmology: properties, preparation, storage and indications for grafting — a review. Cell Tissue Bank **18**: 193–204.

Jonas JB, Rank RM & Budde WM (2001): Tectonic sclerokeratoplasty and tectonic penetrating keratoplasty as treatment for perforated or predescemetal corneal ulcers. Am J Ophthalmol **132**: 14–18.

Kang NH, Lee S & Jun RM (2015): Comparison of epitheliotrophic factors in autologous serum eyedrops from sera of chronic renal failure patients vs. normal controls. Graefe's Arch Clin Exp Ophthalmol **253**: 1705–1712.

Karalezli A, Küçükerdönmez C, Akova YA, Altan-Yaycioglu R & Borazan M (2008): Fibrin glue versus sutures for conjunctival autografting in pterygium surgery: a prospective comparative study. Br J Ophthalmol **92**: 1206–1210.

Kaufman SC, Jacobs DS, Lee WB, Deng SX, Rosenblatt MI & Shtein RM (2013): Options and adjuvants in surgery for pterygium. Ophthalmology **120**: 201–208.

Keyhani K, Seedor JA, Shah MK, Terraciano AJ & Ritterband DC (2005): The incidence of fungal keratitis and endophthalmitis following penetrating keratoplasty. Cornea **24**: 288–291.

Kiatos E, Armstrong JJ, Hutnik CML, Tsioros SM, Malvankar-Mehta MS & Hodge WG (2017): The value of corneoscleral rim cultures in keratoplasty: a systematic review and cost-effectiveness analysis. Clin Outcomes Reseach **9**: 459–474.

Kim KM, Shin YT & Kim HK (2012): Effect of autologous platelet-rich plasma on persistent corneal epithelial defect after infectious keratitis. Jpn J Ophthalmol **56**: 544–550.

Kitzmann AS, Goins KM, Reed C, Padnick-Silver L, Macsai MS & Sutphin JE (2008): Eye bank survey of surgeons using precut donor tissue for Descemet stripping automated endothelial keratoplasty. Cornea **27**: 634–639.

Kitzmann AS, Wagoner MD, Syed N a & Goins KM (2009): Donor-related Candida keratitis after Descemet tripping automated endothelial keratoplasty. Cornea **28**: 825–828.

Kloess PM, Stulting RD, Waring GO & Wilson LA (1993): Bacterial and fungal endophthalmitis after penetrating keratoplasty. Am J Ophthalmol **115**: 309–16.

Kocamis O & Bilgec M (2014): Evaluation of the recurrence rate for pterygium treated with conjunctival autograft. Graefes Arch Clin Exp Ophthalmol **252**: 817–820.

Kojima T, Ishida R, Dogru M, Goto E, Matsumoto Y, Kaido M & Tsubota K (2005): The effect of autologous serum eyedrops in the treatment of severe dry eye disease: A prospective randomized case-control study. Am J Ophthalmol **139**: 242–246.

Küçükerdönmez C, Akova YA & Altinörs DD (2007): Comparison of conjunctival autograft with amniotic membrane transplantation for pterygium surgery. Cornea **26**: 407–413.

Kymionis GD, Mikropoulos DG, Portaliou DM, Boboridis KG, Voudouragkaki IC, Dragoumis ND et al. (2014): New perspectives on lamellar keratoplasty. Adv Ther **31**: 494–511.

Lambert N & Chamberlain W (2017): The structure and evolution of eye banking: a review on eye banks' historical, present, and future contribution to corneal transplantation. J Biorepository Sci Appl Med **Volume 5**: 23–40.

Le R, Yucel N, Khattak S, Yucel YH, Prud'homme GJ & Gupta N (2017): Current indications and surgical approaches to corneal transplants at the University of Toronto: A clinical-pathological study. Can J Ophthalmol **52**: 74–79.

Leveille AS, McMullan FD & Cavanagh HD (1983): Endophthalmitis following penetrating keratoplasty. Ophthalmology **90**: 38–9.

Li J, Koike-Soko C, Sugimoto J, Yoshida T, Okabe M & Nikaido T (2015): Human amnion-derived stem cells have immunosuppressive properties on NK cells and monocytes. Cell Transplant **24**: 2065–2076.

Liang L, Li W, Ling S, Sheha H, Qiu W, Li C et al. (2009): Amniotic membrane extraction solution for ocular chemical burns. Clin Exp Ophthalmol **37**: 855–863.

Lim LS, How AC, Ang LPK & Tan DTH (2009): Gundersen flaps in the management of ocular surface disease in an Asian population. Cornea **28**: 747–751.

Lind JT, Shute TS & Sheybani A (2017): Patch graft materials for glaucoma tube implants. Curr Opin Ophthalmol **28**: 194–198.

Linke SJ, Eddy M, Fricke OH, Bednarz J, Fricke OH, Wulff B et al. (2013): Thirty years of cornea cultivation: long-term experience in a single eye bank. Acta Ophthalmol **91**: 571–578.

Linke SJ, Fricke OH, Eddy M, Bednarz J, Druchkiv V, Kaulfers PM et al. (2013): Risk factors for donor cornea contamination: Retrospective analysis of 4546 procured corneas in a single eye bank. Cornea **32**: 141–148.

Liu T, Liu Y, Xie L, He X & Bai J (2013): Progress in the pathogenesis of pterygium. Curr Eye Res **38**: 1191–1197.

Maier P, Reinhard T & Cursiefen C (2013): Descemet stripping endothelial keratoplasty — rapid recovery of visual acuity. Dtsch Arztebl Int **110**: 365–371.

Malhotra C & Jain AK (2014): Human amniotic membrane transplantation: Different modalities of its use in ophthalmology. World J Transplant **4**: 111–21.

Mamede AC, Carvalho MJ, Abrantes AM, Laranjo M, Maia CJ & Botelho MF (2012): Amniotic membrane: From structure and functions to clinical applications. Cell Tissue Res **349**: 447–458.

Mathes KJ, Tran KD, Mayko ZM, Stoeger CG, Straiko MD & Terry MA (2019): Reports of post-keratoplasty infections for eye bank-prepared and non–eye bank-prepared corneas: 12 Years of Data From a Single Eye Bank. Cornea **38**: 263–267.

Matsumoto M, Suzuma K, Miyamura N, Imamura N & Kitaoka T (2011): Conjunctival swabs and corneoscleral rim cultures from corneal transplantation donors as possible early indicators for posttransplant endopthalmitis. Jpn J Ophthalmol **55**: 321–326.

Matthaei M, Hribek A, Clahsen T, Bachmann B, Cursiefen C & Jun AS (2019): Fuchs endothelial corneal dystrophy: clinical, genetic, pathophysiologic, and therapeutic aspects. Annu Rev Vis Sci **5**: 151–175.

McDonald MB, Sheha H, Tighe S, Janik SB, Bowden FW, Chokshi AR et al. (2018): Treatment outcomes in the DRy Eye Amniotic Membrane (DREAM) study. Clin Ophthalmol **12**: 677–681.

Meller D, Pauklin M, Thomasen H, Westekemper H & Steuhl K-P (2011): Amniotic membrane transplantation in the human eye. Dtsch Aerzteblatt Int **108**: 243–249.

Melles GRJ, Lander F, van Dooren BTH, Pels E & Beekhuis WH (2000): Preliminary clinical results of posterior lamellar keratoplasty through a sclerocorneal pocket incision. Ophthalmology **107**: 1850–1856.

Mentha J & WA F (2002): The sclera, the prion, and the ophthalmologist. Br J Opthtalmology **86**: 587–592.

Mian SI, Aldave AJ, Tu EY, Ayres BD, Jeng BH, Macsai MS et al. (2018): Incidence and outcomes of positive donor rim cultures and infections in the Cornea Preservation Time Study. Cornea **37**: 1102–1109.

Moffatt SL, Cartwright VA & Stumpf TH (2005): Centennial review of corneal transplantation. Clin Exp Ophthalmol **33**: 642–657.

Murri MS, Moshirfar M, Birdsong OC, Ronquillo YC, Ding Y & Hoopes PC (2018): Amniotic membrane extract and eye drops: A review of literature and clinical application. Clin Ophthalmol **12**: 1105–1112.

Nakamura T, Inatomi T, Sekiyama E, Ang LPK, Yokoi N & Kinoshita S (2006): Novel clinical application of membrane to treat patients with pterygium. Acta Ophthalmol Scand **84**: 401–405.

Noble BA, Loh RSK, MacLennan S, Pesudovs K, Reynolds A, Bridges LR et al. (2004): Comparison of autologous serum eye drops with conventional therapy in a randomised controlled crossover trial for ocular surface disease. Br J Ophthalmol **88**: 647–652.

O'Neil EC, Henderson B, Massaro-Giordano M & VY Bunya (2019): Advances in dry eye disease treatment. Curr Opin Ophthalmol **30**: 166–178.

Ogawa Y, Okamoto S, Mori T, Yamada M, Mashima Y, Watanabe R et al. (2003): Autologous serum eye drops for the treatment of severe dry eye in patients with chronic graft-versus-host disease. Bone Marrow Transplant **31**: 579–583.

Organización Nacional de Trasplantes (2018): Memoria de actividad de donación y trasplante de tejidos, España 2018. ONT. Madrid.

Palma-Carvajal F, Morales P, Salazar-Villegas A, Figueroa-Vercellino JP, Spencer F, Peraza-Nieves J et al. (2020): Trends in corneal transplantation in a single center in Barcelona, Spain. Transitioning to DMEK. J Fr Ophtalmol **43**: 1–6.

Pan HW, Zhong JX & Jing CX (2011): Comparison of fibrin glue versus suture for conjunctival autografting in pterygium surgery: a meta-analysis. Ophthalmology **118**: 1049–1054.

Pan Q, Angelina A, Zambrano A, Marrone M, Stark WJ, Heflin T et al. (2017): Autologous serum eye drops for dry eye. Cochrane Darabase Syst Rev **27**: CD009327.

Pant OP, Hao JL, Zhou DD, Pant M & Lu CW (2020): Tectonic keratoplasty using small incision lenticule extraction-extracted intrastromal lenticule for corneal lesions. J Int Med Res **48**: 300060519897668.

Park CY, Lee JK, Gore PK, Lim C & Chuck RS (2015): Keratoplasty in the United States: A 10-year review from 2005 through 2014. Ophthalmology **122**: 2432–2442.

Park E, Lewis K & Alghoul MS (2017): Comparison of efficacy and complications among various spacer grafts in the treatment of lower eyelid retraction: A systematic review. Aesthetic Surg J **37**: 743–754.

Patel S V (2012): Graft survival and endothelial outcomes in the new era of endothelial keratoplasty. Exp Eye Res **95**: 40–47.

Pezzotta S, Del Fante C, Scudeller L, Rossi GC, Perotti C, Bianchi PE et al. (2017): Long-term safety and efficacy of autologous platelet lysate drops for treatment of ocular GvHD. Bone Marrow Transplant **52**: 101–106.

Pikkel J, Porges Y & Ophir A (2001): Halting pterygium recurrence by postoperative 5-fluorouracil. Cornea **20**: 168–171.

Ple-plakon PA & Shtein RM (2014): Trends in corneal transplantation: indications and techniques. Curr Opin Ophthalmol **25**: 300–305.

Prabhasawat P, Barton K, Burkett G & Tseng SCG (1997): Comparison of conjunctival autografts amniotic membrane grafts, and primary closure for pterygium excision. Ophthalmology **104**: 974–985.

Price MO, Mehta JS, Jurkunas U V. & Price FW (2020): Corneal endothelial dysfunction: Evolving understanding and treatment options. Prog Retin Eye Res 100904.

Rahman I, Said DG, Maharajan VS & Dua HS (2009): Amniotic membrane in ophthalmology: Indications and limitations. Eye **23**: 1954–1961.

Rauen MP, Goins KM, Sutphin JE, Kitzmann AS, Schmidt GA & Wagoner MD (2012): Impact of eye bank lamellar tissue cutting for endothelial keratoplasty on bacterial and fungal corneoscleral donor rim cultures after corneal transplantation. Cornea **31**: 376–379.

Rauz S & Saw VP (2010): Serum eye drops, amniotic membrane and limbal epithelial stem cells-tools in the treatment of ocular surface disease. Cell Tissue Bank **11**: 13–27.

Röck D, Wude J, Bartz-Schmidt KU, Yoeruek E, Thaler S & Röck T (2017): Factors influencing the contamination rate of human organ-cultured corneas. Acta Ophthalmol **95**: e706–e712.

Röck T, Landenberger J, Bramkamp M, Bartz-schmidt KU & Röck D (2017): The Evolution of corneal transplantation. Ann Transplant **22**: 749–754.

Saad S, Saad R, Jouve L, Kallel S, Trinh L, Goemaere I, et al. (2020): Corneal crosslinking in keratoconus management. J Fr Ophtalmol **43**: 1078–1095.

Sabater-Cruz N & Casaroli-Marano RP (2019): Actualización en hemoderivados para el manejo de alteraciones de la superficie ocular. Ann d'Oftalmologia **27**: 17–23.

Safi H, Kheirkhah A, Mahbod M, Molaei S, Hashemi H & Jabbarvand M (2016): Correlations between histopathologic changes and clinical features in pterygia. J Ophthalmic Vis Res **11**: 153–158.

Sainz de la Maza M, Tauber J & Foster CS (1989): Scleral grafting for necrotizing scleritis. Ophthalmology **96**: 306–310.

Sanchez-Avila RM, Merayo-Lloves J, Riestra AC, Anitua E, Muruzabal F, Orive G et al. (2017): The effect of immunologically safe plasma rich in growth factor eye drops in patients with Sjögren syndrome. J Ocul Pharmacol Ther **33**: 391–399.

Sanchez-Avila RM, Merayo-Lloves J, Riestra AC, Fernandez-Vega Cueto L, Anitua E, Begoña L, et al. (2018): Treatment of patients with neurotrophic keratitis stages 2 and 3 with plasma rich in growth factors (PRGF-Endoret) eye-drops. Int Ophthalmol **38**: 1193–1204.

Sandri G, Bonferoni MC, Rossi S, Delfino A, Riva F, Cornaglia AI et al. (2016): Platelet lysate and chondroitin sulfate loaded contact lenses to heal corneal lesions. Int J Pharm **509**: 188–196.

Sangwan VS, Jain V & Gupta P (2007): Structural and functional outcome of scleral patch graft. Eye **21**: 930–935.

Seitz B, Das S, Sauer R, Mena D & Hofmann-Rummelt C (2009): Amniotic membrane transplantation for persistent corneal epithelial defects in eyes after penetrating keratoplasty. Eye **23**: 840–848.

Shahriari HA, Famil Tokhmehchi MR & Hashemi NF (2008): Comparison of the effect of amniotic membrane suspension and autologous serum on alkaline corneal epithelial wound healing in the rabbit model. Cornea **27**: 1148–1150.

Sharma N, Kaur M, Agarwal T, Sangwan VS & Vajpayee RB (2018): Treatment of acute ocular chemical burns. Surv Ophthalmol **63**: 214–235.

Sharma RA, Park JSY, Wang Y, Zhang T, Sharpen L, Dixon W et al. (2018): Association between positive corneal rim cultures and microbiology screening protocols in Ontario. Can J Ophthalmol **53**: 272–277.

Shtein RM, Shen JF, Kuo AN, Hammersmith KM, Li JY & Weikert MP (2020): Autologous serum-based eye drops for treatment of ocular surface disease: A report by the American Academy of Ophthalmology. Ophthalmology **127**: 128–133.

Şimşek C, Doğru M, Kojima T & Tsubota K (2018): Current management and treatment of dry eye disease. Turkish J Ophthalmol **48**: 309–313.

Singh NP, Said DG & Dua HS (2018): Lamellar keratoplasty techniques. Indian J Ophthalmol **66**: 1239–1250.

Singh R, Gupta N, Vanathi M & Tandon R (2019): Corneal transplantation in the mordern era. Indian J Med Res **150**: 7–22.

Siu GDJY, Kam KW & Young AL (2019): Amniotic membrane transplant for bullous keratopathy: Confocal microscopy & anterior segment optical coherence tomography. Semin Ophthalmol **34**: 163–167.

Soh YQ, Kocaba V, Pinto M & Mehta JS (2020): Fuchs endothelial corneal dystrophy and corneal endothelial diseases: East meets West. Eye **34**: 427–441.

Solans-Perez de Larraya, AM Salgado-Miranda A, Ortega-Molina M & Chaves-Samaniego J (2015): Plasma enriquecido en plaquetas: Una alternativa como tratamiento de la patología oftalmológica. Ann d'oftalmologia **1**:1-9.

Spierer O, Waisbourd M, Golan Y, Newman H & Rachmiel R (2016): Partial thickness corneal tissue as a patch graft material for prevention of glaucoma drainage device exposure. BMC Ophthalmol **16**: 5–10.

Sridhar MS, Mahesh S, Bansal AK, Nutheti R & Rao GN (2004): Pellucid marginal corneal degeneration. Ophthalmology **111**: 1102–1107.

Stefan P, Sanziana I, Liliana V, Vanessa P & Ciuluvica R (2017): Pseudophakic bullous keratopathy. Rom J Ophthalmol **61**: 90–94.

Stenwall PA, Bergström M, Seiron P, Sellberg F, Olsson T, Knutson F et al. (2015): Improving the anti-inflammatory effect of serum eye drops using allogeneic serum permissive for regulatory T cell induction. Acta Ophthalmol **93**: 654–657.

Sun YC, Kam J & Shen T (2018): Modified conjunctival flap as a primary procedure for nontraumatic acute corneal perforation. Tzu Chi Med J **30**: 24–28.

Tan DT, Chee SP, Dear KB & Lim AS (1997): Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision. Arch Ophthalmol **115**: 1235–1240.

Tan JCH, Holland SP, Dubord PJ, Moloney G, Mccarthy M & Yeung SN (2014): Evolving Indications for and trends in keratoplasty in British Columbia, Canada, from 2002 to 2011: A 10-year review. Cornea **33**: 252–256.

Terry MA (2012): Endothelial keratoplasty: Why aren't we all doing Descemet membrane endothelial keratoplasty? Cornea **31**: 469–471.

Than J, Balal S, Wawrzynski J, Nesaratnam N, Saleh GM, Moore J et al. (2017): Finger-prick autologous blood: A novel treatment for dry eye syndrome. Eye **31**: 1655–1663.

Thomasen H, Pauklin M, Klaus-Peter S & Meller D (2009): Comparison of cryopreserved and air-dried human amniotic membrane for ophthalmologic applications. Graefe's Arch Clin Exp Ophthalmol **247**: 1691–1700.

Tighe S, Moein HR, Chua L, Cheng A, Hamrah P & Tseng SCG (2017): Topical cryopreserved amniotic membrane and umbilical cord eye drops promote re-epithelialization in a murine corneal abrasion model. Investig Ophthalmol Vis Sci **58**: 1586–1593.

Toker E & Eraslan M (2016): Recurrence after primary pterygium excision: Amniotic membrane transplantation with fibrin glue versus conjunctival autograft with fibrin glue. Curr Eye Res **41**: 1–8.

Tourtas T, Laaser K, Bachmann BO, Cursiefen C & Kruse FE (2012): Descemet membrane endothelial keratoplasty versus Descemet stripping automated endothelial keratoplasty. Am J Ophthalmol **153**: 1082–1090.

Tsoukanas D, Xanthopoulou P, Charonis AC, Theodossiadis P, Kopsinis G & Filippopoulos T (2016): Heterologous, fresh, human donor sclera as patch graft material in glaucoma drainage device surgery. J Glaucoma **25**: 558–564.

Tsubota K, Goto E, Shimmura S & Shimazaki J (1999): Treatment of persistent corneal epithelial defect by autologous serum application. Ophthalmology **106**: 1984–1989.

Tsui E, Luong PM, Fogel J, Fogel ES & Zegans ME (2019): Microbial analysis of donor corneoscleral rims and storage media. Ocul Immunol Inflamm **27**: 817–820.

Urzua CA, Vasquez DH, Huidobro A, Hernandez H & Alfaro J (2012): Randomized double-blind clinical trial of autologous serum versus artificial tears in dry eye syndrome. Curr Eye Res **37**: 684–688.

Versura P, Buzzi M, Giannaccare G, Grillini M, Terzi A, Pagliaro P et al. (2014): Cord blood serum-based eye drops: The impact of donor haematological and obstetric factors on the variability of epidermal growth factor levels. Blood Transfus **12**:.

Vislisel JM, Goins KM, Wagoner MD, Schmidt GA, Aldrich BT, Skeie JM et al. (2017): Incidence and outcomes of positive donor corneoscleral rim fungal cultures after keratoplasty. Ophthalmology **124**: 36–42.

Walkden A (2020): Amniotic membrane transplantation in ophthalmology: An updated perspective. Clin Ophthalmol **14**: 2057–2072.

Wang L, Cao K, Wei Z, Baudouin C, Labbé A & Liang Q (2020): Autologous serum eye drops versus artificial tear drops for dry eye disease: a systematic review and meta-analysis of randomized controlled trials. Ophthalmic Res **63**: 443–451.

Wang X, Zhang Y, Zhou L, Wei R & Dong L (2017): Comparison of fibrin glue and Vicryl sutures in conjunctival autografting for pterygium surgery. Mol Vis **23**: 275–285.

Wei Y & Asbell PA (2014): The core mechanism of dry eye disease is inflammation. Eye Contact Lens **40**: 248–256.

Wen JC, Lam J & Banitt MR (2018): Scleral patch graft with a suture reinforcement Technique in surgical management of necrotizing scleritis with ectasia. Cornea **37**: 933–935.

Wilhelmus KR & Hassan SS (2007): The prognostic role of donor corneoscleral rim cultures in corneal transplantation. Ophthalmology **114**: 440–445.

Woodward MA, Titus M, Mavin K & Shtein RM (2012): Corneal donor tissue preparation for endothelial keratoplasty. J Vis Exp **64**: e3847.

Yaisawang S & Piyapattanakorn P (2003): Role of post-operative topical corticosteroids in recurrence rate after pterygium excision with conjunctival autograft. J Med Assoc Thai **86 Suppl 2**: S215--23.

Yeu E, Goldberg DF, Mah FS, Beckman KA, Luchs JI, Solomon JD et al. (2019): Safety and efficacy of amniotic cytokine extract in the treatment of dry eye disease. Clin Ophthalmol **13**: 887–894.

Yilmaz U, Küçük E, Koç Ç & Gökler E (2017): Comparison of autologous serum versus preservative free artificial tear in patients with dry eyes due to systemic isotretinoin therapy. Curr Eye Res **42**: 827–831.

Yoon KC (2014): Use of umbilical cord serum in ophthalmology. Chonnam Med J **50**: 82–85.

Yoon KC, Heo H, Im SK, You IC, Kim YH & Park YG (2007): Comparison of autologous serum and umbilical cord serum eye drops for dry eye syndrome. Am J Ophthalmol **144**:86-92.

Yoon KC, Jeong IY, Im SK, Park YG, Kim HJ & Choi J (2007): Therapeutic effect of umbilical cord serum eyedrops for the treatment of dry eye associated with graft-versus-host disease. Bone Marrow Transplant **39**: 231–235.

Yu AC, Mattioli L & Busin M (2020): Optimizing outcomes for keratoplasty in ectatic corneal disease. Curr Opin Ophthalmol **31**: 268–275.

Zanetti E, Bruni A, Mucignat G, Camposampiero D, Frigo AC & Ponzin D (2005): Bacterial contamination of human organ-cultured corneas. Cornea **24**: 603–607.

Zare M, Javadi MA, Einollahi B, Karimian F, Rafie AR, Feizi S et al. (2012): Changing indications and surgical techniques for corneal transplantation between 2004 and 2009 at a tertiary referral center. Middle East Afr J Ophthalmol **19**: 323–329.

Zemba M, Stamate A-C, Tataru C, Branisteanu D & Balta F (2020): Conjunctival flap surgery in the management of ocular surface disease (Review). Exp Ther Med 3412–3416.

Zhang AQ, Rubenstein D, Price AJ, Elie C, Levitt M, Sharpen L et al. (2013): Evolving surgical techniques of and indications for corneal transplantation in Ontario: 2000 - 2012. Can J Ophthalmol **48**: 153–159.

Zhang X, Munir SZ, Sami Karim SA & Munir WM (2021): A review of imaging modalities for detecting early keratoconus. Eye **35**: 173–187.