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# Dérégulation de l'expression du Récepteur aux Produits Avancés de Glycation (RAGE) dans la pathologie de la Surface oculaire

Valentin Navel

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UNIVERSITÉ CLERMONT AUVERGNE

ÉCOLE DOCTORALE DES SCIENCES DE LA VIE, SANTÉ, AGRONOMIE, ENVIRONNEMENT

THÈSE

Présentée à l'Université Clermont Auvergne  
Pour l'obtention du grade de Docteur  
(Décret du 5 Juillet 1984)

NAVEL Valentin

Spécialité Sciences de la Vie et Santé

Présentée et soutenue publiquement le 30 Mai 2023

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(RAGE) dans la pathologie de la Surface oculaire.

**Rapporteurs :**

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Equipe « Approches translationnelles des lésions épithéliales et de leur réparation »  
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# Liste des abréviations

(par ordre d'apparition)

**RAGE** : Receptor for Advanced Glycation End products

**PRRs** : Pattern-Recognition Receptors

**fl-RAGE** : full-length RAGE

**kDa** : kilo Dalton

**ctRAGE** : domaine cytoplasmique de RAGE

**FH1** : Formin Homology domain 1

**DIAPH1** : mammalian form of diaphanous 1

**sRAGE** : soluble RAGE

**esRAGE** : endogenous secretory RAGE

**MMPs** : Matrix Metalloproteinases

**ITG  $\alpha$**  : Intégrines  $\alpha$

**AGE** : Advanced Glycation End products

**HMGB1**: High mobility group box 1

**ADN** : Acide désoxyribonucléique

**ARNm** : Acide ribonucléique messenger

**Ca<sup>2+</sup>** : Calcium

**FGFR1** : Fibroblast growth factor receptor 1

**TNF**: Tumor Necrosis factor

**IL**: Interleukin

**LPA** : acide lysophosphatidique

**A $\beta$**  : protéine amyloïde beta

**C1q** : protéines du complément

**DIAPH1** : Protein diaphanous homolog 1

**AKT** : sérine/thréonine kinase

**NADPH** : Nicotinamide adenine dinucleotide phosphate

**Ras/MEK/ERK** : Ras/mitogen-activated protein kinase

**MAPK** : Mitogen-activated protein kinase

**JAK/STAT** : Janus kinase/ transducteur de signal et activation de la transduction

**AP-1** : protéine activatrice 1

**HES** : Hématoxyline-éosine-safran

**MUC** : mucine

**$\mu$ m** : micromètre

**CD8** : cluster de différenciation 8

**$\mu$ l** : microlitre

**EGF** : Epithelial Growth Factor

**TGF- $\beta$**  : Transforming growth factor-beta

**KC** : kératocône

**CH** : Corneal Hysteresis

**CRF** : Corneal Resistance Factor

**INF- $\gamma$**  : Interféron  $\gamma$

**CXL** : Cross-linking cornéen

**UV** : ultra-violet

**DED** : Dry Eye Disease

**DEWS** : International Dry Eye Workshop

**LFU** : Lacrymal Functional Unit

**DMLA** : Dégénérescence maculaire liée à l'âge

**NVU** : Neurovascular Unit

**VEGF** : Vascular Endothelial Growth Factor

**GPAO** : Glaucome primitif à angle ouvert

**MIP** : Macrophage Inflammatory Proteins

**HCE** : Human corneal epithelial

**MD** : Membrane de Descemet

**HSV** : Herpes simplex virus

**NFAT5** : Nuclear factor of activated T-cells

5

**KO** : Knock Out

**Règle des 3R** : Réduire, Raffiner, Remplacer

**°C** : degrés Celsius

**mg/kg** : milligramme par kilogramme

**SOD** : Superoxyde dismutase

**RT-qPCR** : Reverse transcription quantitative polymerase chain reaction

**LTF** : Lactoferrine

**mg/ml** : milligramme par millilitre

**Fe<sup>3+</sup>** : Fer

**ALDHases** : protéines aldéhyde oxydo-réactions

**CXCL8** : IL-8

**Vit A** : vVitamine A

**TIMP-1** : Metallopeptidase inhibitor 1

**K<sub>max</sub>** : Kératométrie maximale

**D** : Dioptrie

**RAP** : RAGE antagonist peptide

**COVID-19** : Coronavirus 19

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# 1. Le Récepteur aux Produits Avancés de Glycation : RAGE

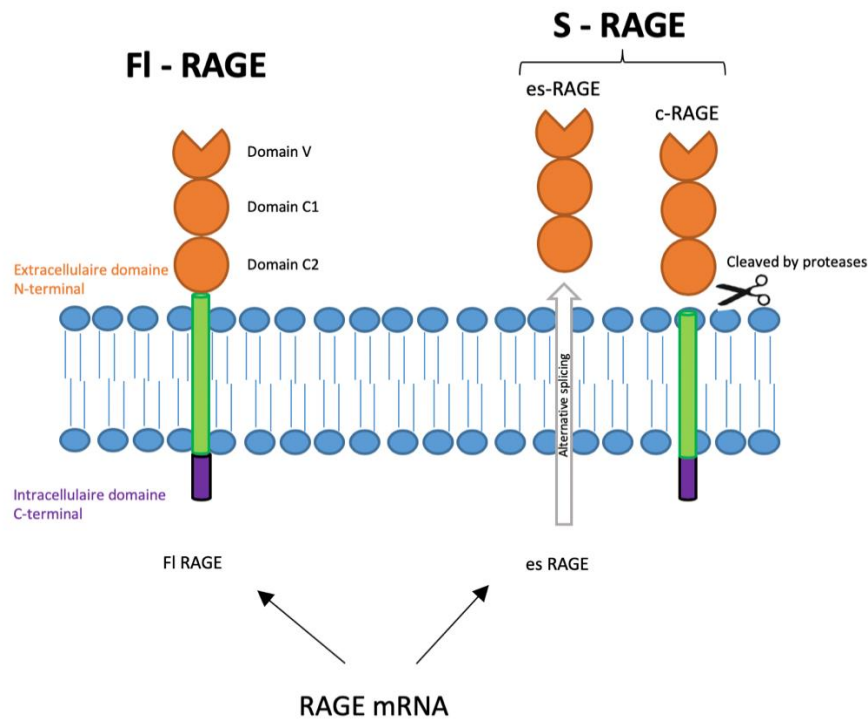
## 1.1 Les différentes isotypes de RAGE

Le Récepteur aux Produits Avancés de Glycation (Receptor for Advanced Glycation End products, RAGE) est un récepteur transmembranaire non spécifique, ubiquitaire, de la famille des immunoglobulines, qui se présente sous de multiples isoformes.<sup>1</sup> Il a été décrit comme reconnaissant des motifs moléculaires pro-inflammatoires et appartient, de ce fait, au groupe des « pattern-recognition receptors » (PRRs).<sup>2,3</sup> Dans le génome humain, le gène RAGE est situé dans le locus du complexe majeur d'histocompatibilité dans la région de classe III du chromosome 6 (6p21.3), une région connue pour être dense en gènes impliqués dans les réactions pro-inflammatoires.<sup>4</sup>

Dans sa forme complète nommée full-length RAGE (fl-RAGE), variant de 35 à 55 kDa selon les tissus, il est constitué de trois domaines majeurs : un domaine extracellulaire (comprenant un sous-domaine variable V et deux domaines constants C1 et C2) permettant la fixation des ligands, un domaine transmembranaire et un domaine cytoplasmique permettant la transduction intracellulaire du signal.<sup>5</sup> Le domaine cytoplasmique de RAGE (ctRAGE) joue un rôle majeur dans la transduction du signal intracellulaire via le domaine FH1 (formin homology domain 1) de DIAPH1 (mammalian form of diaphanous 1), l'un de ses adaptateurs.<sup>6</sup>

Il existe des formes tronquées de RAGE : il s'agit de formes solubles produites par clivage de la partie extracellulaire du récepteur transmembranaire fl-RAGE (dénommées soluble RAGE ou sRAGE), ou grâce à un épissage alternatif pour la forme esRAGE (pour endogenous secretory RAGE). Les formes clivées par mécanisme protéolytique (sRAGE) impliquent des protéases de la famille des matrix metalloproteinases (MMPs), des metalloproteinases (ADAM-10), ou des intégrines  $\alpha$  (ITG  $\alpha$ ). Ces formes solubles de RAGE peuvent jouer le rôle de "leurre" en séquestrant les ligands, empêchant ainsi l'interaction de

ceux-ci avec la forme transmembranaire fl-RAGE et prévenant ainsi son activation.<sup>7</sup> (Figure 1)



**Figure 1.** RAGE et ses isoformes (d'après Navel *et al.* Dysregulation of Receptor for Advanced Glycation End Products (RAGE) in Keratoconus).<sup>8</sup>

## 1.2 Les ligands de RAGE

Les ligands de RAGE sont issus de processus biologiques divers, tant exogènes qu'endogènes, souvent secondaires à une agression cellulaire, et appelés de façon générique alarmines. Il en existe un très grand nombre, aux caractéristiques et origines moléculaires divers, mais il est classique d'en décrire trois grandes familles : les produits de glycation avancée (Advanced Glycation End products, AGE), les amphotérines dont HMGB1, et les calgranulines dont les protéines S100.

Les AGEs sont générés par la glycosylation non enzymatique et l'oxydation de protéines, d'acides nucléiques ou de lipides par la réaction de Maillard.<sup>9</sup> Les AGEs peuvent ainsi être générés dans les tissus par excès d'apport exogène en glucides et protéines, ou encore

par les fumées de tabac.<sup>10</sup> Les AGEs ainsi produits vont altérer l'intégrité et les fonctions d'échange des membranes cellulaires, favoriser la réponse inflammatoire non-spécifique via l'activation de récepteur multi-ligands comme RAGE, et induire in-fine la production de cytokines pro-inflammatoires et d'espèces réactives de l'oxygène (stress oxydant).<sup>11</sup> De fait, un lien fort a été établi entre l'accumulation d'AGEs dans l'organisme et la survenue de pathologies telles que le diabète, les pathologies cardiovasculaires, les pathologies neurodégénératives, et les néoplasies cancéreuses.<sup>9,12-14</sup>

HMBG1, pour High Mobility Group Box 1 en lien avec sa forte mobilité dans les gels d'électrophorèse, a été le premier ligand identifié non-AGE pouvant interagir avec RAGE.<sup>15</sup> Il est retrouvé de manière ubiquitaire dans le corps humain (cerveau, cœur, poumon, foie, système vasculaire etc..), et peut être mis en évidence aussi bien dans le noyau que dans le cytoplasme des cellules, ou dans le milieu extra-cellulaire.<sup>16</sup> Dans le noyau cellulaire, HMGB1 est impliqué dans les processus de réparation de l'ADN, la stabilité chromosomique et, via Beclin-1, l'autophagie.<sup>17</sup> Dans le milieu extra-cellulaire, HMGB-1 est un ligand des isoformes de RAGE, et des récepteurs Toll-like, impliqués dans les réponses inflammatoires non-spécifiques (libération de cytokines pro-inflammatoires et apoptose) et spécifiques (maturation des cellules de l'immunité).<sup>18</sup>

La famille des protéines S100 (S100A1, S100A18, S100B, S100G ...) regroupe un très large groupe de protéines de liaison au calcium (Ca<sup>2+</sup>), impliquées dans la régulation de divers processus intra et extracellulaire.<sup>19</sup> En intracellulaire, les protéines S100 ont pour action de changer la conformation moléculaire de facteurs de transcription, via l'adjonction ou le retrait de Ca<sup>2+</sup>, régulant ainsi l'expression du génome, l'activation enzymatique, le cycle cellulaire, et la réponse inflammatoire.<sup>20</sup> En extracellulaire, les protéine S100 ont un rôle autocrine et paracrine, interagissant avec de nombreux récepteurs tels que RAGE, les récepteurs couplés aux protéines G, les récepteurs Toll-like, les récepteurs des facteurs de croissance des

fibroblastes de type 1 (fibroblast growth factor receptor 1 (FGFR1)). De fait, elles agissent sur la transcription de médiateurs de l'inflammation (TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and IL-8), le stress oxydant, et l'apoptose cellulaire.<sup>21</sup>

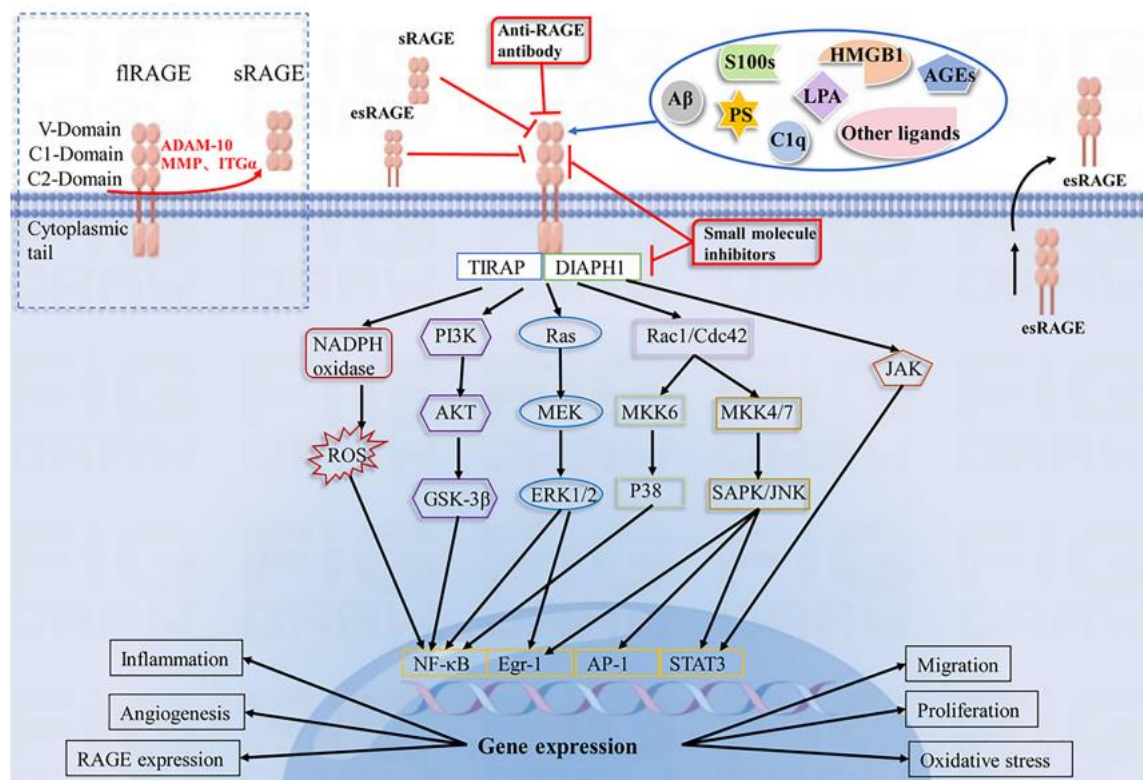
On citera également comme ligands de RAGE : l'acide lysophosphatidique (LPA) issue de la dégradation des acides gras saturés et insaturés ;<sup>22</sup> la protéine amyloïde beta (A $\beta$ ) impliquées dans la pathophysiologie de la maladie d'Alzheimer ;<sup>23</sup> la phosphatidylsérine jouant un rôle de reconnaissance au niveau des membranes cellulaires dans les processus de phagocytose des macrophages ;<sup>24</sup> et enfin les protéines du complément (C1q) sécrétées en majorité par les phagocytes et régulant l'activité des cellules de l'immunité adaptative.<sup>25</sup>

### **1.3 Les voies d'activation intracellulaire de RAGE**

L'activation de la voie intracellulaire de RAGE passe par la reconnaissance du couple fl-RAGE–ligand au niveau de la portion extracellulaire du récepteur transmembranaire.<sup>26</sup> La deuxième étape clé de la transduction du signal intervient au niveau du couple ctRAGE-DIAPH1, impliquant des mécanismes complexes de phosphorylation par des protéines kinases comme AKT (sérine/thréonine kinase, appelée également protéine kinase B).<sup>6</sup> Une fois activée par phosphorylation, le couple ctRAGE-DIAPH1 enclenche la cascade d'activation intracellulaire via de nombreuses voies telles que : NADPH oxydase, Ras/mitogen-activated protein kinase (Ras/MEK/ERK1/2), mitogen-activated protein kinase (MAPK/P38), PI3K/AKT, janus kinase/ transducteur de signal et activation de la transduction (JAK/STAT).<sup>27,28</sup> Il en résulte l'activation de facteurs de transcription (i.e. NF- $\kappa$ B, STAT-3, protéine activatrice 1 (AP-1)), induisant la synthèse de molécules pro-inflammatoire type IL-1, IL-6 ou TNF- $\alpha$ .<sup>26</sup> (Figure 2)

In fine, l'activation de la voie RAGE via la libération extracellulaire de facteurs pro-

inflammatoires, pro-thrombotiques, pro-apoptotiques, et vasoconstricteurs, entraîne un rétrocontrôle positif sur sa propre voie d'activation (e.g. augmentation de l'expression de fl-RAGE).<sup>1</sup> A contrario, dans certains tissus, un rétrocontrôle négatif peut se mettre en place via le clivage protéolytique de fl-RAGE à la surface des cellules (création de sRAGE), ou par la sécrétion de es-RAGE obtenue après traduction d'un ARNm découlant de l'épissage alternatif, augmentant alors la concentration extracellulaire de leurs solubles de RAGE (sRAGE et es-RAGE).<sup>29</sup> Ces différents mécanismes d'activations ou de rétrocontrôle négatif de la voie RAGE rendent compte de la complexité de cette voie d'activation cellulaire, tantôt pro-inflammatoire dans certaines conditions physiopathologiques (i.e. diabète, pathologies cardiovasculaires, cancers),<sup>30-32</sup> tantôt anti-inflammatoire (i.e. bronches et poumons de fumeurs).<sup>33</sup>



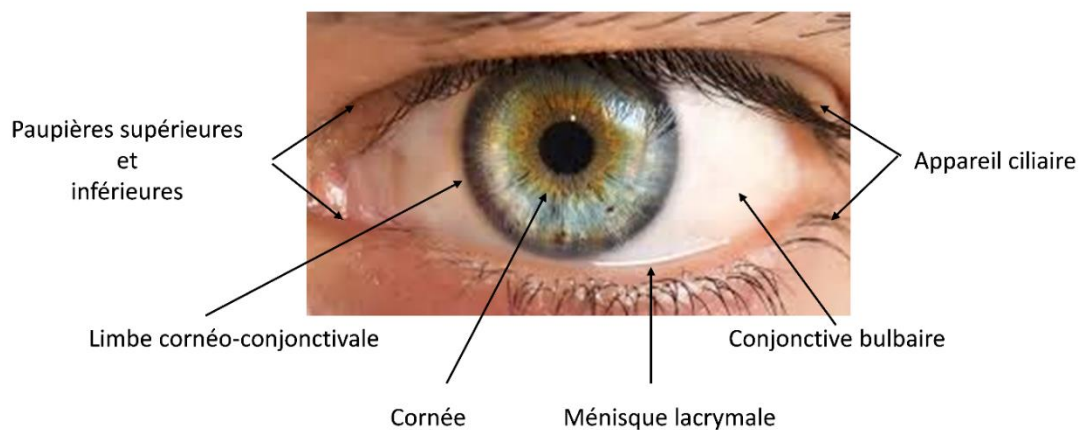
**Figure 2.** RAGE et ses voies d'activation intracellulaire (d'après Dong *et al.* Pathophysiology of RAGE in inflammatory diseases, *Frontiers in Immunology*, 2022).<sup>26</sup>

## 2. Kératocône et syndrome sec oculaire : des pathologies de la Surface Oculaire

### 2.1 Le concept de Surface Oculaire

La notion de surface oculaire regroupe un ensemble de tissus barrières de l'œil et de la face, aux origines et fonctions très divers, interreliés par une fonction anatomo-physiologique commune qui est celle de protéger l'œil des agressions du monde extérieur. A l'instar d'organes « frontières » du corps humain, comme la peau ou le tube digestif, le concept de surface oculaire renvoie aux notions fondamentales de surface d'échange avec notre environnement, de protection des agressions microbiologiques ou physico-chimiques, ou encore de qualité de vision via la nature du dioptré oculaire.<sup>34</sup>

Elle présente à décrire la conjonctive, la cornée, le limbe, le film lacrymal, et les paupières. (Figure 3)

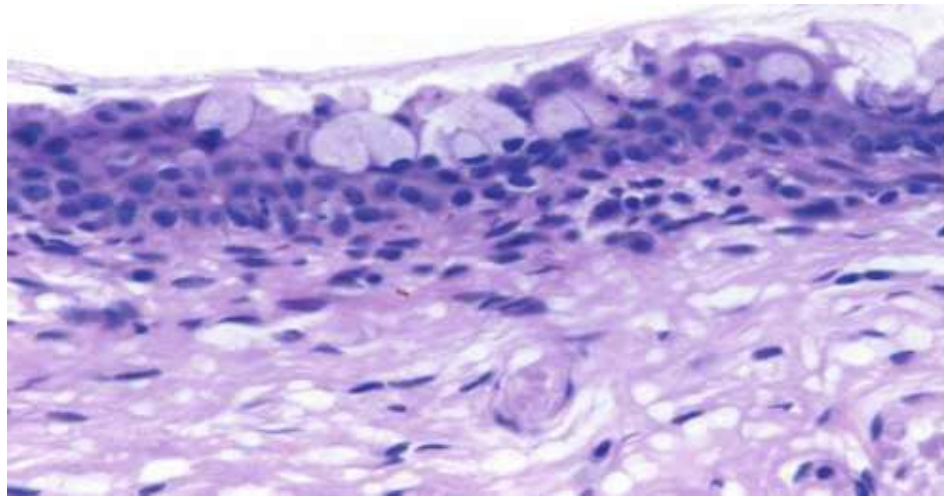


**Figure 3.** Les composants de la surface oculaire.

#### 2.1.1 La conjonctive

La conjonctive est une structure muqueuse disposée à la face antérieure du globe oculaire et à la face postérieure des paupières. Elle est richement vascularisée, contient les cellules à mucus essentielles au film lacrymal et participe à la veille immunitaire de l'organisme en hébergeant les cellules de l'immunité innée et acquise.<sup>35</sup>

Elle est subdivisée en trois parties : la conjonctive palpébrale, tendue de l'arrière des orifices des glandes de Meibomius au fornix, le fornix ou culs de sacs conjonctivaux, zone de transition entre paupière et globe oculaire, et la conjonctive bulbaire, tendue du fornix au limbe. Histologiquement, la conjonctive est formée par un épithélium pluristratifié contenant des cellules à mucus, reposant sur une membrane basale et un stroma à tissu conjonctif lâche.<sup>34</sup>



**Figure 4** : Coupe histologique (HES  $\times 25$ ) conjonctive bulbaire, Surface Oculaire, rapport de la Société française d'Ophthalmologie, 2015.<sup>34</sup>

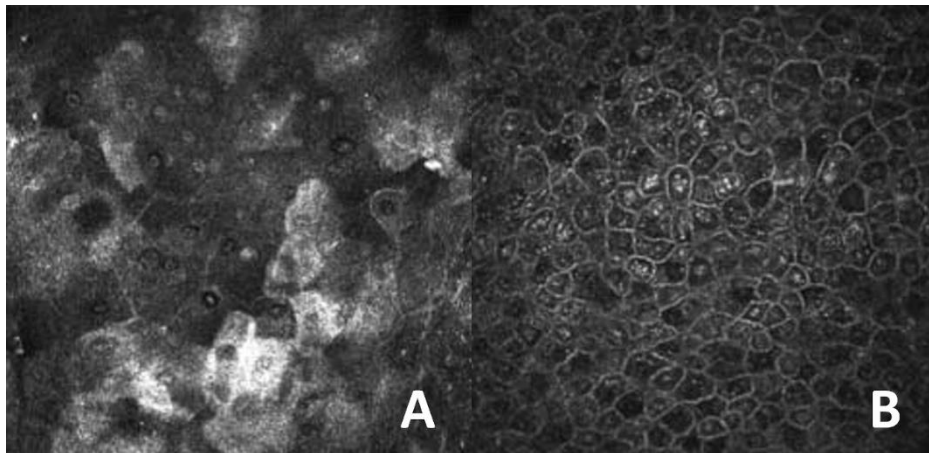
Les cellules à mucus sont responsables de la synthèse de mucines, notamment MUC5AC et MUC2, dont le rôle fondamental au sein du film lacrymal est de recouvrir la surface hydrophobe des cellules épithéliales cornéennes afin de les relier à la phase aqueuse des larmes et d'en assurer la propriété visqueuse.<sup>36</sup>

### 2.1.2 La cornée

La cornée, composante majeure du dioptré oculaire avec le film lacrymal, est en continuité avec la conjonctive bulbaire via le limbe cornéo-conjonctivale. Elle se compose de 5 couches : l'épithélium cornéen, la membrane de Bowman, le stroma, la membrane de

Descemet, et l'endothélium cornéen. Certains décrivent une sixième couche située entre le stroma postérieur et la membrane de Descemet, appelée couche de Dua.<sup>37</sup>

L'épithélium cornéen s'organise en 5 à 7 couches cellulaires, il est de type pavimenteux stratifié. La couche basale cylindrique monocellulaire repose sur une fine membrane basale séparant l'épithélium de la membrane de Bowman. La couche intermédiaire représente un état de transition avec des cellules polygonales réparties en 2 à 4 assises en cours de différenciation dans leur migration vers la surface. La couche superficielle compte 2 à 3 couches cellulaires différenciées, de forme allongée et aplatie. En contact intime avec le film lacrymal, l'épithélium cornéen est une surface d'échange importante et constitue le dioptré principal du système oculaire.<sup>15</sup>



**Figure 5.** Coupe en microscopie confocale de la couche superficielle (A) et de la couche basale (B) de l'épithélium cornéen, Surface Oculaire, rapport de la Société française d'Ophtalmologie, 2015.<sup>34</sup>

Le stroma cornéen représente la plus grande partie de l'épaisseur cornéenne, avec presque 400µm soit 90% de son épaisseur totale. A sa partie la plus superficielle, la membrane de Bowman est dépourvue de cellules et a une orientation de ses fibres de collagènes différentes du reste du stroma.<sup>38</sup> Le stroma, avasculaire, est composé presque exclusivement de collagène de type I et V, organisé en fibrilles de même taille et parallèles entre elles. L'orientation



géométrique des fibrilles de collagènes, ainsi que leur diamètre inférieur à la moitié de la longueur d'onde de la lumière, revêtent une importance fondamentale pour la transparence de la cornée et la transmission de la lumière. Rares et disséminés entre les fibres de collagènes, les kératocytes ont pour fonctions la biosynthèse du collagène et de la substance fondamentale.<sup>34</sup>

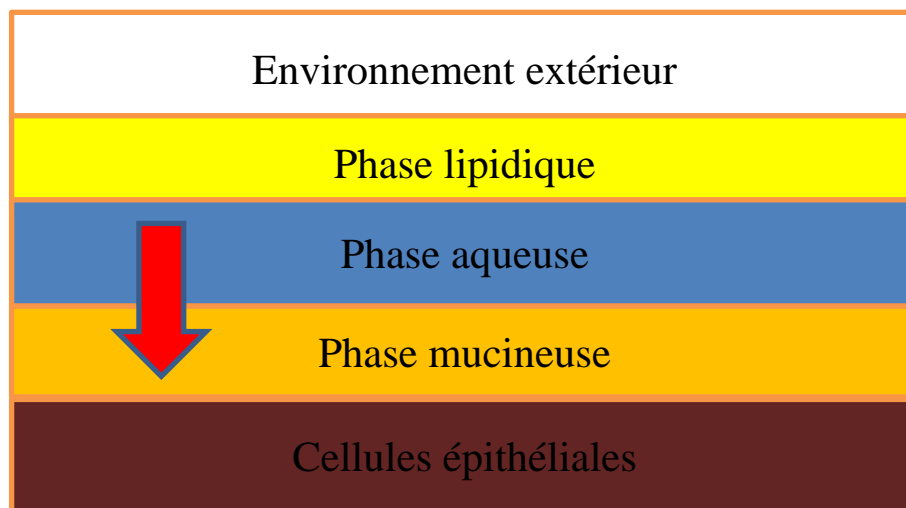
L'endothélium cornéen est une couche unique de cellules polygonales reposant sur la membrane de Descemet. Cette couche cellulaire, bloquée en phase G2 de la mitose, a un rôle fondamental de régulation des mouvements d'eau dans la cornée, permettant un état de déturgescence cornéenne propice à sa transparence.<sup>34</sup>

### **2.1.3 Le limbe**

Anatomiquement, le limbe constitue la jonction entre les tissus conjonctivo-scléaux, vascularisés, et la cornée, avasculaire. L'importance du limbe se résume par ses fonctions fondamentales de renouvellement de l'épithélium cornéen, de réservoir immunitaire, et d'apport nutritif pour la cornée via sa richesse en anastomose vasculaire. Le limbe abriterait ainsi un grand nombre de cellules souches de l'épithélium cornéen au niveau de cryptes situées dans les palissades de Vogt.<sup>39</sup> La théorie actuelle du renouvellement des cellules épithéliales cornéennes implique une répartition des cellules souches sur l'ensemble de la surface cornéenne au niveau de la membrane basale, associée à une réserve limbique au niveau des cryptes.<sup>40</sup> Sa richesse en apport vasculaire et lymphatique lui permet une fonction de veille immunitaire de la surface oculaire : les cellules de l'immunités innées (macrophages, cellules dendritiques) et spécifiques (lymphocytes CD8+) sont retrouvées en grand nombre dans l'épithélium cornéoscléral.<sup>41</sup>

### **2.1.4 Le film lacrymal**

Le film lacrymal est organisé schématiquement en 3 couches : la couche lipidique en superficialité, la couche aqueuse, puis la couche mucineuse donc la concentration en mucines augmente vers la profondeur pour former le glycocalyx, étroitement lié aux cellules superficielles cornéo-conjonctivales. Cette description en 3 couches est évidemment schématique, puisqu'un large continuum existe entre les différentes phases du film lacrymal.<sup>42</sup> (Figure 6)



**Figure 6.** Répartition schématique des différentes couches du film lacrymal. Gradient de concentration en mucines (flèche rouge).

D'un volume d'un peu moins de 10 $\mu$ l, la qualité réfractive et protectrice du film lacrymal dépend de la sécrétion des glandes lacrymales, de son étalement par le balayage palpébral, de sa composition, de son évaporation et de son évacuation par les voies excrétrices lacrymonasales.<sup>42,43</sup> La phase superficielle est de composition lipidique, avec une partie interne faite d'acides gras à longues chaînes et une partie externe faite d'esters de cholestérol et de dérivés des triglycérides. L'ensemble confère à cette couche hydrophobe un rôle mécanique résistif à la rupture et de stabilisation.<sup>42</sup> Cette phase lipidique est alimentée en continue par la sécrétion des glandes de Meibomius, contenues à l'intérieur même du tarse et au nombre d'une

trentaine par paupière, dont l'orifice excrétoire est situé en arrière de l'implantation des cils. Les glandes de Zeiss et de Moll sont, quant à elles, annexées aux follicules pileux et ont un rôle secondaire dans cette sécrétion.<sup>44</sup>

La couche mucino-aqueuse représente la majeure partie des 4µm d'épaisseur en centrale du film lacrymal. Elle est riche en protéines dont des enzymes et des mucines, des hormones, des facteurs de croissance, des cellules desquamées et d'autres cellules inflammatoires et immunitaires.<sup>45</sup> La protection immunitaire de la surface oculaire est permise par les substances dissoutes comme les IgA, la lactoferrine, le lysozyme et les cystatines. C'est la glande lacrymale principale, située en supéro-externe de la cavité orbitaire dont le canal excréteur s'abouche par 4 à 7 canaux dans le fornix supérieur, qui est responsable de la sécrétion de 99% de ces protéines.<sup>34</sup> On décrit également les glandes accessoires de Krause et Wolfring réparties dans le stroma conjonctival. Considérant le rôle fondamental antimicrobien de la phase aqueuse, elle possède un rôle trophique non moins majeur sur l'épithélium cornéen et conjonctival par la présence de facteurs de croissance comme l'EGF et le TGF-β.<sup>42</sup> La couche muqueuse permet l'amarrage de la phase hydrophile de la couche aqueuse au niveau de la surface hydrophobe des membranes cellulaires de l'épithélium conjonctival et cornéen superficiel. Elle est formée de mucines formant un gel de glycocalyx au contact intime avec ces cellules. Cette phase profonde est sécrétée par les cellules à mucus réparties dans l'épithélium conjonctival.<sup>46</sup>

### **2.1.5 Les paupières**

Les paupières concourent à l'étalement et au renouvellement du film lacrymal. Assurant la fermeture de la fente palpébrale, elles protègent de la dessiccation et des agressions mécaniques l'ensemble du globe oculaire et permet l'évacuation du trop-plein de larmes par la pompe lacrymale vers les points lacrymaux qui constituent le début du canal lacrymo-nasal.

Anatomiquement, on lui décrit une lamelle antérieure, musculo-cutanée, et une lamelle postérieure, tarso-muqueux, dont la limite est visible en la présence de la ligne grise au niveau du bord libre qui n'est autre que le muscle de Riolan vu par transparence.<sup>34</sup> Une notion importante en dynamique palpébrale est celle de pompe lacrymale : elle permet l'évacuation harmonieuse de l'excédent de larmes du lac lacrymal au niveau du canthus médial à chaque clignement par effet de succion des points lacrymaux. Ceci est permis par l'insertion des chefs profonds de la portion pré-tarsale de l'orbiculaire en arrière de la crête lacrymale postérieure, se dirigeant vers les canalicules en les enserrant. Toute anomalie musculaire ou d'élasticité des paupières va inmanquablement retentir de façon négative sur la qualité du film lacrymal et la trophicité de la surface oculaire.<sup>47</sup>

## **2.2 Le Kératocône**

### **2.2.1 Définition & Epidémiologie**

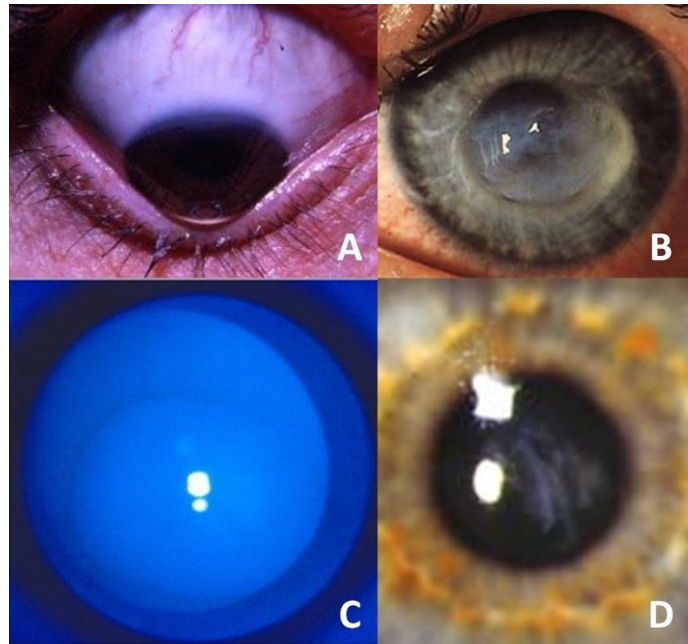
Le kératocône (KC) est historiquement défini comme une pathologie non inflammatoire et ectasique de la cornée.<sup>48</sup> Caractérisé par une déformation en cône et un amincissement progressif de la cornée, le KC est à l'origine de l'apparition d'une protrusion cornéenne et d'un astigmatisme irrégulier conduisant à une diminution de l'acuité visuelle de façon chronique, progressive et irréversible sans correction optique spécifique.<sup>49</sup>

Il s'agit d'une maladie le plus souvent bilatérale (dans plus de 95 % des cas) et asymétrique, avec un délai de survenue et d'aggravation variable entre les deux yeux d'un individu atteint. Cette affection touche indifféremment les deux sexes, et concerne environ 1 personne sur 2 000, avec une légère prédominance masculine et une incidence stable.<sup>50</sup> Même s'il s'agit d'une maladie rare, le KC est toutefois désigné comme la maladie de la cornée la plus fréquente en population générale. La prévalence rapportée du KC varie considérablement en fonction de

l'emplacement géographique, des critères de diagnostic utilisés et de la cohorte de patients sélectionnés. Ainsi, la prévalence du KC dans les études peut varier de 0.2 cas pour 100.000 habitants en Russie, à 3300 cas pour 100.000 habitants en Iran.<sup>51</sup> En France, la prévalence est estimée à environ 50 cas pour 100 000 habitants.<sup>52</sup>

L'âge moyen de découverte du KC est de  $27,3 \pm 9,5$  ans avec près de 90% des patients diagnostiqués entre 10 et 39 ans. Classiquement, la maladie est diagnostiquée durant l'adolescence et atteint son stade le plus avancé vers 20-30 ans. Dans la grande majorité des cas, la pathologie se stabilise pour ne plus évoluer vers la troisième ou quatrième décennie.<sup>53</sup>

Les signes cliniques d'un KC modéré à sévère sont : l'amincissement cornéen (souvent en inféro-temporale), une protrusion cornéenne (e.g. signe de Munson), un anneau de dépôt ferrique à la base du cône (anneau ou signe de Fleischer) et des stries de tension dans le stroma profond (stries de Vogts).<sup>48</sup> Dans les formes extrêmes de la pathologie, il existe des ruptures spontanées (ou provoquées par frottements oculaires) de la membrane de Descemet entraînant un œdème subaigüe de la cornée (on parle alors d'hydrops cornéen) évoluant vers, ou précédant, une opacification cornéenne.<sup>49</sup> (Figure 7) Ces dernières décennies, le diagnostic du KC a beaucoup évolué avec l'émergence des topographies cornéennes dites spéculaires (e.g. réflexion des mires de Placido) ou d'élévation (e.g. balayage d'une fente lumineuse), permettant un dépistage des formes frustres (ou pré-cliniques) sans signes cliniques ni retentissement fonctionnel.<sup>54</sup>



**Figure 7.** Signes cliniques du KC (A. signe de Munson, B. hydrops, C. anneau de Fleischer à la base du cône, D. stries de Vogt avec opacités cornéennes).

### 2.2.2 Etiopathogénie

L'étiologie du KC reste méconnue, mais semble multifactorielle. Plusieurs facteurs de risque sont impliqués dans la physiopathologie du KC : génétiques,<sup>29,55</sup> environnementaux (atopie),<sup>56</sup> géographiques (climat tropical, ethnie asiatique) ou mécaniques (frottement oculaire).<sup>57</sup> Usuellement la découverte d'un KC est isolée, même s'il existe des associations pathologiques avec la trisomie 21, l'amaurose congénitale de Leber, la kérato-conjonctivite vernale, l'eczéma, ou encore le prolapsus de la valve mitrale.<sup>58-60</sup>

La physiopathologie du KC ne fait pas consensus, probablement par manque de compréhension des modifications biomoléculaires princeps de la pathologie. Cependant, l'apparition d'un KC semble être la manifestation finale de divers processus pathologiques décrits comme étant impliqués dans la modification de la biomécanique cornéenne. L'étude de la biomécanique cornéenne rend compte du comportement viscoélastique des tissus cornéens

soumis à une force de déformation. Dans le KC, cette biomécanique cornéenne est diminuée par une diminution de l'hystérèse cornéenne (corneal hysteresis, CH) et du facteur de résistance cornéen (corneal resistance factor, CRF).<sup>61</sup> Ces modifications biomécaniques sont en lien avec des modifications ultra-structurales de l'épithélium et du stroma cornéen : ruptures de la membrane de Bowman, apoptose des kératocytes du stroma antérieur, fibrose focale, et réduction du nombre de lamelles de fibrilles de collagène impliquant des modifications architecturales du collagène cornéen.<sup>62,63</sup> L'origine de ces modifications ultra-structurales cornéennes, à la base de la formation et de la progression de l'ectasie kératoconique, pourrait être en lien avec un déséquilibre inflammatoire au sein de la surface oculaire. En effet, plusieurs études ont montré des taux élevés de facteurs pro-inflammatoires dans les larmes de patients KC : IL-1, IL-6, TNF- $\alpha$ , métalloprotéinases (MMP-9), et interféron  $\gamma$  (INF- $\gamma$ ).<sup>64-66</sup> De plus, un déséquilibre de la balance oxydative a été montré dans les larmes et les tissus de la surface oculaire dans le KC par rapport aux sujets sains.<sup>67</sup> Considérant les liens étroits d'activation entre inflammation, stress oxydatif et autophagie, le remodelage cornéen menant au KC pourrait être l'aboutissement de ces mécanismes biomoléculaires complexes.<sup>68</sup>

### **2.2.3 Prise en charge thérapeutique**

Le KC est une pathologie cornéenne handicapante, puisque touchant des adolescents et des jeunes adultes durant des périodes de vies cruciales pour leur avenir socio-professionnel. C'est autour de cette notion de handicap visuel que s'élabore la prise en charge des patients KC.

La découverte d'un KC chez un enfant ou adolescent de moins de 18 ans motive souvent l'indication chirurgicale de cross-linking cornéen (CXL).<sup>69</sup> En effet, le risque d'un KC sévère ou nécessitant une greffe de cornée est plus important lorsque l'on diagnostique un KC à un jeune âge.<sup>70</sup> Il est en effet plus complexe de suivre un enfant en consultation régulière avec réalisation d'examen complémentaires, et délicat de contrôler un frottement oculaire compulsif (e.g.

allergies oculaires) ou faisant partie de troubles du comportement (troubles obsessionnels compulsifs).<sup>69</sup> Chez l'adulte, l'indication de CXL se fait plus mesurée, dans un contexte de soins personnalisés, après avoir stabilisé ou stoppé d'éventuels frottements oculaires, et après avoir attesté d'une progression de la maladie (évolution de l'ectasie sur des examens de topographies cornéennes espacés dans le temps).<sup>71</sup> Schématiquement, l'intervention de CXL consiste à imprégner le stroma cornéen de riboflavine (vitamine B2) et d'exposer celui-ci à une source de rayonnement UV pour augmenter la résistance biomécanique du tissu cornéen.<sup>72</sup> A ce jour, considérant les préceptes de l'Evidence Based Medicine, il n'existe que l'intervention de CXL pour stopper ou limiter la progression d'un KC.<sup>71</sup> En effet, toutes les autres stratégies chirurgicales (pose d'anneaux intracornéens, implants réfractifs intraoculaires, greffes de cornée lamellaire ou transfixiante) ou optiques (adaptation en lunettes, lentilles souples hydrophiles, lentilles rigides perméables aux gaz, lentilles rigides à appui sclérale) n'ont pour objectif que la réhabilitation visuelle des patients KC.<sup>73</sup>

## **2.3 Le syndrome sec oculaire**

### **2.3.1 Définition & Epidémiologie**

Le syndrome sec oculaire (Dry Eye Disease, DED) est défini par le rapport du « International Dry Eye Workshop (DEWS) » comme une pathologie multifactorielle des larmes et de la surface oculaire entraînant une sensation d'inconfort oculaire, une perturbation de la vision, et une instabilité du film lacrymal provoquant de potentiels dommages au niveau de la surface oculaire.<sup>42</sup> De plus, le DED est fondamentalement relié aux notions d'hyperosmolarité du film lacrymale, ainsi qu'à une inflammation chronique de la surface oculaire.<sup>42</sup> On décrit schématiquement 2 grands types de DED, selon que la déficience se situe au niveau de la phase lipidique (i.e. DED évaporatif) ou au niveau de la phase mucino-aqueuse



(i.e. DED quantitatif). En clinique, la plupart des patients ont des formes mixtes, associant à des degrés divers une déficience qualitative et quantitative.<sup>43</sup>

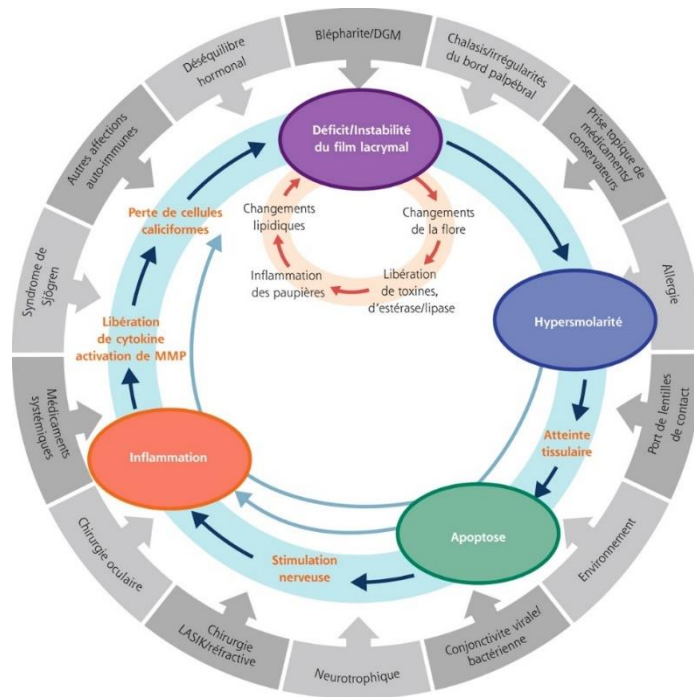
Il s'agit d'une pathologie fréquente, puisqu'on évalue la prévalence du DED à 5-34% de la population générale.<sup>74</sup> Evidemment, à l'instar du KC, ces chiffres de prévalence varient beaucoup selon les classes d'âge et les zones géographiques étudiées. Ainsi, il est commun de retrouver des prévalences de DED supérieure à 50% dans les classes d'âges supérieures à 40-50 ans.<sup>75</sup> Les facteurs de risque de DED sont nombreux : sexe féminin, exposition à la pollution environnementale,<sup>76,77</sup> travail sur écran,<sup>78</sup> les climats en hydrométrie basse et/ou température élevée, les traitements au long court par collyres conservés (e.g. glaucome),<sup>79</sup> les pathologies inflammatoires ou allergiques de la surface oculaire,<sup>80</sup> les chirurgies oculaires (e.g. chirurgie réfractive),<sup>81</sup> les pathologies systémiques inflammatoires (connectivites, syndrome de Goujerot-Sjögren).<sup>82</sup>

Le retentissement fonctionnel du DED (douleurs oculaires, inconfort visuel, sensation de « corps étrangers » ou « grain de sable ») est classiquement plus important que les signes cliniques retrouvés à l'examen oculaire (instabilité du film lacrymal, hyperhémie conjonctivale, kératite ponctuée superficielle, inflammation palpébrale).<sup>83</sup>

### **2.3.2 Etiopathogénie**

Le DED n'est pas seulement une pathologie du film lacrymal, mais bien une perte globale de l'homéostasie de la surface oculaire. On définit ainsi l'unité fonctionnelle lacrymale (lacrymal functional unit, LFU) regroupant le film lacrymal et ses glandes excrétrices, le système d'évacuation lacrymonasal, et les épithéliums conjonctivaux et cornéens. Une atteinte d'un des composants du LFU (e.g. une perturbation hormonale ou un défaut d'innervation) va entraîner un déséquilibre dans la constitution du film lacrymal, induisant une perte de trophicité et de

protection de la surface oculaire.<sup>84</sup> C'est cette notion de « perte d'homéostasie » de la surface oculaire qui sous-tend la compréhension moderne du DED, avec celle du « cercle vicieux » qui relie : instabilité et hyperosmolarité du film lacrymal – inflammation locale – apoptose cellulaire précoce. (Figure 8)



**Figure 8.** Cercle vicieux dans le DED (d'après Baudouin *et al.*, Un nouveau schéma pour mieux comprendre les maladies de la surface oculaire, Journal Français d'Ophtalmologie, 2007).<sup>34,85</sup>

L'élément princeps du DED est l'instabilité du film lacrymal, par anomalies de sa composition ou de ses constituants (couche lipidique, électrolytes, mucines). De l'instabilité du film lacrymal va découler la notion centrale d'hyperosmolarité des larmes. A ce stade, l'osmolarité du film lacrymal dépasse celui des cellules épithéliales, induisant une souffrance cellulaire et des dommages tissulaires de la surface oculaire.<sup>80</sup> L'apoptose cellulaire précoce déclenchée par l'hyperosmolarité des larmes, conduit à son tour à une cascade d'activation pro-inflammatoire médiée par la libération de cytokines (IL-1, IL-8, TNF- $\alpha$ ) et de métalloprotéinase (MMPs).<sup>74</sup> On parle alors de stress cellulaire hyperosmotique, interrelié avec le stress oxydant et les altérations de l'ADN.<sup>86</sup> Nous avons d'ailleurs montré qu'il existait un déséquilibre du

stress oxydant, en faveur des espèces radicalaires, dans les larmes et les tissus de la surface oculaire de patients DED par rapport aux sujets sains.<sup>87</sup> Cette inflammation chronique va retentir négativement sur les glandes excrétrices du film lacrymal (notamment par apoptose des cellules à mucus), aggravant l'instabilité de ce dernier et alimentant ainsi le cercle vicieux du DED.<sup>85</sup>

### **2.3.3 Prise en charge thérapeutique**

Le primum movens de la prise en charge du DED repose sur l'exclusion/réduction des facteurs de risque modifiables de la pathologie (e.g. ammonium quaternaire des collyres conservés, allergènes, molécules cytotoxiques, travail sur écran, hydrométrie ambiante, pollution atmosphérique). Il s'agit d'une étape fondamentale, motivée par une éducation thérapeutique régulière, qui doit impliquer le patient et son entourage personnel et professionnel.<sup>88</sup> L'hygiène des paupières, couplée à des solutions antiseptiques, fait partie intégrante de cette éducation thérapeutique dans les dysfonctions des glandes de Meibomius associées aux blépharites chroniques.<sup>89</sup> C'est également le cas, en milieu professionnel, pour l'adaptation du poste de travail des salariés devant écran.<sup>90</sup>

Le deuxième pan thérapeutique repose sur l'introduction de larmes artificielle, instillées régulièrement tout au long du nyctémère, afin de compenser la déficience qualitative et/ou quantitative du film lacrymal.<sup>88</sup> Très souvent, des collyres anti-inflammatoire (e.g. hydrocortisone, fluorométholone) sont nécessaires en cures courtes pour briser le cercle vicieux auto-inflammatoire du DED.<sup>91</sup> Dans les atteintes sévères de la surface oculaire (e.g. DED dans la maladie du greffon contre l'hôte), les cures courtes d'anti-inflammatoires ne suffisent pas à le contrôler efficacement, et sur le long court, l'inflammation chronique est alors responsable de lésions cornéo-conjonctivales. On introduit alors des collyres à bases de molécules

immunosuppressives (e.g. cyclosporine, tacrolimus), souvent en formulation hospitalière.<sup>92</sup> Il existe également des collyres à base de sérum autologue, riche en facteurs anti-inflammatoires et facteurs de croissance.<sup>93</sup>

Deux classes d'antibiotiques, les macrolides et les cyclines, sont utilisés dans les dysfonctions des glandes de Meibomius, par voies locale ou systémique, à visée anti-inflammatoire et anti-métalloprotéinase (MMPs). Leurs actions en cures courtes visent à éradiquer une colonisation bactérienne à l'œuvre dans les blépharites chroniques, et à stopper la production de toxines (i.e. staphylocoques, streptocoques) à l'origine de l'instabilité du film lacrymal et d'une toxicité de la surface oculaire.<sup>94</sup>

Les déficits purement quantitatifs (i.e. DED lié au syndrome de Gougerot-Sjögren) répondent très bien à l'occlusion des méats lacrymaux via des dispositifs stériles implantables type bouchons lacrymaux.<sup>95</sup>

### **3. RAGE et les pathologies oculaires**

RAGE est un récepteur ubiquitaire dans le corps humain, on peut ainsi l'identifier dans les myocytes cardiaques,<sup>96</sup> les neurones et cellules gliales,<sup>97</sup> les cellules épithéliales du système respiratoire,<sup>98</sup> et les cellules hépatiques ou rénales.<sup>99,100</sup> L'œil et ses annexes n'échappent pas à cette description, puisque RAGE a été décrit dans la physiopathologie de la rétinopathie diabétique, et dans des pathologies liées au vieillissement oculaire comme la dégénérescence maculaire liée à l'âge (DMLA) et la cataracte. La littérature est plus anecdotique, ou controversée, concernant le rôle de RAGE dans d'autres pathologies ou tissus oculaires (e.g. glaucome, dystrophie endothéliale de Fuchs, surface oculaire).

### **3.1 RAGE et la rétinopathie diabétique**

L'atteinte rétinienne dans le diabète se focalise sur l'ensemble neurovasculaire rétinien (neurovascular unit, NVU), à savoir les cellules endothéliales et les péricytes de la microcirculation, les cellules gliales (cellules de Müller, astrocytes, microglie), et les cellules neuronales (cellules ganglionnaires, amacrines, cellules bipolaires et horizontales) de la neurorétine.<sup>101</sup> L'hyperglycémie chronique du diabète entraîne la production de facteurs de stress cellulaires tel que les AGEs, les espèces radicalaires du stress oxydant, et in-fine une inflammation chronique. De fait, ces facteurs de stress cellulaire entraînent une autophagie et une apoptose précoce des cellules de la microcirculation rétinienne et de la microglie, altérant les fonctions du NVU.<sup>102</sup> Cette cascade d'évènements pro-apoptotiques et pro-ischémiques au sein des tissus rétiniens va aboutir à la sécrétion de cytokines pro-inflammatoires et de facteur de croissances pro-angiogéniques (vascular endothelial growth factor, VEGF). L'implication de la voie d'activation de RAGE dans la rétinopathie diabétique, via le couple AGEs – fl-RAGE, est ainsi au centre des altérations neurorétiniennes menant aux complications cécitantes de l'ischémie rétinienne.<sup>103</sup>

### **3.2 RAGE et les pathologies liées au vieillissement oculaire**

La physiopathologie de la DMLA repose sur l'accumulation, lié à l'âge, de lipoprotéines (issues d'ester de cholestérol) et de particules oxydées de cholestérols au sein de l'épithélium pigmentaire et de la membrane de Bruch. Il semblerait que ces dépôts pathologiques de l'épithélium pigmentaire, mais plus globalement les mécanismes de vieillissement rétinien (e.g. accumulation de déchets cellulaires en lien avec le cycle cellulaire), puissent entraîner la formation d'AGEs et/ou d'espèces radicalaires, et ainsi activer la cascade d'activation de la voie RAGE.<sup>104,105</sup>

L'atteinte du cristallin liée à l'âge est un processus lent et progressif qui aboutit à une cataracte (dite sénile). Il existe plusieurs descriptions cliniques de cataracte en lien avec le siège des opacités cristalliniennes (e.g. corticale, nucléaire, sous capsulaire). La capsule cristallinienne est une membrane basale qui est sécrétée par les cellules épithéliales du cristallin. Il s'agit d'un tissu à faible renouvellement, ce qui en fait un tissu exposé aux modifications post-traductionnelles des protéines liées à l'âge (i.e. oxydation et glycation protéique).<sup>106</sup> Ces phénomènes d'oxydation et de glycation protéique de la capsule et du cristallin lui-même entraîne l'accumulation intratissulaire d'AGEs, à la base des voies d'activation de RAGE. Il a ainsi été montré que l'accumulation d'AGEs dans le cristallin et la capsule menait à l'activation de la voie RAGE dans les cellules de l'épithélium cristallinien, induisant une fibrose tissulaire.<sup>107,108</sup>

### **3.3 RAGE et glaucome**

La physiopathologie du glaucome primitif à angle ouvert (GPAO) n'est pas encore totalement élucidée, même s'il existe un faisceau d'arguments pour évoquer une pathologie multifactorielle (hypertension intraoculaire, stress oxydatif, anomalies de la lame criblée, neurodégénérescence).<sup>109</sup> Il ne semblerait pas y avoir de liens significatifs entre l'expression de RAGE et la survenue d'un GPAO, sous réserve de l'origine des prélèvements étudiés (on peut se poser la question de la pertinence de prélèvements sanguins dans l'exploration d'une pathologie oculaire comme le glaucome).<sup>110</sup> Cependant, une augmentation des taux d'AGEs a été attestée dans des tissus oculaires (rétines et nerfs optiques) de patients glaucomateux décédés par rapport à des sujets sains. De plus, RAGE était surexprimé dans les cellules de Müller et la microglie des tissus neurorétiniens des patients glaucomateux.<sup>111</sup> Par ailleurs, il semble établi que le stress oxydant joue un rôle pathologique dans la neurodégénérescence glaucomateuse.<sup>112,113</sup> Considérant l'intrication des voies d'activation de RAGE et du stress

oxydant, et l'expression de RAGE dans les cellules gliales et neuronales, il semble licite de penser que la voie RAGE concoure aux mécanismes physiopathologiques du GPAO.

### **3.4 RAGE et les pathologies de la surface oculaire**

La surface oculaire, à l'instar des autres tissus barrières de l'organisme, est continuellement agressée par notre environnement extérieur (i.e. radiation solaire, pollution atmosphérique, flore bactérienne, facteurs exogènes physico-chimiques). A ce titre, la conjonctive et la cornée sont particulièrement exposés aux rayonnements UV.<sup>114</sup> Les altérations tissulaires des rayons UV sont en lien avec la formation d'espèces radicalaires (i.e. stress oxydatif), entraînant une cascade d'activation pro-inflammatoire (MIP-1, IL-8, IL-17, HMGB-1) et pro-apoptotique (altération du cycle cellulaire via altération de p53, p63 ou p73).<sup>115</sup> Ainsi, l'exposition aux UV provoquerait une sécrétion de protéines S100A dans la conjonctive, en plus des espèces radicalaires, activant l'expression des MMPs et provoquant du coup des altérations épithéliales et de la fibrose tissulaire. Un mécanisme similaire a été montré dans la surface oculaire de patients DED, avec sécrétion de protéines S100 par les cellules de l'immunité innée (i.e. macrophages).<sup>116</sup> Une pathologie de la conjonctive en lien avec l'exposition solaire, le ptérygion, a ainsi été associé à une surexpression de RAGE.<sup>117</sup> Il a également été démontré que la voie d'activation de RAGE était surexprimée dans les processus complexes de cicatrisation cornéenne après brûlures chimiques (via le couple protéines S100 – fl-RAGE).<sup>118</sup> Dans un modèle cellulaire (culture de cellules épithéliales cornéenne HCE) d'ulcère traumatique, l'activation de la cicatrisation cornéenne semblait être médiée par le couple AGEs – fl-RAGE, de manière dose dépendante.<sup>119</sup> Contrairement à de nombreux autres tissus du corps humain, HMGB1 ne semblait pas être impliqué dans la cicatrisation cornéenne.<sup>118,119</sup>

Similairement, l'accumulation d'AGEs au niveau de l'endothélium cornéen et de la membrane de Descemet (MD) serait un facteur physiopathologique de dysfonction endothéliale. Cette accumulation serait majorée dans la dystrophie endothéliale de Fuchs, et notamment dans la partie la plus antérieure (et donc la plus anciennement synthétisée) de la MD.<sup>120</sup> Cette constatation irait dans le sens que les AGEs s'accumulent dans la MD tout au long de la vie, sous l'influence des taux de glucose et d'acide ascorbique dissouts dans l'humeur aqueuse, du stress oxydant dépendant du rayonnement UV, ou encore du métabolisme cellulaire endothéliale.<sup>121</sup> Dans un contexte différent, en cas d'infection des cellules endothéliales cornéennes par le virus HSV-1, il y aurait une surexpression de RAGE à la surface cellulaire. Cette suractivation de la voie RAGE dans un contexte d'infection à HSV-1 serait un mécanisme cellulaire de lutte antivirale pour stimuler la sécrétion de cytokines pro-inflammatoires (IL-6) et d'interféron.<sup>122</sup>

Au moment de l'écriture de ce manuscrit, il n'y avait pas de données de la littérature sur l'expression de RAGE dans le kératocône et le syndrome sec oculaire.



## 4. Objectifs de thèse & Résultats

Initialement défini comme une pathologie cornéenne ectasique et non inflammatoire,<sup>48,49</sup> notre conception du KC a évolué ces dernières décennies avec les travaux portant sur les marqueurs de l'inflammation au sein de la surface oculaire.<sup>51,64,65,123</sup> Comme décrits plus haut, de nombreux biomarqueurs de l'inflammation (i.e. IL-1, IL-6, TNF- $\alpha$ , MMP-9) et un déséquilibre de la balance oxydative (i.e. produits réactifs de l'oxygène) semblent dérégulés dans les tissus de patients KC.

Considérant l'hypothèse que le développement et la progression du KC sont probablement médiés par des mécanismes d'activation inflammatoire complexes au sein des tissus et fluides de la surface oculaire, nous avons étudié l'expression de RAGE et ses principaux ligands dans différents tissus et larmes de sujets KC comparés à des sujets sains (publication n°1). Dans un deuxième temps, nous avons cherché à faire une synthèse systématique et exhaustive de la littérature scientifique portant sur les biomarqueurs du stress oxydant et antioxydants dans le KC comparé au sujet sain (publication n°2).

Un autre axe de recherche dans notre équipe considère l'étude de RAGE et ses ligands dans la physiopathologie du DED. Plus spécifiquement, il s'agit d'un travail de thèse de Doctorat sur l'étude *in vitro* de l'implication de NFAT5 et RAGE dans un contexte hyperosmolaire de la surface oculaire (travaux non publiés à ce jour mais dont je serai co-signataires). Ainsi, considérant les liens étroits d'activation entre la voie RAGE et les biomarqueurs du stress oxydatif, nous avons également cherché à faire une synthèse systématique et exhaustive de la littérature scientifique portant sur les biomarqueurs du stress oxydant et antioxydants dans le DED comparé au sujet sain (publication n°3).

Finalement, en parallèle avec ce travail de recherche sur modèle cellulaire de DED, nous avons développé un modèle animal sur souris pour étudier l'influence des conditions environnementales connues pour être à risque de syndrome sec oculaire (attention et concentration visuelle soutenue, condition venteuse et hygrométrie de l'air faible). Notre objectif par ce travail « *in-vivo* » consiste donc, chez des souris « Knock Out » (KO) RAGE -/- comparées à des souris "sauvages" RAGE +/+, à caractériser RAGE et ses voies cellulaires en provoquant la survenue d'un syndrome sec oculaire dans plusieurs tissus et fluides de l'œil (cornée, conjonctive, cellules à mucus, larmes). Au moment de la rédaction de ce manuscrit, ce travail de recherche est en cours.



## **Publication N°1**

### **Dysregulation of Receptor for Advanced Glycation End products (RAGE) expression as biomarker of keratoconus**

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## Research Article

# Dysregulation of Receptor for Advanced Glycation End Products (RAGE) Expression as a Biomarker of Keratoconus

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**Background.** Because of the implications of Receptor for Advanced Glycation End Products (RAGE) in keratoconus (KC), we describe a differential expression of RAGE transcripts and proteins in corneal tissues and tears of KC and healthy patients. **Methods.** Using a case-controlled study, corneal epitheliums and tears of KC and healthy subjects were obtained during corneal collagen cross-linking and photorefractive keratectomy (PKR) and during usual consultations. Quantitative reverse transcription (RT-qPCR) and Western-Blot were performed to analyze RAGE transcripts and proteins' expression in corneal tissues and tears. **Results.** One hundred and six patients were included in this study. The characteristics of the patients were as follows: 56 KC (25 corneal epithelium and 31 tears) and 50 control subjects (25 corneal epithelium and 25 tears). Transcripts of RAGE, HMGB1, and S100 family ligands were quantified by RT-qPCR, identifying a significantly higher expression of RAGE and HMGB1 in the healthy group than in the KC group ( $p = 0.03$  and  $0.04$ , respectively). Western Blot showed a significantly higher fl-RAGE expression in KC corneal epithelium than control ( $p < 0.001$ ) and lower s-RAGE expression in KC tears than control ( $p = 0.04$ ). **Conclusions.** Linked with the inflammatory process occurring in KC pathophysiology, we propose for the first time that the RAGE expression (total and truncated forms of receptor and ligands) in KC corneal tissues and tear samples provides viable biomarkers.

## 1. Introduction

Keratoconus (KC) is a progressive vision-threatening corneal disease classically beginning in the second decade of life, causing deformation of the structure of the cornea [1–4]. Because the regular corneal curvature has a predominant role in refraction and quality of vision [5], this disease causes a visual impairment despite therapeutic strategies involving the use of rigid contact lenses [6], corneal collagen cross-

linking [7, 8], refractive surgeries [9], or advances in corneal grafting [10, 11]. The prevalence of KC is approximately 5.3/2000 in the general population [12], with certain ethnic groups, such as Asians being more at risk [13]. Well-known environmental factor such as eye rubbing [14, 15] or atopy [16, 17] also increases the frequency. There is some evidence for genetic transmission [18–20]. However, the physiopathology of this disease is still poorly understood, and many questions persist in relation to triggering and

progression of ectasia. To our knowledge, there are no biomarkers clearly described in the ocular surface (e.g., corneal tissues or tears) of KC. If KC was initially defined as a non-inflammatory corneal disease, several studies during the past decade have described inflammatory process in corneal tissues and tears. In fact, proinflammatory cytokines (IL-1, IL-6, TNF- $\alpha$ , and TGF- $\beta$ ), oxidative stress, or degradation of collagens by metalloproteases (MMP-1, MMP-3, and MMP-9) has been implicated in keratoconus pathophysiology [3, 21–25].

Receptor for Advanced Glycation End Products (RAGE) is a multiligand transmembrane receptor (member of the immunoglobulin superfamily) implicated in inflammation and cell migration processes [26–28]. The pre-mRNA of RAGE can be subjected to alternative splicing causing truncated RAGE isoforms from the same gene: full-length RAGE (fl-RAGE) and soluble forms RAGE (s-RAGE) including endogenous secretory RAGE (es-RAGE) and cleaved RAGE (c-RAGE), with molecular weights of 35–55 kD varying between isoforms, cellular types, and tissues. fl-RAGE is a transmembrane receptor (able to transduce the signal) whereas s-RAGE corresponds to soluble truncated isoforms of fl-RAGE, comprising an extracellular domain, and plays a role as a decoy to prevent ligands from interacting with fl-RAGE receptors [29–31] (Figure 1). Expression of RAGE, described in several human tissues, is upregulated in response to its natural and historical ligand, i.e., advanced glycation end products (AGEs), but also HMGB1 and S100 proteins that are well-known in aseptic inflammation and oxidative stress of diabetes patients, atherosclerosis, pulmonary and auto-immune diseases, cancer, and chronic neurodegenerative like Alzheimer's disease [30–34]. In ophthalmology, we recently demonstrated the implications of RAGE in the first steps of corneal epithelial wound healing [35].

In addition, previous studies described the role of RAGE in primary open-angle glaucoma [36], diabetic retinopathy [37–39], age-related macular degeneration [40], endothelial Fuchs's dystrophy [41], cataract, and posterior capsule opacification [42] or pterygium [43]. However, to our knowledge, there is no description in KC. Therefore, the purpose of this study was to highlight the role of the RAGE pathway in the cornea and tears of KC compared to healthy patients. A further aim was to describe a putative candidate viable biomarker in the ocular surface of KC.

## 2. Material and Methods

**2.1. Subjects.** One hundred and six subjects, over 18 years of age, were enrolled in the study. Fifty healthy subjects and 56 patients with clinically and topographic KC were considered for the study. The exclusion criteria were ocular surgery performed during the past 3 months, ocular allergy or any ocular disease, dry eye syndrome, any systemic disease, and local or systemic medication (anti-inflammatory) that could interfere with the interpretation of the results. The diagnosis of KC was performed by clinical examination (objective and subjective refraction, slit lamp with fluorescein testing, and tonometry) and topographic evaluation using a Pentacam

(Oculus Optikgeräte GmbH, Wetzlar, Germany). The research received approval from the University Hospital Ethics Committees and was conducted in accordance with the recommendations of the Declaration of Helsinki on Biomedical Research Involving Human Subjects. Prior to any data collection, all patients were orally informed about the nature of the study and the working hypothesis and therapeutic education concerning keratoconus. Signed informed consent was obtained from all patients prior to collection of tears or epithelium samples.

**2.2. Corneal and Tear Sampling.** Corneal epitheliums of KC were obtained during surgical intervention of collagen cross-linking with UVA irradiation (A-CXL) and corneal de-epithelialization (Epi-Off technique). After topical anaesthesia using one drop of Oxybuprocaine (Oxybuprocaine THEA 1.6 mg/0.4 ml, single-dose eye drop), we used a disposable scarifier to deepithelialize 6.5 to 8.5 mm of cornea. Epithelial samples were stored at  $-80^{\circ}\text{C}$  in 1.5 ml Eppendorf tubes. The same protocol was used for healthy subjects undergoing PRK surgery.

Tear samples were collected without topical anaesthesia, using calibrated  $20\ \mu\text{l}$  glass micropipettes (BLAUBRAND intraMark, Wertheim, Germany), from the inferior temporal tear meniscus, taking care to minimize the irritation of the ocular surface. The samples (from 5 to  $8\ \mu\text{l}$  per eye) were placed in 0.2 ml Eppendorf tubes using a pipetting aid for capillaries (HIRSCHMANN Laborgeräte GmbH & Co., Eberstadt, Germany) and stored at  $-80^{\circ}\text{C}$ .

**2.3. RNA Extraction and Reverse Transcriptase Quantitative PCR Assays.** Total RNA was extracted from corneal epithelium using RNeasy<sup>®</sup> Mini Kit (Qiagen, 74106). The RNA concentration was determined by spectrophotometry at 260 nm with the Denovix DS-11 FX NanoDrop. The cDNA was synthesized from  $1\ \mu\text{g}$  of RNA using oligo-(dT) primers (Promega, C1101), 10 mM dNTP (Invitrogen, 10297-018), SuperScript IV Reverse Transcriptase (Invitrogen, 18090050), and rRNasin<sup>®</sup> RNase Inhibitor (Promega, N2515). The reaction mixture ( $20\ \mu\text{l}$ ) was incubated at  $65^{\circ}\text{C}$  for 5 min,  $50^{\circ}\text{C}$  for 10 min,  $80^{\circ}\text{C}$  for 10 min, and, after addition of RNase H (Promega, M4281),  $37^{\circ}\text{C}$  for 20 min. The primer sequences used for classic or quantitative RT-PCR are indicated in Table 1. Recombinant Taq Polymerase (Invitrogen, 10342-020) and 5 mM dNTP (Invitrogen, 10297-018) were used. The reaction mixture ( $50\ \mu\text{l}$ ) was incubated for 10 min at  $95^{\circ}\text{C}$ , followed by 35 amplification cycles—comprising 45 sec at  $94^{\circ}\text{C}$ , 45 sec at the annealing temperature specific to each primer pair, indicated in Table 1, and 45 sec at  $72^{\circ}\text{C}$  and terminated by 10 min at  $72^{\circ}\text{C}$ . A negative control for amplicon contamination was set up using a complete PCR mix without cDNA. Quantitative PCR reactions were performed using LightCycler<sup>®</sup> 480 SYBR Green I Master (Roche, 04887352001). We applied the following program on the LightCycler<sup>®</sup> 480 Instrument II (Roche): 10 min at  $95^{\circ}\text{C}$ , followed by 45 amplification cycles comprising 10 sec at  $95^{\circ}\text{C}$ , 10 sec at the annealing temperature specific to each primer pair, indicated in Table 1, and 15 sec at  $72^{\circ}\text{C}$ . PCR reactions were assessed using Taq

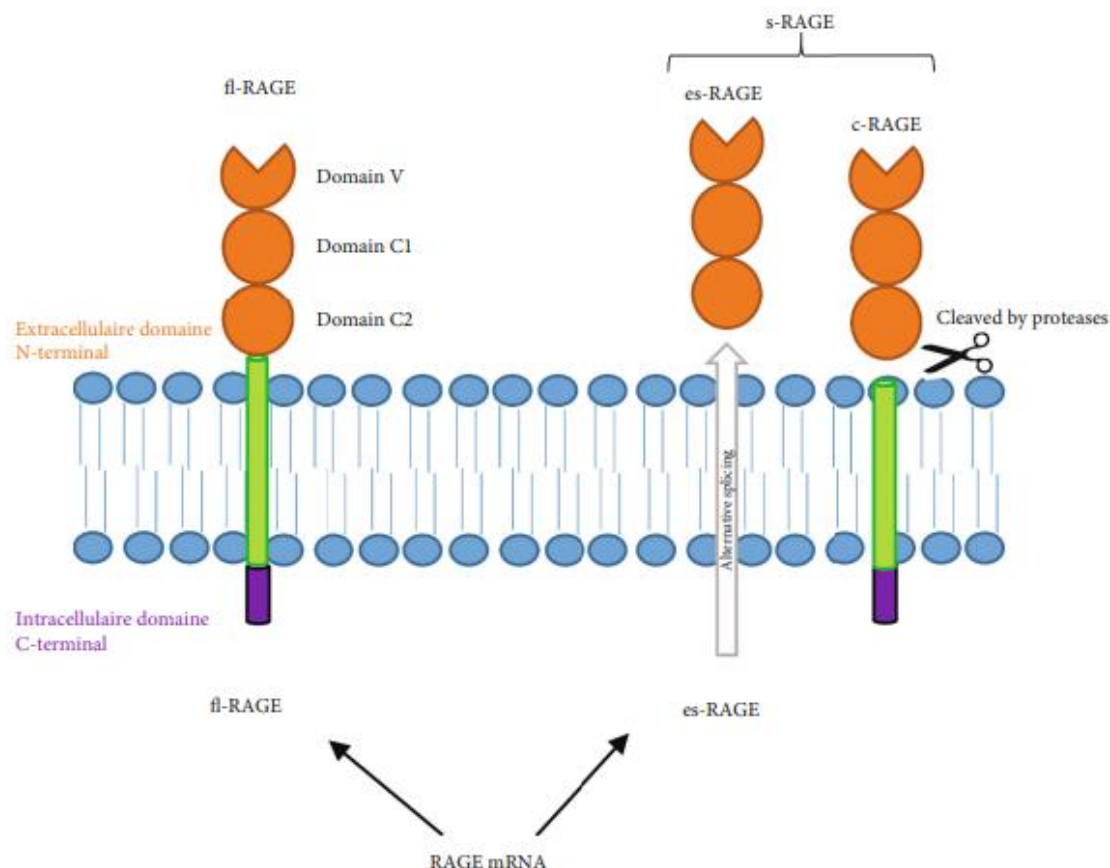


FIGURE 1: RAGE isoforms: transmembrane receptor and soluble forms.

TABLE 1: Forward and reverse primer sequences used for RT-PCR and RT-qPCR. These primer sequences were used in order to amplify human cDNAs of indicated genes obtained after reverse transcription of total mRNAs, extracted from the corneal epithelium of keratoconus and healthy subjects.

Gene	Sequence 5'-3' (F: forward, R: reverse)	Product size (bp)	Hybridization temperature (°C)
Total RAGE	F: TGTGCTGATCCTCCCTGAGA R: CGAGGAGGGGCCAACTGCA	139	61
es/fl-RAGE	F: TGTCAGCATCAGCATCATCG R: TCCTGGTTTTCTGGGGCC	fl-RAGE: 195 es-RAGE: 150	56
hsRPLP0	F: AGGCTTTAGGTATCACCCT R: TATCACAGAGGAACTCTGC	219	61
hsRSP17	F: TGCGAGGAGATCGCCATTATC R: AAGGCTGAGACCTCAGGAAC	169	61
hsHMGB1	F: ACCTATATCCCCTCCCAAAGGG R: TTTTGGGCGATACTCAGAGC	109	61
hsS100A8	F: TAAAGGGGAATTTCCATGCCGT R: GTTAACTGCACCATCAGTGTG	137	61
hsS100B	F: AAGGGAGGGAGACAAGCACA R: TCCTGGAAGTCACATTCCGC	159	61

DNA polymerase (Thermo Fisher Scientific, EP0405). We applied the following procedure: 56°C for hybridization, 5 min at 95° followed by 35 amplification cycles—comprising 45 s at 94°C, 45 sec at the annealing temperature specific

to each primer pair, indicated in Table 1, and 45 sec at 72°C and terminated by 10 min at 72°C. Results were analyzed on a 2% agarose gel. (Figure 2) Quantification of two house-keeping genes, *RPLP0* and *RPS17*, and transcripts was

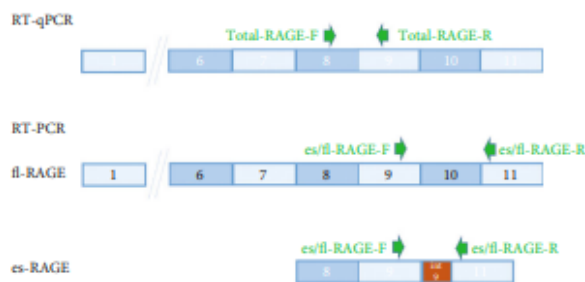


FIGURE 2: Amplification of total RAGE and its two principal isoforms (fl-RAGE and es-RAGE) by RT-qPCR. Numbers represent exons; the green arrows involve the forward (F) and reverse (R) transcriptions.

performed for all samples as an internal control of the amount and quality of cDNA. These two genes were giving the same results when used separately to standardize. Standard curves were used to quantify the number of amplified transcripts. The results are given as the ratio between the amount of each *transcript of interest* and the geometric mean of these two housekeeping genes (*RPLP0* and *RPS17*) as recommended by the MIQE guidelines. All experiments were performed in triplicate.

**2.4. Protein Extraction and Quantification.** The corneal epithelium was, respectively, lysed with either 500  $\mu$ l or 250  $\mu$ l RIPA buffer (20 mM Tris (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 0.5% Sodium Deoxycholate, 1 mM EDTA, and 0.1% SDS), supplemented with 10% Protease inhibitor cocktail (Roche, 04693159001). For the tissues, a preliminary disruption step was performed using ceramic beads (Precellys, KT03961-1-009.2) and a tissue lyser (Qiagen) (three lysing steps during 25 sec with a 30 Hz oscillation frequency, separated by two break steps during 30 sec). Samples were then vortex-mixed for 5 sec and kept on ice for 10 min, one in three occasions. After centrifugation (5 min, 8000 rpm), supernatants were collected. The Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, 23225) was used to measure the protein concentration according to the manufacturer's guidelines.

**2.5. Western Blot Assays.** Forty micrograms of proteins were separated a 4–15% Mini-PROTEAN™ TGX Stain-Free™ Protein gel (Bio-Rad) and transferred to nitrocellulose membranes (Bio-Rad, 1704271) using the Trans-Blot® Turbo™ Transfer System (Bio-Rad). After saturation with 5% skimmed-milk in 1X Tris-Buffered Saline (TBS) buffer (blocking buffer) for 2h, membranes were incubated with primary antibody, anti-RAGE polyclonal goat IgG (1:5000 dilution in blocking buffer supplemented with 0.1% Tween-20) (Biotechnie, R&D Systems France, AF1179), overnight at 4°C. Membranes were washed three times for 10 min with 1X TBS-0.1% Tween-20 (TBST) and incubated with a 1:5000 dilution of peroxidase-conjugated polyclonal anti-IgG goat (Abliance, BI2403) for 2 h. Blots were washed with TBST on three occasions for 10 min, rinsed with 1X TBS, and developed with the Clarity Max™ Western ECL Blotting Substrates (Bio-Rad, 1705062) according to the

manufacturer's protocols. The All Blue Standard (Bio-Rad, 161-0373) was used as a protein ladder. The relative intensities of protein bands were analyzed using Image Lab™ software (BIO-RAD), and the results were presented as a ratio between the protein of interest and the total protein on the same blot. The use of stain-free imaging allows for the normalization of bands to the total protein on a blot, without requesting the use of housekeeping proteins or stripping and reprobing.

**2.6. Statistical Analysis.** All data were analyzed using the GraphPad Prism Program version 5.02. When the distribution of samples in each group respected the normal distribution, we used the parametric *t*-tests to compare two independent groups. When the distribution of samples did not respect a Gaussian distribution or was too small ( $n < 15$ ), we used a nonparametric Mann-Whitney test to compare two independent groups. When more than two groups were compared, the nonparametric Kruskal-Wallis test was applied, followed by multiple comparison with Dunn's correction. In all cases, a *p* value  $< 0.05$  was considered statistically significant.

### 3. Results

**3.1. Clinical Features.** One hundred and six patients were included in the study: 56 KC (25 corneal epithelium and 31 tears) and 50 healthy subjects (25 corneal epithelium and 25 tears). In the KC group, males represented 77.1%, with a mean age of  $26.8 \pm 8.5$  years. KC rubbed eyes in 69.9%, with corneal opacity in 7.2%. In Amsler-Krumeich classification, grade 1, grade 2, grade 3, and grade 4 represented 22.9%, 45.8%, 9.6%, and 12.1%, respectively. In addition, forme fruste keratoconus represented 9.6%. In the control group, males represented 70.6%, with a mean age of  $30.6 \pm 8.1$  years. No age-related or sex-related statistical differences were detected between the groups ( $p = 0.2$ ). Statistical analysis stratified by age, gender, and KC severity did not reveal significant results (data not shown). More details of clinical and topographic features in each group are presented in Table 2.

**3.2. Transcript Expression Levels of RAGE Isoforms and Ligands in KC and Healthy Epithelium.** The mRNAs were quantified in corneal samples for RAGE, HMGB1, and S100 family ligands and normalized with the geometric mean of two housekeeping genes' expressions (*RPLP0* and *RPS17*). Total RAGE showed a significant difference between normal and KC (Figure 3(a)). This was statistically higher in the normal group than in the KC group ( $p = 0.03$ ). HMGB1, one of the most important ligands of RAGE, showed a similar expression profile ( $p = 0.04$ ) (Figure 3(b)), whereas there were no significant differences for two S100 family ligands (Figures 3(c) and 3(d)). The ratio between fl-RAGE (functional transmembrane receptor responsible of inflammatory signaling) (Figure 3(e)) and es-RAGE (decoy of fl-RAGE involving anti-inflammatory response) (Figure 3(f)) was significantly higher in KC than in the control ( $p = 0.01$ ) (Figure 3(g)). This difference was explained by a statistically

TABLE 2: Clinical features of keratoconus and healthy subjects.

Samples	Population (N)	Age (years)	Sex (% men)	Clinical features			Topography			Amsler-Krumelch classification					
				Eye rubbing (%)	Corneal opacity (%)	BCVA (logMar)	Km (D)	Kmax (D)	Topography Astigmatism (D)	Thinnest P ( $\mu$ m)	Suspect (%)	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
<i>Keratoconus</i>															
Corneal epithelium	25	24.3 (8.6)	78.8	69.7	3.0	0.23 (0.23)	45.8 (2.4)	56.9 (8.3)	1.33 (1.63)	476.2 (45.7)	0.0	30.3	57.6	6.1	6.7
Tears	31	28.5 (8.1)	72.1	70.2	10.0	0.20 (0.25)	48.6 (4.2)	51.3 (4.7)	6.50 (2.12)	453.5 (72.2)	1.60	18.0	38.0	12.0	16.0
Total	56	26.8 (8.5)	77.1	69.9	7.2	0.22 (0.24)	47.2 (3.5)	51.3 (4.7)	4.13 (2.17)	462.5 (63.7)	9.6	22.9	45.8	9.6	12.1
<i>Normal</i>															
Corneal epithelium	25	32.4 (6.4)	65.4	32.6	0.0	0.0	—	—	—	—	—	—	—	—	—
Tears	25	28.7 (9.8)	75.8	26.4	0.0	0.0	—	—	—	—	—	—	—	—	—
Total	50	30.6 (8.1)	70.6	29.6	0.0	0.0	—	—	—	—	—	—	—	—	—

Values are expressed as the mean (SD). BCVA: Best-Corrected Visual Acuity; D: Diopter.



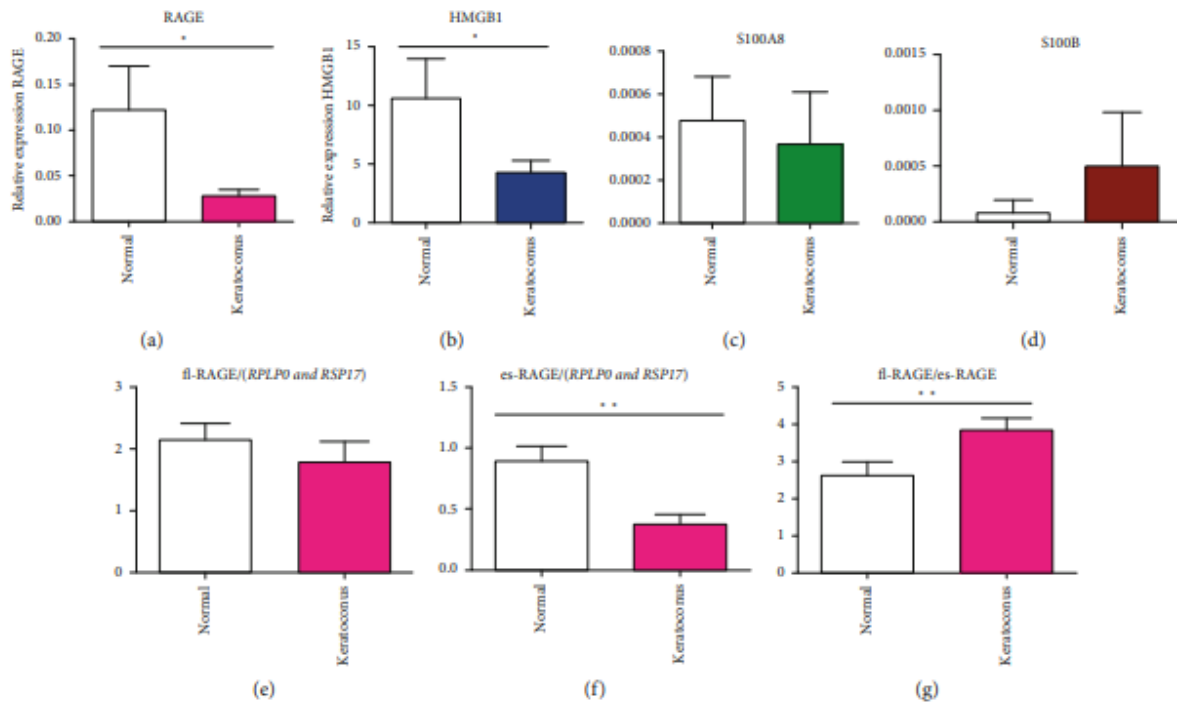


FIGURE 3: Transcript expression levels (mRNA) of total RAGE and its two principal isoforms (fl-RAGE and es-RAGE) and ligands in KC (25 corneal epithelium) and healthy subjects (25 corneal epithelium). RT-qPCR were performed to identify total RAGE isoforms (a) and his three major ligands (HMGB1 (b), S100A8 (c), and S100B (d)). In addition, we described the ratio between fl-RAGE (e) (transmembrane functional receptor responsible of inflammatory signaling) and es-RAGE (f) (soluble form of RAGE with an action of decoy, produced by alternative splicing) (g). Quantification of two housekeeping genes, RPLP0 and RPS17, and transcripts was performed for all samples as an internal control of the amount and quality of cDNA. The results are given as the ratio between the amount of each transcript of interest and the geometric mean of these two housekeeping genes (RPLP0 and RPS17) transcripts. \*  $p < 0.05$ ; \*\*  $p < 0.01$ .

lower es-RAGE expression in KC than control whereas there were no differences in fl-RAGE expression ( $p = 0.03$ ).

**3.3. Protein Expression Levels of RAGE Isoforms and Ligands in KC and Healthy Epithelium and Tears.** fl-RAGE (transmembrane forms of RAGE) proteins were quantified in the corneal epithelium of KC and control. Quantification of the attempted band (molecular weight around 45-50 kD) showed a higher expression of fl-RAGE in the KC group than in the control group ( $p < 0.001$ ) (Figure 4(a)). The s-RAGE (soluble forms of RAGE, decoy of fl-RAGE involving anti-inflammatory response) proteins were also quantified in tears of KC and compared with controls. Using the quantification of the attempted band (molecular weight around 30-35 kD) corresponding to s-RAGE, we established that the concentration of s-RAGE in tears was significantly higher in the normal group than in the KC group ( $p = 0.04$ ). (Figure 4(b)).

#### 4. Discussion

Keratoconus has been classically described as a noninflammatory corneal ectatic disorder. However, recent evidence suggests a possible role of inflammation in the pathogenesis of KC. Indeed, several articles have described an upregulation of proinflammatory cytokines, oxidative stress mediators, and enzymes in KC, without evidence in relation to

biomarkers. The RAGE transmembrane receptor has been implicated in aseptic inflammation by reaction with AGEs, actors well-known in diabetes and cardiovascular disease. We describe here, for the first time in cornea and tears of KC, the implication of the RAGE-inflammatory pathway, with potential use of the related findings as potential biomarkers for this pathology.

**4.1. Keratoconus: An Aseptic Inflammatory Disease?** KC was initially admitted to be a noninflammatory disease without cellular infiltration and vascularisation [2]. In the last few decades, many theories have been proposed for the pathogenesis of keratoconus such as incorrect development of cornea, genetic mutations, enzymes released by degeneration cells, and collagen disruption ([44-46]; Yaron S. [47]). Interestingly, extracellular matrix degradation is involved in the production of inflammatory cytokines and enzymes, and vice versa. Proinflammatory cytokines (IL-1, IL-6, TNF- $\alpha$ , and TGF- $\beta$ ) and enzymes, involved in degradation of collagens such as metalloproteinases (MMP-1, MMP-3, and MMP-9), were described to be upregulated in KC when looking at tears and corneal samples [3, 21, 24, 25]. These proinflammatory cytokines seem to play a role in the protease cascade, along with the plasmin pathway, cyclooxygenase, and metalloproteinases, which may explain the observed changes in the extracellular matrix [48]. Considering that the production of MMPs is regulated by IL-1, IL-6, and IL-7 and that

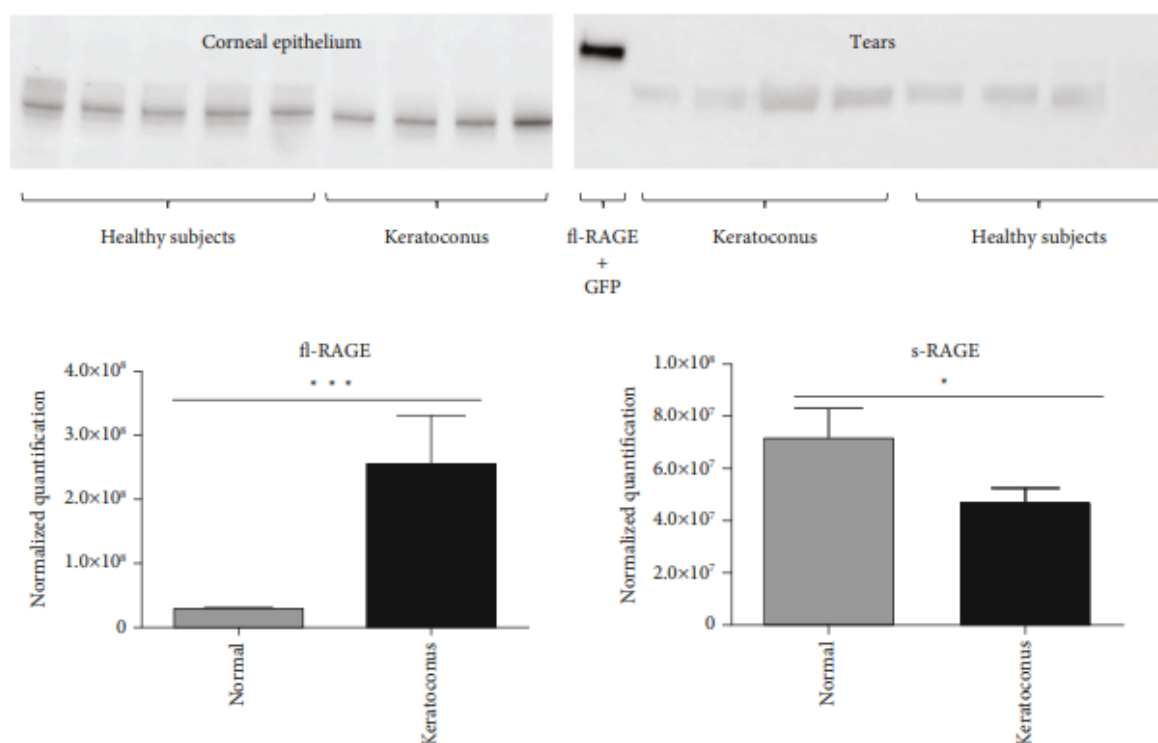


FIGURE 4: Western Blot of RAGE isoforms in keratoconus (25 corneal epithelium and 31 tears) and healthy subjects (25 corneal epithelium and 25 tears). Normalized quantification of fl-RAGE (transmembrane functional receptor of RAGE) in corneal epithelium samples (a) and s-RAGE (soluble forms of RAGE, with an action of decoy) in tears (b). Positive control chosen is transfected corneal cellular with fl-RAGE tagged GFP (expected molecular weight at 80kD). The relative intensities of protein bands were analyzed using Image Lab™ software (BIO-RAD), and the results were presented as a ratio between the protein of interest and the total protein on the same blot. \* $p < 0.05$ ; \*\*\* $p < 0.001$ .

TNF- $\alpha$  increases MMP-1, MMP-3, and MMP-9, it was proposed that there is an inflammatory vicious circle established at the onset or during the progression of ectasia. In addition, evidence-based data showed that oxidative stress imbalance (in tears, cornea, aqueous humour, and blood) seemed to be a central process in the pathophysiology of KC [23]. Therefore, it appears that inflammation is clearly an integral part of the complex pathophysiology of KC.

**4.2. RAGE: A Proinflammatory Pathway.** RAGE is a glycoprotein transmembrane receptor binding AGEs or Damage-Associated Molecular Patterns (DAMPs also called alarmins): HMGB1/amphoterin family, S100/calgranulin family. RAGE is involved in various inflammatory processes like diabetic complications, chronic inflammatory diseases, atherosclerosis, and chronic neurodegenerative disease such as Alzheimer's disease [49]. A variety of tissues and cells express this pattern recognition receptor: vascular endothelial, bronchial, and pulmonary cells, vascular smooth muscle cells, neurons, and many others [29, 31]. The involvement of the RAGE pathway in corneal epithelial wound healing, as well as diseases of conjunctiva and retina and trabecular meshwork, has been demonstrated previously [35–41, 43]. The activation of fl-RAGE stimulates NF- $\kappa$ B, a transcription factor residing at the inactive state in the cytosol of cells, which can modulate the expression of proinflammatory

cytokines, vasoconstrictive and prothrombotic products, and adhesion molecules [29]. Truncated forms of RAGE are described as soluble decoys to prevent ligands from interacting with cell fl-RAGE surface receptor: es-RAGE (an alternative splicing product) and c-RAGE (a cleavage product by metalloproteases). Consequently, s-RAGE indirectly inhibits the fl-RAGE receptor. Thus, an inflammatory reaction mediated by fl-RAGE can be downregulated by these soluble decoys. This anti-inflammatory regulation has been described in bronchi and lungs of smokers, provoked by an underexpression of fl-RAGE with overexpression of es-RAGE isoforms [30, 50]. The inflammatory RAGE pathway is complex and still poorly understood because its activation can induce inflammatory cascades as well as anti-inflammatory retro control by soluble forms.

**4.3. Dysregulation of RAGE Pathway in Keratoconus.** To our knowledge, our study described for the first time the implication of the RAGE pathway in KC. Results of RT-qPCR showed a significant underexpression of global RAGE transcripts in the corneal epithelium of keratoconus compared to healthy subjects. Nevertheless, this is emphasised by the ratio fl-RAGE/es-RAGE which highlighted a decrease of es-RAGE in KC ( $p = 0.01$ ). Indeed, we did not observe any difference in fl-RAGE expression in the two groups using RT-qPCR whereas we highlighted a significant under-

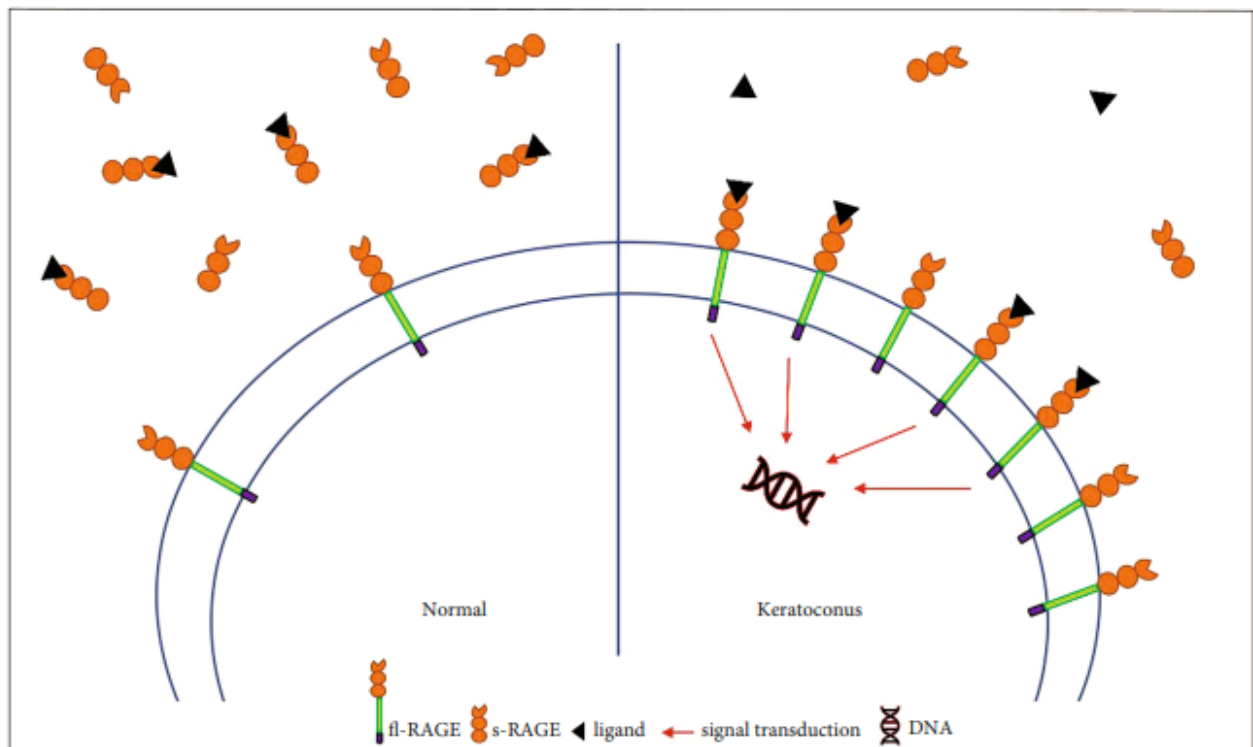


FIGURE 5: Feedback loop of inflammatory RAGE pathway: interaction between ligands, soluble decoys, and transmembrane receptors, in normal and KC cornea.

expression of es-RAGE in KC compared to controls. The results of Western Blotting showed a significant higher quantity of fl-RAGE protein in the corneal epithelium of KC compared to healthy subjects. These results were comforted by a significant lower quantity of s-RAGE protein (soluble forms) in tears of KC compared to healthy subjects. As explained above, the RAGE pathway can be implicated in activating inflammatory cascades as well as in an anti-inflammatory retro control, depending on tissues and pathologies [50]. In KC, the RAGE pathway seems to be upregulated in the corneal epithelium without upregulation of soluble decoys in tears. Interestingly, we highlighted a lower expression of RAGE transcripts concerning es-RAGE in KC, which could result from an alternative splicing mechanism. These results were comforted by RAGE-protein expression with a higher concentration of fl-RAGE in the corneal epithelium of KC and a lower concentration of s-RAGE (considered like decoys) in tears of KC compared with healthy patients. Our study shows that a proinflammatory dysregulation similar to that described for smokers in the lung is present in keratoconic corneas [30, 50]. In normal corneas, the activation of fl-RAGE (transmembrane receptor) can be downregulated by the soluble RAGE decoys while in KC there is an upregulation of the RAGE pathway without retro control of decoys in tears (Figure 5). Thus, the RAGE pathway is dysregulated in KC, potentially generating a number of proinflammatory signals in the ocular surface.

It is worth noting that the majority of KC in our study was grades 1 and 2 in Krumeich severity scale, which are

the two lower stages of its ranking. It is possible that the RAGE pathway is present at an earlier stage of KC causing or fuelling the vicious circle of tissue remodelling. The modification of the expression of RAGE in early forms of keratoconus is particularly interesting: RAGE could be an easy access biomarker in tears particularly useful in keratoconus diagnosis. Indeed, KC is difficult to diagnose for nonspecialist ophthalmologists, requiring clinical and topographical evidences. In addition, the need for viable biomarkers in the early stages of KC is particularly interesting in preoperative of refractive surgery (where KC is contraindication) [51]. Further studies are needed to explore the inflammatory pathways resulting in the dysregulation of RAGE in KC and their putative implication in the progression of corneal ectasia. The biomarkers based on the RAGE pathway could become an interesting biological tool to evaluate KC progression. Moreover and interestingly, some blocking peptides like inhibitor ligands named RAP or SAGE and monoclonal specific antibodies anti-RAGE have been used in an attempt to block inflammatory RAGE pathway in different inflammation experimental models [32, 52–56]. The established biomarkers described in this study could also take a place for the future monitoring of RAGE-based therapeutics.

## 5. Conclusion

To our knowledge, we described for the first time the implication of RAGE pathway in KC. Other studies will compare these results and try to better specify the role that RAGE

plays in KC pathophysiology and determine how the RAGE mRNA and protein isoforms (pro- or anti-inflammatory) could be used as future biomarkers for the diagnosis, prognosis, and treatment of the KC.

## Abbreviations

AGEs:	Advanced glycation end products
c-DNA:	Complementary-deoxyribonucleic acid
°C:	Celsius degree
c-RAGE:	Cleaved-RAGE
DAMPs:	Damage-Associated Molecular Patterns
D:	Diopter
dNTP:	Deoxyribonucleotide
EDTA:	Ethylenediaminetetraacetic acid
es-RAGE:	Endogenous secretory-RAGE
fl-RAGE:	Full length-RAGE
HMGB1:	High-mobility group box 1
IL:	Interleukin
KC:	Keratoconus
Kmax:	Maximal keratometry
ml:	Milliliters
MMP:	Matrix metalloproteinase
NaCl:	Sodium Chloride
OCT:	Optical cutting temperature compound
PBS:	Phosphate-Buffered Saline
PRK:	Photorefractive keratectomy
RAGE:	Receptor for Advanced Glycation End Products
RNA:	Ribonucleic acid
RT-qPCR:	Reverse Transcription Quantitative Polymerase Chain Reaction
SDS:	Sodium dodecyl sulfate
s-RAGE:	Soluble-RAGE
TBS:	Tris-Buffered Saline
TBST:	TBS and polysorbate 20 (Tween 20)
TGF:	Tumoral growth factor
TNF:	Tumoral necrosis factor
UVA:	Ultraviolet A.

## Data Availability

All the data used to support the findings of this study are included within the article and figures.

## Conflicts of Interest

The authors declare that they have no competing interests.

## Authors' Contributions

VN, JM, FC, LB, and VS were responsible for the design and conception of the study. VN and JM collected the samples and performed the laboratory manipulations. VN, JM, LB, and CB analyzed and interpreted data. VN, JM, LB, and CB were in charge of statistical analyses. VN and JM wrote the manuscript. All authors made critical revisions to the article. All authors gave their final approval of the article. Valentin Navel and Jean Malecaze contributed equally.

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## References

- [1] M. L. Khaled, I. Helwa, M. Drewry, M. Seremwe, A. Estes, and Y. Liu, "Molecular and histopathological changes associated with keratoconus," *BioMed Research International*, vol. 2017, 16 pages, 2017.
- [2] J. H. Krachmer, R. S. Feder, and M. W. Belin, "Keratoconus and related noninflammatory corneal thinning disorders," *Survey of Ophthalmology*, vol. 28, no. 4, pp. 293–322, 1984.
- [3] E. di Martino, M. Ali, and C. F. Inglehearn, "Matrix metalloproteinases in keratoconus—too much of a good thing?," *Experimental Eye Research*, vol. 182, pp. 137–143, 2019.
- [4] Y. S. Rabinowitz, "Keratoconus," *Survey of Ophthalmology*, vol. 42, no. 4, pp. 297–319, 1998.
- [5] T. T. McMahon, T. B. Edrington, L. Szczotka-Flynn et al., "Longitudinal changes in corneal curvature in keratoconus," *Cornea*, vol. 25, no. 3, pp. 296–305, 2006.
- [6] A. N. Jinabhai, "Customised aberration-controlling corrections for keratoconic patients using contact lenses," *Clinical and Experimental Optometry*, vol. 103, no. 1, pp. 31–43, 2019.
- [7] A. C. Ferdi, V. Nguyen, D. M. Gore, B. D. Allan, J. J. Rozema, and S. L. Watson, "Keratoconus natural progression," *Ophthalmology*, vol. 126, no. 7, pp. 935–945, 2019.
- [8] W. Li and B. Wang, "Efficacy and safety of transepithelial corneal collagen crosslinking surgery versus standard corneal collagen crosslinking surgery for keratoconus: a meta-analysis of randomized controlled trials," *BMC Ophthalmology*, vol. 17, no. 1, 2017.
- [9] K. Allard and M. Zetterberg, "Toric IOL implantation in a patient with keratoconus and previous penetrating keratoplasty: a case report and review of literature," *BMC Ophthalmology*, vol. 18, no. 1, 2018.
- [10] M. Matthaeci, H. Sandhaeger, M. Hermel et al., "Changing indications in penetrating keratoplasty: a systematic review of 34 years of global reporting," *Transplantation*, vol. 101, no. 6, pp. 1387–1399, 2017.
- [11] J. S. Parker, K. van Dijk, and G. R. J. Melles, "Treatment options for advanced keratoconus: a review," *Survey of Ophthalmology*, vol. 60, no. 5, pp. 459–480, 2015.
- [12] D. A. Godefrooij, G. A. De Wit, C. S. Uiterwaal, S. M. Imhof, and R. P. Wisse, "Age-specific incidence and prevalence of keratoconus: a nationwide registration study," *American Journal of Ophthalmology*, vol. 175, pp. 169–172, 2017.
- [13] A. R. Pearson, B. Soneji, N. Sarvananthan, and J. H. Sandford-Smith, "Does ethnic origin influence the incidence or severity of keratoconus?," *Eye*, vol. 14, no. 4, pp. 625–628, 2000.
- [14] D. Gatinel, "Eye rubbing, a sine qua non for keratoconus?," *International Journal of Keratoconus and Ectatic Corneal Diseases*, vol. 5, no. 1, pp. 6–12, 2016.
- [15] B. Jafri, H. Lichter, and R. D. Stulting, "Asymmetric keratoconus attributed to eye rubbing," *Cornea*, vol. 23, no. 6, pp. 560–564, 2004.
- [16] A. M. Bawazeer, "Atopy and keratoconus: a multivariate analysis," *British Journal of Ophthalmology*, vol. 84, no. 8, pp. 834–836, 2000.

- [17] H. Hashemi, S. Heydarian, E. Hooshmand et al., "The prevalence and risk factors for keratoconus: a systematic review and meta-analysis," *Cornea*, vol. 39, no. 2, pp. 263–270, 2019.
- [18] H. Valgaeren, C. Koppen, and G. Van Camp, "A new perspective on the genetics of keratoconus: why have we not been more successful?," *Ophthalmic Genetics*, vol. 39, no. 2, pp. 158–174, 2018.
- [19] K. H. Weed, C. J. MacEwen, and C. N. J. McGhee, "The variable expression of keratoconus within monozygotic twins: Dundee University Scottish Keratoconus Study (DUSKS)," *Contact Lens and Anterior Eye*, vol. 29, no. 3, pp. 123–126, 2006.
- [20] J. Wheeler, M. A. Hauser, N. A. Afshari, R. Rand Allingham, and Y. Liu, "The genetics of keratoconus: a review," *Reproductive System & Sexual Disorders: Current Research*, vol. 1, Supplement 6, no. 2, 2012.
- [21] V. Galvis, T. Sherwin, A. Tello, J. Merayo, R. Barrera, and A. Acera, "Keratoconus: An Inflammatory Disorder?," *Eye*, vol. 29, no. 7, pp. 843–859, 2015.
- [22] I. Lema, T. Sobrino, J. A. Duran, D. Brea, and E. Diez-Feijoo, "Subclinical keratoconus and inflammatory molecules from tears," *British Journal of Ophthalmology*, vol. 93, no. 6, pp. 820–824, 2009.
- [23] V. Navel, J. Malecaze, B. Pereira et al., "Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis," *Acta Ophthalmologica*, vol. 99, no. 6, pp. e777–e794, 2020.
- [24] K. Nishtala, N. Pahuja, R. Shetty, R. M. Nuijts, and A. Ghosh, "Tear biomarkers for keratoconus," *Eye and Vision*, vol. 3, no. 1, 2016.
- [25] R. P. L. Wisse, J. J. W. Kuiper, R. Gans, S. Imhof, T. R. D. J. Radstake, and A. Van der Lelij, "Cytokine expression in keratoconus and its corneal microenvironment: a systematic review," *The Ocular Surface*, vol. 13, no. 4, pp. 272–283, 2015.
- [26] S. Ahmad, H. Khan, Z. Siddiqui et al., "AGEs, RAGEs and s-RAGE; friend or foe for cancer," *Seminars in Cancer Biology*, vol. 49, pp. 44–55, 2018.
- [27] S. Ahmad, M. Y. Khan, Z. Rafi et al., "Oxidation, glycation and glycoxidation—the vicious cycle and lung cancer," *Seminars in Cancer Biology*, vol. 49, pp. 29–36, 2018.
- [28] S. Tabrez, K. Z. Al-Shali, and S. Ahmad, "Lycopene powers the inhibition of glycation-induced diabetic nephropathy: a novel approach to halt the AGE-RAGE axis menace: lycopene powers the inhibition of glycation-induced DN," *BioFactors*, vol. 41, no. 5, pp. 372–381, 2015.
- [29] A. Bierhaus, P. M. Humpert, M. Morcos et al., "Understanding RAGE, the receptor for advanced glycation end products," *Journal of Molecular Medicine*, vol. 83, no. 11, pp. 876–886, 2005.
- [30] N. Vazzana, F. Santilli, C. Cuccurullo, and G. Davi, "Soluble forms of RAGE in internal medicine," *Internal and Emergency Medicine*, vol. 4, no. 5, pp. 389–401, 2009.
- [31] J. Xie, J. D. Méndez, V. Méndez-Valenzuela, and M. M. Aguilar-Hernández, "Cellular signalling of the receptor for advanced glycation end products (RAGE)," *Cellular Signalling*, vol. 25, no. 11, pp. 2185–2197, 2013.
- [32] J. Audard, T. Godet, R. Blondonnet et al., "Inhibition of the receptor for advanced glycation end-products in acute respiratory distress syndrome: a randomised laboratory trial in piglets," *Scientific Reports*, vol. 9, no. 1, p. 9227, 2019.
- [33] T.-W. Lee, Y.-H. Kao, T.-I. Lee, and Y.-J. Chen, "ADAM10 modulates calcitriol-regulated RAGE in cardiomyocytes," *European Journal of Clinical Investigation*, vol. 47, no. 9, pp. 675–683, 2017.
- [34] S. J. Park, T. Kleffmann, and P. A. Hessian, "The G82S polymorphism promotes glycosylation of the receptor for advanced glycation end products (RAGE) at asparagine 81: comparison of wild-type rage with the g82s polymorphic variant," *Journal of Biological Chemistry*, vol. 286, no. 24, pp. 21384–21392, 2011.
- [35] C. Gross, C. Belville, M. Lavergne et al., "Advanced glycation end products and receptor (RAGE) promote wound healing of human corneal epithelial cells," *Investigative Ophthalmology & Visual Science*, vol. 61, no. 3, p. 14, 2020.
- [36] M. M. Moschos, I. Chatziralli, A. Sioziou, and M. Gazouli, "Receptor of advanced glycation end products gene polymorphism and primary open-angle glaucoma," *Ophthalmic Research*, vol. 58, no. 2, pp. 81–84, 2017.
- [37] D. Tao, X. Mai, T. Zhang, and Y. Mei, "Association between the RAGE (receptor for advanced glycation end-products) -374T/A gene polymorphism and diabetic retinopathy in T2DM," *Revista da Associação Médica Brasileira*, vol. 63, no. 11, pp. 971–977, 2017.
- [38] J. Xu, L.-J. Chen, Y. Jian et al., "Involvement of advanced glycation end products in the pathogenesis of diabetic retinopathy," *Cellular Physiology and Biochemistry*, vol. 48, no. 2, pp. 705–717, 2018.
- [39] W. Yu, J. Yang, W. Sui, Q. Bin, P. Huang, and Y. Chen, "Association of genetic variants in the receptor for advanced glycation end products gene with diabetic retinopathy: a meta-analysis," *Medicine*, vol. 95, no. 39, article e4463, 2016.
- [40] M. Banevicius, A. Vilkeviciute, L. Kriauciuniene, R. Liutkeviciene, and V. P. Deltuva, "The association between variants of receptor for advanced glycation end products (RAGE) gene polymorphisms and age-related macular degeneration," *Medical Science Monitor*, vol. 24, no. janvier, pp. 190–199, 2018.
- [41] Z. Wang, J. T. Handa, W. Richard Green, W. J. Stark, R. S. Weinberg, and S. Albert, "Advanced glycation end products and receptors in Fuchs' dystrophy corneas undergoing Descemet's stripping with endothelial keratoplasty," *Ophthalmology*, vol. 114, no. 8, pp. 1453–1460, 2007.
- [42] C. T. Raghavan and R. H. Nagaraj, "AGE-RAGE interaction in the TGF $\beta$ 2-mediated epithelial to mesenchymal transition of human lens epithelial cells," *Glycoconjugate Journal*, vol. 33, no. 4, pp. 631–643, 2016.
- [43] S. Al-Swailem, X. Zhenhua, W. Lijuan, M. J. Hartsock, S. C. Yiu, and E. J. Duh, "Induction of endothelial RAGE expression in pterygium," *Molecular Vision*, vol. 20, pp. 1740–1748, 2014.
- [44] K. Nielsen, J. Hjortdal, M. Pihlmann, and T. J. Corydon, "Update on the keratoconus genetics," *Acta Ophthalmologica*, vol. 91, no. 2, pp. 106–113, 2013.
- [45] D. W. Sabiston, "The association of keratoconus, dermatitis and asthma," *Transactions of the Ophthalmological Society of New Zealand*, vol. 18, pp. 66–71, 1966.
- [46] C. C. Teng, "Electron microscope study of the pathology of keratoconus: part I\*," *American Journal of Ophthalmology*, vol. 55, no. 1, pp. 18–47, 1963.
- [47] Y. S. Rabinowitz, "The genetics of keratoconus," *Ophthalmology Clinics of North America*, vol. 16, no. 4, pp. 607–620, 2003.

- [48] Y. Poulighen, J. Bureau, M. Mirshahi, S. S. Mirshahi, M. Assouline, and G. Lorens, "Keratoconus and inflammatory processes," *Bulletin De La Societe Belge D'ophtalmologie*, vol. 262, pp. 25–28, 1996.
- [49] L. Zhang, M. Bukulin, E. Kojro et al., "Receptor for advanced glycation end products is subjected to protein ectodomain shedding by metalloproteinases," *Journal of Biological Chemistry*, vol. 283, no. 51, pp. 35507–35516, 2008.
- [50] A. Faiz, M. van den Berge, C. J. Vermeulen, N. H. T. ten Hacken, V. Guryev, and S. D. Pouwels, "AGER expression and alternative splicing in bronchial biopsies of smokers and never smokers," *Respiratory Research*, vol. 20, no. 1, 2019.
- [51] M. W. Belin, H. S. Jang, and B. M. Keratoconus, "Keratoconus: diagnosis and staging," *Cornea*, vol. 41, no. 1, 2021.
- [52] R. Blondonnet, J. Audard, C. Belville et al., "RAGE inhibition reduces acute lung injury in mice," *Scientific Reports*, vol. 7, no. 1, p. 7208, 2017.
- [53] E. Charrin, C. Faes, A. Sotiaux et al., "Receptor for advanced glycation end products antagonism blunts kidney damage in transgenic Townes sickle mice," *Frontiers in physiology*, vol. 10, p. 880, 2019.
- [54] S. Lee, C. Piao, G. Kim, J. Y. Kim, E. Choi, and M. Lee, "Production and application of HMGB1 derived recombinant RAGE-antagonist peptide for anti-inflammatory therapy in acute lung injury," *European Journal of Pharmaceutical Sciences*, vol. 114, pp. 275–284, 2018.
- [55] J. B. Lewis, C. Mejia, C. Jordan et al., "Inhibition of the receptor for advanced glycation end-products (RAGE) protects from secondhand smoke (SHS)-induced intrauterine growth restriction IUGR in mice," *Cell and Tissue Research*, vol. 370, no. 3, pp. 513–521, 2017.
- [56] H. Yang, H. Liu, Q. Zeng et al., "Inhibition of HMGB1/RAGE-mediated endocytosis by HMGB1 antagonist box a, anti-HMGB1 antibodies, and cholinergic agonists suppresses inflammation," *Molecular Medicine*, vol. 25, no. 1, p. 13, 2019.

## **Publication N°2**

### **Oxidative and Anti-Oxidative Stress Markers in Keratoconus: A Systematic Review and Meta-Analysis**

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# Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis

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## ABSTRACT.

**Purpose:** To conduct a systematic review and meta-analysis on the levels of oxidative stress markers and antioxidants in keratoconus compared to healthy subject.

**Method:** The PubMed, Cochrane Library, Embase, Science Direct and Google Scholar databases were searched on 1st June 2020 for studies reporting oxidative and antioxidative stress markers in keratoconus and healthy controls. Main meta-analysis was stratified by type of biomarkers, type of samples (tears, cornea, aqueous humour and blood) and type of corneal samples (stromal cells, epithelium and endothelium).

**Results:** We included 36 articles, for a total of 1328 keratoconus patients and 1208 healthy controls. There is an overall increase in oxidative stress markers in keratoconus compared with healthy controls (standard mean deviation (SMD) = 0.94, 95% confidence interval (95% CI) 0.55–1.33), with a significant increase in reactive oxygen and nitrogen species (1.09, 0.41–1.78) and malondialdehyde (1.78, 0.83–2.73). There is an overall decrease in antioxidants in keratoconus compared with healthy controls (–0.63, –0.89 to –0.36), with a significant decrease in total antioxidant capacity/status (–1.65, –2.88 to –0.43), aldehyde/NADPH dehydrogenase (–0.77, –1.38 to –0.17), lactoferrin/transferrin/albumin (–1.92, –2.96 to –0.89) and selenium/zinc (–1.42, –2.23 to –0.61). Oxidative stress markers were higher in tears and in cornea of keratoconus than in aqueous humour, and antioxidants were decreased in tears, aqueous humour and blood without difference between sample type. Oxidative stress markers increased in stromal cells and antioxidants decreased in endothelium.

**Conclusion:** Oxidative stress markers and antioxidants were dysregulated in keratoconus, involving an imbalance of redox homeostasis in tears, cornea, aqueous humour and blood.

**Key words:** antioxidants – cornea – inflammation – keratoconus – oxidative damage – oxidative stress – reactive oxygen species – stroma – tears

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## Introduction

Keratoconus (KC) is classically described like a non-inflammatory vision-threatening disease beginning at adolescence or in young adult (Krachmer et al. 1984; Bron 1988; Rabinowitz 1998). The reported incidence of KC is estimated at 1 to 5.3/2000 in the general population (Pouliquen 1987; Rabinowitz 1998; Godefrooij et al. 2017; Bak-Nielsen et al. 2019a,b). The cornea, transparent and avascular structure, is a crucial part of the optical system involved in 2/3 of the refractive power of the eye. Its function and transparency are intimately linked to its regular dome-shaped that are progressively disturbing during KC (Andreasen et al. 1980; McMonnies 2005; McMonnies & Boneham 2010), despite of the innovative therapeutics involving the use of rigid contact lenses (Rico-Del-Viejo et al. 2017; Jinabhai 2020), corneal collagen cross-linking (Wen et al. 2018a,b; Jiang et al. 2019), refractive surgeries (Allard & Zetterberg 2018; Benoist d'Azy et al. 2019) or corneal graft (Dragnea et al. 2018; Song et al. 2019). KC seems to be a



multifactorial disease associated with environmental factors, such as atopy (Shajari et al. 2016; Naderan et al. 2017) or eye rubbing (Gordon-Shaag et al. 2012; Balasubramanian et al. 2013; Bak-Nielsen et al. 2019a,b), and genetic predispositions (Rabinowitz 2003; Rong et al. 2017; Kabza et al. 2019). However, the pathogenic processes of the disease are still unknown, and many questions persist on the triggering and progression of ectasia. During past decades, the identification of pro-inflammatory cytokines (IL-1, IL-6, TNF- $\alpha$  and TGF- $\beta$ ), degradation of collagens by metalloproteases (MMP-1, MMP-3 and MMP-9) and lysyl oxidase (LOX) in cornea tissues and tears provided news arguments to consider KC, at least part, as an inflammatory condition (Kusano et al. 1998; Abalain et al. 2000; Collier 2001; Lema & Duran 2005; Clark et al. 2008; Lema et al. 2009; Balasubramanian et al. 2013; Dudakova & Jirsova 2013; Galvis et al. 2015; Wisse et al. 2015; Nishtala et al. 2016; Khaled et al. 2017; Ionescu et al. 2018; di Martino et al. 2019). However, the role of the oxidative stress as a stakeholder in the vicious circle of KC is still unresolved (Chen et al. 2009; Khaled et al. 2017). The ocular surface is particularly exposed to the solar ultraviolet radiations, free radicals of atmospheric pollution and oxygen involving reactive oxygen species (ROS) that induced lipids peroxidation of membranes, mitochondrial DNA damages, tissue remodelling and cells apoptosis mediated by autophagy (Chen et al. 2009; Čejka & Čejkova 2015; Čejková & Čejka 2015; Shetty et al. 2017). Evidence data tended to describe an imbalance of redox homeostasis in the blood of KC as well as in the ocular surface, but the variability of test methods, sample sizes and biomarkers induced sometimes conflicting conclusions (Karamichos et al. 2015; Cantemir et al. 2016a, 2016b; Atilano et al. 2019; Zarei-Ghanavati et al. 2019; Abdul-Maksoud et al. 2020). Thereby, levels of oxidative stress markers and antioxidants seem to be increased or decreased in KC compared to healthy subjects according to studies and the type of samples (cornea, tears, aqueous humour or blood) (Behndig et al. 2001; Cantemir et al. 2016a, 2016b; Balmus et al. 2020). Thus, it seemed to be necessary to summarize the literature to

clarify the role of oxidative stress involved in the pathological process of KC. Therefore, we aimed to compute a systematic review and meta-analysis to compare the level of oxidative stress markers and antioxidants in KC than healthy controls.

## Methods

### Method of literature search

We searched all articles in PubMed, Cochrane Library, Embase, ScienceDirect and Google Scholar databases to 1st June 2020 with the following keywords: ('reactive oxygen species' OR 'oxidative stress' OR 'nitrate stress' OR 'nitrate damage' OR 'oxidant stress' OR 'oxidative damage' OR 'antioxidant effect' OR 'antioxidants' OR 'antioxidative stress' OR 'antioxidant stress' OR 'antinitrate stress') AND keratoconus (details of search strategy are presented in Appendix S1). The search was not limited to specific years, language restrictions, and no minimal sample size was applied. To be included, articles needed to evaluate a level of oxidative and/or antioxidative stress markers in sample of KC and healthy controls. We excluded articles studying on animals. We imposed no limitation of regional origin. In addition, references list of all publications was manually searched to identify any other ones not found with electronic search. The search strategy is presented in Fig. 1. One author conducted all literature searches (Valentin Navel) and collated the abstracts. Two authors (Valentin Navel and Frédéric Dutheil) separately reviewed the abstracts and based on the selection criteria, decided the suitability of the articles for inclusion. A third author (Frédéric Chiambaretta) was asked to review the articles where consensus on suitability was debated. Finally, all authors reviewed eligible articles. We followed the PRISMA guidelines (Appendix S2).

### Data collection

The data collected included first author's name, publication year, study design (case-control, cross-sectional or cohort study), type of study (laboratory or field study), aims and outcomes of included articles, inclusion and exclusion criteria, sample size, mean

age, percentage of male sex, type of sample (blood, cornea, aqueous humour or tears), origin of samples (during penetrating keratoplasty, cornea cross-linking, refractive surgery as refractive photo-keratectomy or laser-assisted in situ keratomileusis, cataract surgery, or immediately after the death), type of cornea tissues (stroma cells, epithelium, stroma or endothelium), follow-up, best-corrected visual acuity (logMar), maximal keratometry (diopetre), central pachymetry ( $\mu$ m), type of biomarkers (oxidants or antioxidants), method of determination (such as enzymatic assay and reaction, Western blot, ELISA, mass spectrometry) and putative adjustment/explaining factors (such as geographic zone, body mass index (BMI), smoking, atopy, contact lenses users).

### Outcomes

We studied the levels of oxidative stress markers and antioxidants in keratoconus comparing with healthy controls as primary outcome. Secondary outcomes were the difference of oxidative/antioxidative biomarkers levels according to the type of samples, type of biomarkers, geographic zones and their association with covariates.

### Quality of assessment

Although not created for that, the 'Strengthening the Reporting of Observational studies in Epidemiology' (STROBE) criteria may be inappropriately used as an assessment tool to judge study quality. STROBE is a checklist of 22 items. We attributed one point per items and then converted into percentage to give a quality score for each included study (da Costa et al. 2011; von Elm et al. 2014). We also used the Newcastle-Ottawa Quality Assessment Scale (NOS) model to also judge observational studies with the dedicated evaluation grids. The following nine items were assessed in all cohort studies: four items on selection bias (representativeness of the exposed and non-exposed cohort, ascertainment of expose), two items on comparability bias (design and analysis) and three items on outcome bias (assessment of outcome, longer and adequacy of follow-up). Similar items were used to evaluate case-control studies. Each item was assigned a judgement of

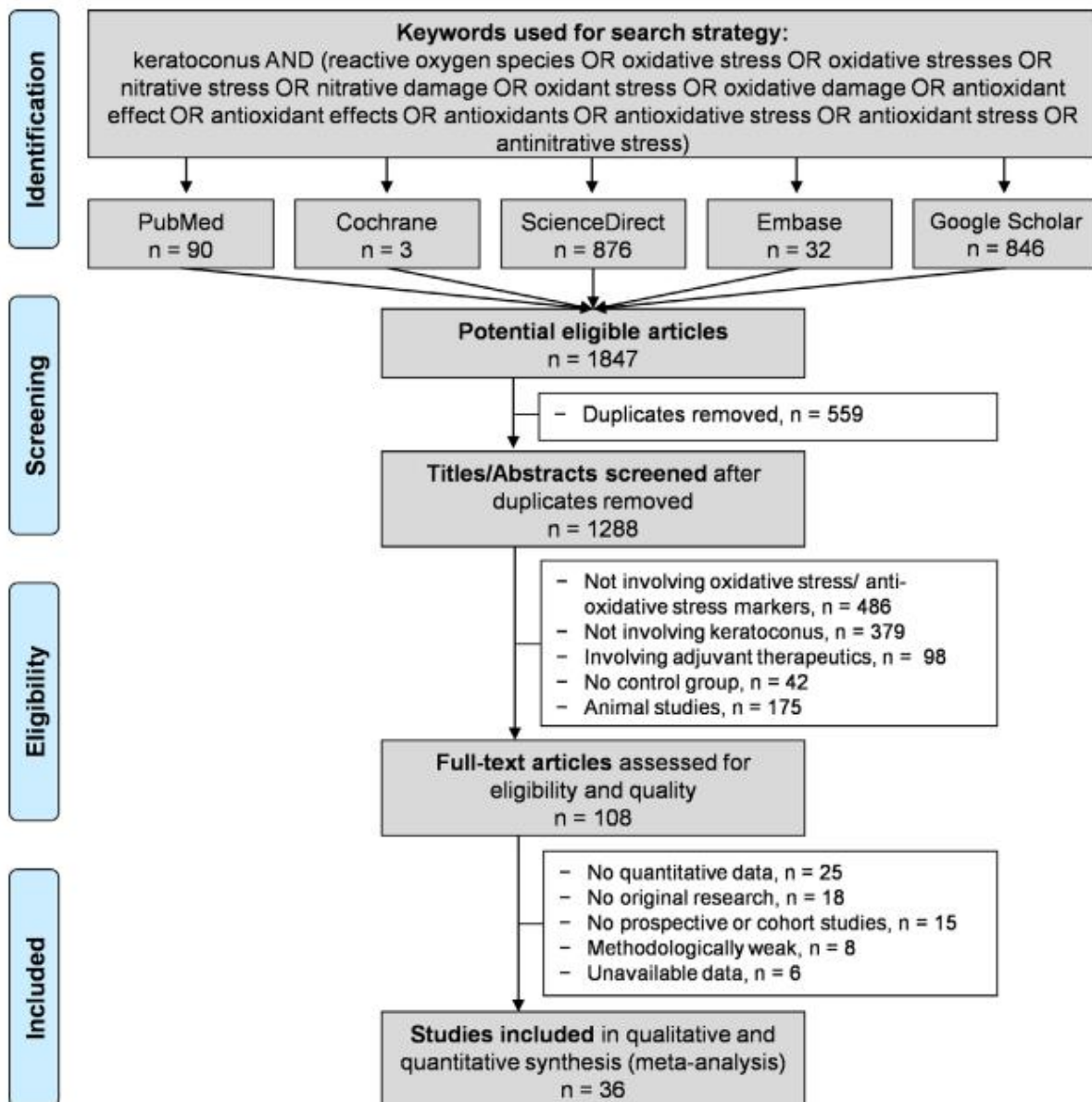


Fig. 1. Flow diagram according with PRISMA guidelines.

‘Yes’, ‘No’, ‘Can’t say’ or ‘Not applicable’ (Wells et al. 2014). The methodological quality of the studies was evaluated by two authors (Valentin Navel and Frédéric Dutheil). Disagreements were addressed by obtaining a consensus with a third author (Bruno Pereira) (Fig. 2).

**Statistical considerations**

Statistical analyses were conducted using Comprehensive Meta-analysis

software (version 2, Biostat Corporation) and Stata software (version 13, StataCorp, College Station, TX, USA) (Ollier et al. 2014; Lanhers et al. 2015; Benoit d’Azy et al. 2016a,b; Courtin et al. 2016; Lanhers et al. 2017; Benoit d’Azy et al. 2019; Navel et al. 2019). Characteristics of the biomarkers and subjects (keratoconus and controls) were summarized for each study sample and reported as mean ± standard deviation (SD) and number (%) for continuous and categorical variables,

respectively. For rigour, effect sizes were defined as standard mean differences (SMD) of oxidants/antioxidants levels. First, we conducted meta-analyses on oxidants/antioxidants levels. Standard difference in means between groups (SMD, difference between means divided by the pooled standard deviation, with a correction for small sample bias) and 95% confidence interval (95% CI) were estimated using DerSimonian and Laird random-effects models (assuming that the true effect

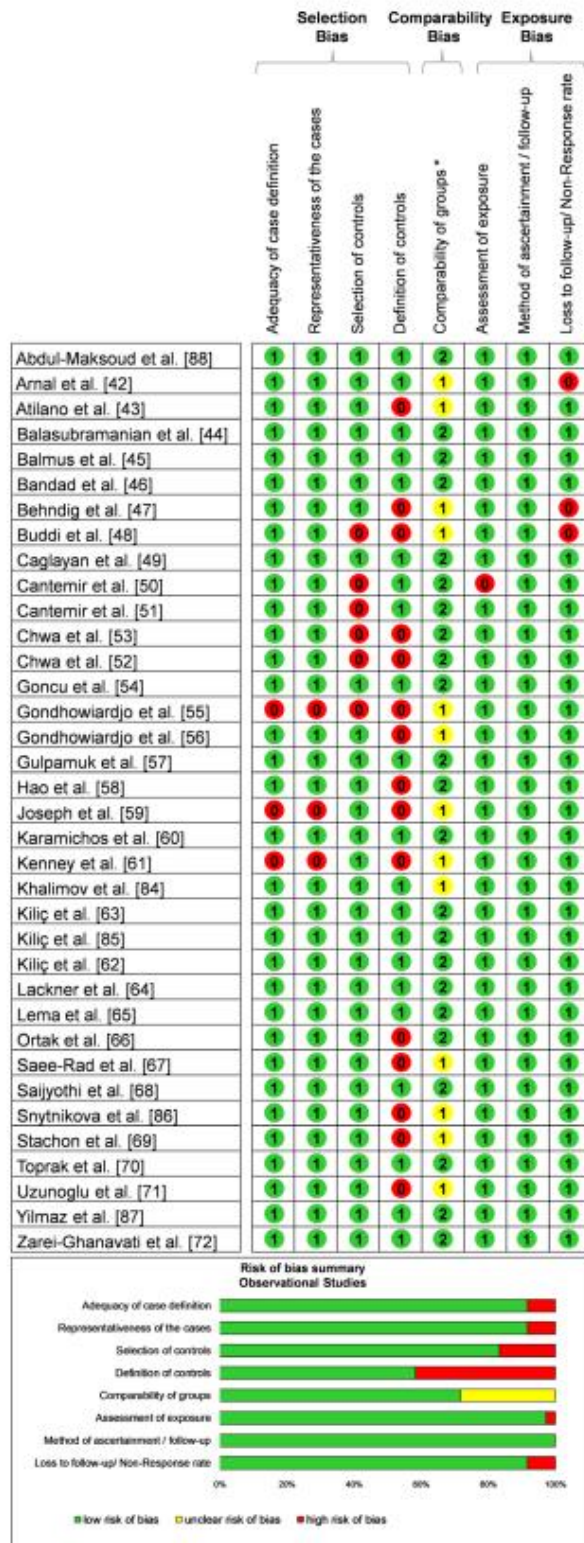


Fig. 2. Methodological quality of included articles using Newcastle–Ottawa Quality Assessment Scale. For each item, criteria fulfilled: no: 0, yes: 1 (\*: 2 subitems), unclear: ? and not applicable: NA.

**Table 1.** Table Characteristics of included studies (more details available in Appendix S3 including study designs, *in vivo* or *in vitro* studies, and characteristics of keratoconus, i.e.  $K_{max}$  and pachymetry).

Study	Methodology				Keratoconus				Controls				Oxidants/Antioxidants			
	Country	Follow-up years	Material tissues	n	Age mean ± SD	Sex %men	Sex %women	Age mean ± SD	Sex %men	Sex %women	Biomarkers	Status	Methods	Status	Methods	
																n
Abdul-Maksoud 2020 [1]	Egypt	-	Blood	150	22.8 ± 3.2	72.7	74.0	100	22.6 ± 2.6	74.0	Catalase	Antioxidant	Colorimetry/method of Aebi	Antioxidant	Colorimetry/method of Aebi	
Arnal 2011 [2]	Spain	2011	Cornea	150	22.8 ± 3.2	72.7	74.0	100	22.6 ± 2.6	74.0	Glutathione peroxidase	Antioxidant	Enzyme activity assay	Antioxidant	Enzyme activity assay	
				7	33.6	71.4	66.7	6	34.8	66.7	Total antioxidant capacity	Antioxidant	Enzyme activity assay	Antioxidant	Enzyme activity assay	
				7	33.6	71.4	66.7	6	34.8	66.7	Total Nitrites	Oxidant	Total nitrite assay	Oxidant	Total nitrite assay	
				7	33.6	71.4	66.7	6	34.8	66.7	Malondialdehyde	Oxidant	Lipid peroxidation microplate assay	Oxidant	Lipid peroxidation microplate assay	
Arlano 2019 [3]	USA	2018	Cornea (stromal cells)	7	33.6	71.4	66.7	6	34.8	66.7	4-hydroxy-2,3-trans-nonenal	Oxidant	Immunohistochemistry	Oxidant	Immunohistochemistry	
				7	33.6	71.4	66.7	6	34.8	66.7	Glutathione	Antioxidant	Glutathione assay	Antioxidant	Glutathione assay	
				5	34.8	-	-	6	40.2	-	Reactive oxygen species/reactive nitrogen species	Oxidant	RT-qPCR	Oxidant	RT-qPCR	
Balasubramanian 2012 [4]	Australia	2011	Tears	26	-	50.0	46.4	28	-	46.4	Lactoferrin	Antioxidant	ELISA	Antioxidant	ELISA	
Baltus 2020 [5]	Romania	2019	Tears	18	-	44.4	46.7	15	-	46.7	Albumin	Antioxidant	ELISA	Antioxidant	ELISA	
				20	29.9 ± 1.3	75.0	75.0	20	29.4 ± 1	75.0	Malondialdehyde	Oxidant	Thio-barbituric acid-reactive substances	Oxidant	Thio-barbituric acid-reactive substances	
				20	29.9 ± 1.3	75.0	75.0	20	29.4 ± 1	75.0	Superoxide dismutase	Antioxidant	Spectrophotometry/	Antioxidant	Spectrophotometry/	
Banded 2018 [6]	Iran	2018	Blood	20	29.9 ± 1.3	75.0	75.0	20	29.4 ± 1	75.0	Glutathione peroxidase	Antioxidant	Enzyme activity assay	Antioxidant	Enzyme activity assay	
				50	29.3 ± 3.5	56.0	54.0	50	28.9 ± 5.9	54.0	Selenium	Antioxidant	Atomic absorption	Antioxidant	Atomic absorption	
				50	29.3 ± 3.5	56.0	54.0	50	28.9 ± 5.9	54.0	Zinc	Antioxidant	Colorimetry	Antioxidant	Colorimetry	
Behndig 2001 [7]	Sweden	2000	Cornea	9	-	-	-	11	-	-	Superoxide dismutase	Antioxidant	Spectrophotometry/ELISA	Antioxidant	Spectrophotometry/ELISA	
				9	-	-	-	11	-	-	Superoxide dismutase	Antioxidant	Spectrophotometry/ELISA	Antioxidant	Spectrophotometry/ELISA	
				9	-	-	-	11	-	-	Superoxide dismutase	Antioxidant	Spectrophotometry/ELISA	Antioxidant	Spectrophotometry/ELISA	
				9	-	-	-	11	-	-	Superoxide dismutase	Antioxidant	Spectrophotometry/ELISA	Antioxidant	Spectrophotometry/ELISA	
				9	-	-	-	11	-	-	Superoxide dismutase	Antioxidant	Spectrophotometry/ELISA	Antioxidant	Spectrophotometry/ELISA	
Buddi 2002 [8]	USA	2001	Cornea (epithelium)	13	-	-	-	8	-	-	Malondialdehyde	Oxidant	Immunohistochemistry	Oxidant	Immunohistochemistry	
				26	-	-	-	10	-	-	Nitric oxide synthase	Oxidant	Immunohistochemistry	Oxidant	Immunohistochemistry	
				26	-	-	-	10	-	-	Nitric oxide synthase	Oxidant	Immunohistochemistry	Oxidant	Immunohistochemistry	
				26	-	-	-	11	-	-	Nitric oxide synthase	Oxidant	Immunohistochemistry	Oxidant	Immunohistochemistry	
Caglayan 2018 [9]	Turkey	2017-2018	Cornea (endothelium)	69	24.6 ± 5	53.6	51.9	52	28.4 ± 7.4	51.9	Heme-oxidase 2 enzyme	Antioxidant	Immunohistochemistry	Antioxidant	Immunohistochemistry	
				10	-	-	-	10	-	-	Malondialdehyde	Oxidant	Thio-barbituric acid-reactive substances	Oxidant	Thio-barbituric acid-reactive substances	
Camtemir 2016a [10]	Romania	-	Blood	20	27.1 ± 1.5	75.0	75.0	20	29.3 ± 1.1	75.0	Superoxide dismutase	Antioxidant	Enzyme activity assay	Antioxidant	Enzyme activity assay	
Camtemir 2016b [11]	Romania	-	Blood	20	27.1 ± 1.5	75.0	75.0	20	29.3 ± 1.1	75.0	Glutathione peroxidase	Antioxidant	Enzyme activity assay	Antioxidant	Enzyme activity assay	
Chwa 2006 [12]	USA	2005-2006	Cornea (stromal cells)	10	33.9 ± 7.1	60.0	77.8	9	36.7 ± 9	77.8	Catalase	Antioxidant	Biochemical protein assay	Antioxidant	Biochemical protein assay	

Table 1 (Continued)

Study	Methodology			Keratoconus			Controls			Oxidants/Antioxidants			Status	Methods
	Country	Follow-up years	Material tissues	n	Age mean ± SD	Sex %men	n	Age mean ± SD	Sex %men	Biomarkers	Oxidant	Antioxidant		
Chwa 2008 [13]	USA	2008	Cornea (stromal cells)	10	33.9 ± 7.1	60.0	9	36.7 ± 9	77.8	Reactive oxygen species/ reactive nitrogen species	Oxidant		Fluorescent dye 2', 7'-dichlorodihydrofluorescein diacetate	
Goncu 2015 [14]	Turkey	2013–2014	Blood	31	22.6 ± 9.3	58.1	33	25.1 ± 7.9	48.5	Total antioxidant status	Antioxidant		Spectrophotometry	
Gondhoviardjo 1993a [15]	Indonesia	-	Cornea	18	34.8 ± 6.8	45.5	11	40.1 ± 11.6	72.7	Reactive oxygen species/ reactive nitrogen species	Oxidant		Fluorescent dye 2', 7'-dichlorodihydrofluorescein diacetate	
Gondhoviardjo 1993b [16]	Indonesia	1991–1992	Cornea (epithelium)	23	22.6 ± 9.3	58.1	33	25.1 ± 7.9	48.5	Total antioxidant status	Antioxidant		Spectrophotometry	
Gulpamuk 2017 [17]	Turkey	2016–2017	Cornea (stromal cells)	23	22.6 ± 9.3	58.1	33	25.1 ± 7.9	48.5	Oxidative stress index	Oxidant		Percentage ratio of TOS levels to TAS levels	
Hao 2016 [18]	China	-	Cornea (stroma cells)	18	-	-	44	-	-	Aldehyde dehydrogenase	Antioxidant		Enzyme assay/ spectrophotometry	
Joseph 2011 [19]	USA	-	Cornea (epithelium)	18	-	-	44	-	-	Glutathione reductase	Antioxidant		Enzyme assay/ spectrophotometry	
Karamichos 2016 [20]	USA	-	Tears	18	-	-	44	-	-	Glutathione-S-transferase	Antioxidant		Enzyme assay/ spectrophotometry	
Kenney 2005 [21]	USA	-	Cornea	23	32.2 ± 8.5	-	51	56.8 ± 21.9	-	Aldehyde dehydrogenase	Antioxidant		Enzyme assay/ spectrophotometry	
Khalimov 2019 [22]	Russia	-	Tears	23	32.2 ± 8.5	-	10	64.2 ± 20.7	-	Aldehyde dehydrogenase	Antioxidant		Enzyme assay/ spectrophotometry	
Kılıç 2015a [23]	Turkey	2014–2015	Blood	23	32.2 ± 8.5	-	10	64.2 ± 20.7	-	Aldehyde dehydrogenase	Antioxidant		Enzyme assay/ spectrophotometry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Native thiol	Antioxidant		Colorimetry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Total thiol	Antioxidant		Colorimetry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Native thiol/total thiol	Antioxidant		Colorimetry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Disulphide	Oxidant		Colorimetry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Disulphide/native thiol	Oxidant		Colorimetry	
				28	23.1 ± 6.1	50.0	30	21.6 ± 5.1	56.7	Disulphide/total thiol	Oxidant		Colorimetry	
				3	21 ± 13	-	3	38.0 ± 14.6	-	Reactive oxygen species	Oxidant		Immunohistochemistry	
				4	-	0.0	4	-	-	NADPH dehydrogenase	Antioxidant		Mass spectrophotometry	
				4	-	0.0	4	-	-	Serotransferrin	Antioxidant		Mass spectrophotometry	
				14	29.7 ± 9.3	0.0	15	38.0 ± 7	-	Lactate/pyruvate	Oxidant		Mass spectrophotometry	
				14	29.7 ± 9.3	0.0	15	38.0 ± 7	-	Glutathione	Antioxidant		Mass spectrophotometry	
				10	-	0.0	7	-	-	Catalase	Antioxidant		Enzyme activity assay	
				8	-	0.0	8	-	-	Catalase	Antioxidant		RT-PCR/immunochimistry	
				38	-	63.2	12	-	-	Total oxidant status	Antioxidant		Spectrophotometry	
				38	-	63.2	12	-	-	Superoxide dismutase	Antioxidant		Enzyme activity assay	
				69	25.3 ± 7.3	40.6	72	24.6 ± 6.7	40.3	Total antioxidant capacity	Antioxidant		Total Antioxidant Status assay	
				69	25.3 ± 7.3	40.6	72	24.6 ± 6.7	40.3	Total Oxidant Status	Oxidant		Total Oxidant Status assay	

Table 1 (Continued)

Study	Methodology			Keratoconus			Controls			Oxidants/Antioxidants			Status	Methods
	Country	Follow-up years	Material tissues	n	Age	Sex	n	Age	Sex	Biomarkers	Oxidative stress index	Status		
					mean ± SD	%men		mean ± SD	%men					
Kılıç 2015b [24]	Turkey	2014-2016	Blood	69	25.3 ± 7.3	40.6	72	24.6 ± 6.7	40.3	Oxidative stress index	Oxidant	Percentage ratio of TOS levels to TAS levels		
Kılıç 2016 [25]	Turkey	2016	Blood	58	25.4 ± 7.3	39.7	53	25.3 ± 7.1	35.8	Superoxide dismutase	Antioxidant	Spectrophotometry/Enzyme assay		
				58	25.4 ± 7.3	39.7	53	25.3 ± 7.1	35.8	Malondialdehyde	Oxidant	Thiobarbituric acid-reactive substances		
				58	25.4 ± 7.3	39.7	53	25.3 ± 7.1	35.8	Zinc	Antioxidant	Atomic absorption spectrophotometry		
Lachner 2014 [26]	USA	-	Cornea (epithelium)	59	33.5 ± 8.7	50.8	18	52.7 ± 9.6	55.6	8-Oxo-29-deoxyguanosine	Oxidant	Immunohistochemistry		
Lema 2010 [27]	Spain	2007-2008	Tears	24	31.3 ± 7.8	50.0	24	30.8 ± 7.6	54.5	Lactoferrin	Antioxidant	Mass spectrophotometry		
				20	-	-	20	-	-	Lactoferrin	Antioxidant	Western blot		
Ottak 2012 [28]	Turkey	2011-2012	Blood	36	36.1 ± 7.3	47.2	40	36.8 ± 3.8	50.0	Superoxide dismutase	Antioxidant	Enzyme activity assay		
				36	36.1 ± 7.3	47.2	40	36.8 ± 3.8	50.0	Zinc	Antioxidant	Atomic absorption spectrometry		
Saeed-Rad 2013 [29]	Iran	-	Cornea (stromal cells)	10	-	0.0	1	-	0.0	Superoxide dismutase	Antioxidant	RT-qPCR		
Saijyothi 2012 [30]	India	-	Tears	40	26 ± 2	67.5	29	27.0 ± 6	31.0	Cysteine	Antioxidant	HPLC electrochemical detection		
				40	26 ± 2	67.5	29	27.0 ± 6	31.0	Ascorbic acid	Antioxidant	HPLC electrochemical detection		
				40	26 ± 2	67.5	29	27.0 ± 6	31.0	Glutathione	Antioxidant	HPLC electrochemical detection		
				40	26 ± 2	67.5	29	27.0 ± 6	31.0	Acid uric	Antioxidant	HPLC electrochemical detection		
				40	26 ± 2	67.5	29	27.0 ± 6	31.0	Tyrosine	Antioxidant	HPLC electrochemical detection		
				40	26 ± 2	67.5	29	27.0 ± 6	31.0	Reactive oxygen species	Oxidant	Diclorodihydrofluorescein diacetate method		
				16	-	-	29	27.0 ± 6	31.0	Cysteine	Antioxidant	HPLC electrochemical detection		
				16	-	-	29	27.0 ± 6	31.0	Ascorbic acid	Antioxidant	HPLC electrochemical detection		
				16	-	-	29	27.0 ± 6	31.0	Glutathione	Antioxidant	HPLC electrochemical detection		
				16	-	-	29	27.0 ± 6	31.0	Acid uric	Antioxidant	HPLC electrochemical detection		
				16	-	-	29	27.0 ± 6	31.0	Tyrosine	Antioxidant	HPLC electrochemical detection		
				16	-	-	29	27.0 ± 6	31.0	ROS	Oxidant	Diclorodihydrofluorescein diacetate method		
				24	-	-	29	27.0 ± 6	31.0	Cysteine	Antioxidant	HPLC electrochemical detection		

Table 1 (Continued)

Study	Country	Methodology				Keratoconus				Controls				Oxidants/Antioxidants				Methods
		Follow-up years	Material tissues	Age	Sex	Age	Sex	Age	Sex	Biomarkers	Status	Methods						
				n	mean ± SD	%men	n	mean ± SD	%men									
Snyrnikova 2017 [31]	Russia	-	Cornea	24	-	-	29	27.0 ± 6	31.0	Ascorbic acid	Antioxidant	HPLC electrochemical detection						
				24	-	-	29	27.0 ± 6	31.0	Glutathione	Antioxidant	HPLC electrochemical detection						
				24	-	-	29	27.0 ± 6	31.0	Acid uric	Antioxidant	HPLC electrochemical detection						
				24	-	-	29	27.0 ± 6	31.0	Tyrosine	Antioxidant	HPLC electrochemical detection						
				24	-	-	29	27.0 ± 6	31.0	ROS	Oxidant	Dichlorodihydrofluorescein diacetate method						
				11	31 ± 14.5	54.5	13	48.0	53.8	Ascorbate	Antioxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Citrate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Lactate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Nicotinamide adenine dinucleotide	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Pyruvate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Tyrosine	Antioxidant	Mass spectrophotometry						
Stachon 2017 [32]	Germany	2016	Aqueous humour	11	31 ± 14.5	54.5	13	48.0	53.8	Acid uric	Antioxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Glutathione	Antioxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Ascorbate	Antioxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Citrate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Lactate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Pyruvate	Oxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Tyrosine	Antioxidant	Mass spectrophotometry						
				11	31 ± 14.5	54.5	13	48.0	53.8	Acid uric	Antioxidant	Mass spectrophotometry						
				100	41.9 ± 14.9	69.0	100	71.2 ± 12.4	58.0	Uric acid	Antioxidant	Fossati reaction assay						
				25	26.4 ± 1.7	48.0	25	26.6 ± 1.7	44.0	Total oxidant status	Oxidant	Colorimetry						
Toprak 2014 [33]	Turkey	-	Blood	25	26.4 ± 1.7	48.0	25	26.6 ± 1.7	44.0	Total antioxidant capacity	Antioxidant	Colorimetry						
				25	26.4 ± 1.7	48.0	25	26.6 ± 1.7	44.0	Oxidative stress index	Oxidant	Colorimetry						
Uzunoglu 2013 [34]	Turkey	-	Aqueous humour	13	27.7 ± 5.5	53.8	10	68.0 ± 15.5	0.0	Nitrite	Oxidant	Griess reactive/ELISA method						
				13	27.7 ± 5.5	53.8	10	68.0 ± 15.5	0.0	Nitrate	Oxidant	Griess reactive/ELISA method						
Yilmaz 2020 [35]	Turkey	-	Blood	13	27.7 ± 5.5	53.8	10	68.0 ± 15.5	0.0	Nitric oxide	Oxidant	Calculating (NOx = NO3-NO2)						
				33	34.7 ± 13.3	66.7	47	30.6 ± 8.7	46.8	Homocysteine	Oxidant	Competitive immunoassay						
Zarei-Ghanavati 2019 [36]	Iran	2011	Blood	33	34.7 ± 13.3	66.7	47	30.6 ± 8.7	46.8	Vitamin B12	Antioxidant	Immunoassays						
				100	26.3 ± 7.3	0.0	100	27.8 ± 6.9	0.0	Folic acid	Antioxidant	Immunoassays						
100	26.3 ± 7.3	0.0	100	27.8 ± 6.9	0.0	Selenium	Antioxidant	Colorimetry										
100	26.3 ± 7.3	0.0	100	27.8 ± 6.9	0.0	Zinc	Antioxidant	Colorimetry										

estimates varied among studies) (DerSimonian & Laird 1986). A SMD is defined as a unitless measure of the concentration of oxidants/antioxidants centred at zero if oxidants/antioxidants levels in keratoconus are not different from healthy controls. A positive SMD denoted higher oxidants/antioxidants levels in keratoconus than healthy controls. A scale for SMD has been suggested with 0.8 reflecting a large effect, 0.5 a moderate effect and 0.2 a small effect (DerSimonian & Laird 1986).  $p$  Values less than 0.05 were considered statistically significant. Then, we stratified these meta-analyses on the type of samples (blood, aqueous humour, cornea and tears) and type of biomarkers. Statistical heterogeneity between results was assessed by examining forest plots, confidence intervals, and using  $I^2$ , which is the most common metric for measuring the magnitude of between-study heterogeneity and is easily interpretable.  $I^2$  values range between 0% and 100% and are typically considered low for heterogeneity scores of <25%, modest for 25–50% and high for >50%. For example, a significant heterogeneity may be due to the variability between the characteristics of the studies, such as those of the subjects (age, sex, etc), the type of samples or other covariables. Publication bias was taken into consideration and funnel plots were compiled, with sensitivity analysis excluding studies not evenly distributed around the base of the funnel. When possible (sufficient sample size), meta-regressions were proposed to study the relation between oxidants/antioxidants levels and characteristics of subjects (e.g. age, sex, country, smoking, atopy, contact lenses users, central pachymetry) and other sample characteristics (e.g. type of samples). Results were expressed as regression coefficients and 95% CI.

## Results

An initial search produced a possible for 1847 articles (Fig. 1). We identified 36 eligible articles (Gondhowiardjo & van Haeringen 1993; Gondhowiardjo et al. 1993; Behndig et al. 2001; Buddi et al. 2002; Kenney et al. 2005; Chwa et al. 2006; Chwa et al. 2008; Lema et al. 2010; Arnal et al. 2011; Joseph et al. 2011; Balasubramanian et al. 2012; Ortak et al. 2012; Saijyothi et al. 2012; Saeed-Rad et al. 2013; Uzunoğlu et al. 2013; Lackner et al.

2014; Toprak et al. 2014; Göncü et al. 2015; Karamichos et al. 2015; Hao et al. 2016; Kiliç et al. 2016; Kılıç et al. 2016a,b; Cantemir et al. 2016a,2016b; Gulpamuk et al. 2017; Snytnikova et al. 2017; Stachon et al. 2017; Bammad et al. 2018; Atilano et al. 2019; Caglayan et al. 2019; Khalimov et al. 2019; Abdul-Maksoud et al. 2020; Balmus et al. 2020; Yilmaz et al. 2020) (detailed in Table 1 with associated reference number) including 34 case-control studies [1–9,11–35], one cohort study [10] and one cross-sectional study [36]. Overall, these studies included a total of 1328 keratoconus and 1208 controls (Table 1 and Appendix S3). All articles were written in English, except one in Russian [22].

More details on quality of articles (Figs 1 and 2), population (sample size, gender, age, body mass index, smoking status, atopy, contact lenses users, best-corrected visual acuity, maximal keratometry and central pachymetry), keratoconus diagnosis and severity scales (diagnostic methods and severity scales of keratoconus), samples (type of samples, origins of samples, and sampling methods), oxidative stress markers/antioxidants (levels and methods of quantification), study designs, geographic zones, follow-up, aims and outcomes of included articles are described in Table 1 and Appendix S3 and S4.

### Meta-analysis

#### *Meta-analysis of oxidative stress in keratoconus*

*Stratified by type of oxidative stress markers.* We included 20 studies [2,3,5,8,10,12–14,17,18,20,23–26,30,31,33–35], with eight studies for reactive oxygen and nitrogen species (ROS/RNS) [2,3,8,12,13,18,30,34], four studies for total oxidant status (TOS)/oxidative stress index (OSI) [14,24,25,33], five studies for malondialdehyde (MDA) [2,5,8,10,23], two studies for lactate/pyruvate/citrate [20,31] and five studies for other oxidative stress markers (4-hydroxy-2,3-trans-nonanal(4-HNE) [2], disulphide-thiol [17], 8-Oxo-29-deoxyguanosine (8-OHdG) [26], nicotinamide adenine dinucleotide (NAD) [31] and homocysteine [35]). The overall SMD was 0.94 (95% CI 0.55–1.33,  $p < 0.001$ ) with an important heterogeneity ( $I^2 = 91.4\%$ ), involving a significant increase of oxidative stress markers in KC

compared with healthy controls (Fig. 3, and Appendix S5 and S6). Only reactive oxygen and nitrogen species (ROS/RNS), and malondialdehyde (MDA) were increased in keratoconus compared with healthy controls (SMD = 1.09, 95% CI 0.41–1.78,  $p = 0.002$ ,  $I^2 = 87.8\%$ ; and 1.78, 95% CI 0.83–2.73;  $p < 0.001$ ;  $I^2 = 83.3\%$ , respectively) (Fig. 3 and Appendix S5 and S6). Meta-regressions did not demonstrate any significant results on the socio-demographical parameters, corneal topography measures and between type of oxidative stress markers (Fig. 4).

*Stratified by type of samples.* We included 20 studies [2,3,5,8,10,12–14,17,18,20,23–26,30,31,33–35], with three studies for tears [5,20,30], eight studies for cornea [2,3,8,12,13,18,26,31], two studies for aqueous humour [31,34] and eight studies for blood samples [10,14,17,23–25,33,35]. We highlighted an increase of oxidative stress markers in tears (SMD = 1.94, 95% CI 0.69–3.18,  $p = 0.002$ ,  $I^2 = 93.3\%$ ), in cornea (1.75, 95% CI 0.85–2.64,  $p < 0.001$ ,  $I^2 = 91.2\%$ ) and in blood samples of keratoconus compared with healthy controls (0.55, 95% CI 0.15–0.94,  $p = 0.007$ ,  $I^2 = 87.7\%$ ) (Fig. 3, Appendix S7 and S8). Meta-regressions demonstrated that oxidative stress markers were higher in tears of keratoconus than aqueous humour (coefficient = 2.74; 95% CI 0.30–5.18;  $p = 0.029$ ) and higher in cornea samples of keratoconus than aqueous humour (2.30; 0.36–4.23;  $p = 0.021$ ). We did not retrieve any significant results on the other parameters (Fig. 4).

*Stratified by type of corneal cells.* We included six studies [3,8,12,13,18,26], with four studies for stroma cells [3,12,13,18], two studies for epithelial cells [8,26], and one study for endothelial cells [8]. Only oxidative stress markers in stromal cells were increased in keratoconus compared with healthy controls (SMD = 3.60, 95% CI 2.72–4.48,  $p < 0.001$ ,  $I^2 = 0.0\%$ ) (Fig. 3, Appendix S8 and S9). Meta-regressions demonstrated that oxidative stress markers were higher in stromal cells of KC than epithelial cells (coefficient = 2.95; 95% CI 0.048–5.87;  $p = 0.048$ ). We did not retrieve any significant results on the other parameters (Fig. 4).

*Stratified by geographic zone.* We included 20 studies [2,3,5,8,10,12–14,17,



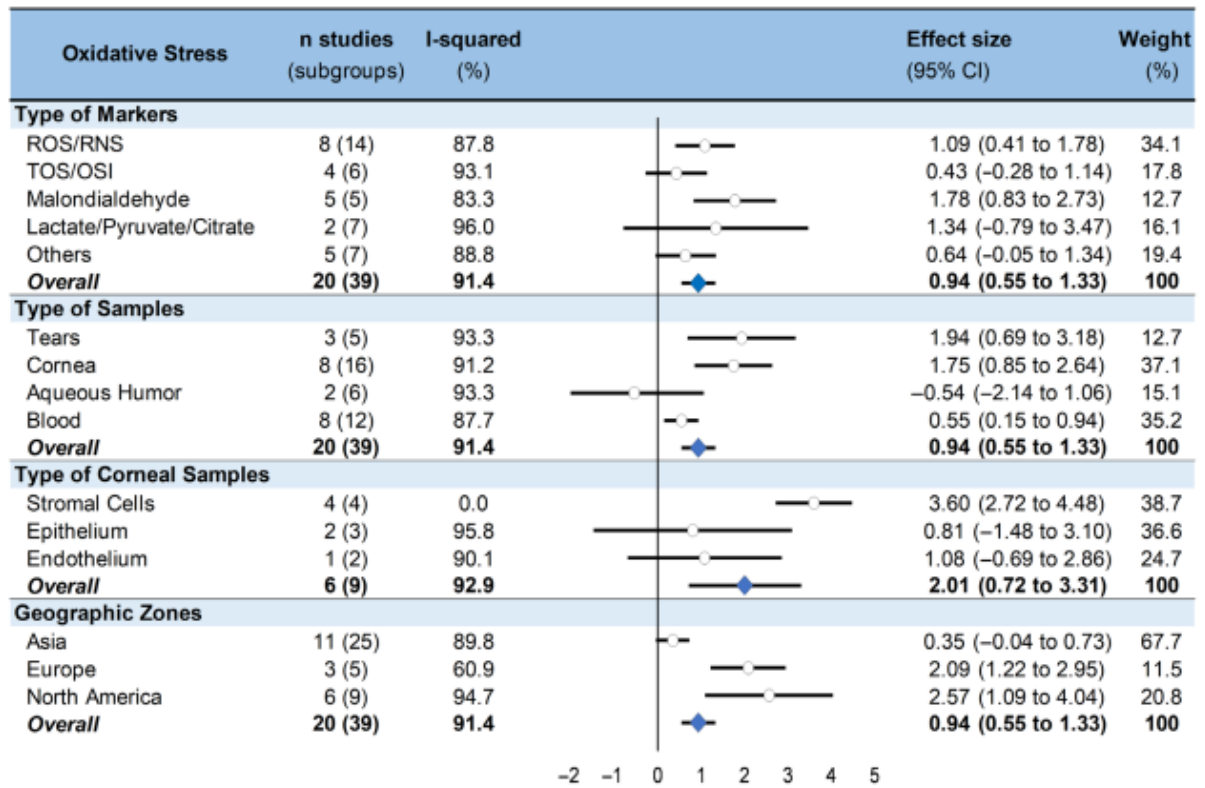


Fig. 3. Summary of meta-analysis on oxidative stress markers in keratoconus comparing with healthy controls, stratified by type of markers, by type of samples (tears, cornea, aqueous humour and blood), by type of corneal samples (stromal cells, epithelium and endothelium) and by geographic zones (Asia, Europe and North America).

18,20,23–26,30,31,33–35], with 11 studies for Asia [14,17,18,23–25,30,31,33–35], three studies for Europe [2,5,10] and six studies in North America [3,8,12,13,20,26]. Studies taking place in Europe and North America showed an increase of oxidative stress markers in keratoconus compared with healthy controls (SMD = 2.09, 95% CI 1.22–2.95,  $p < 0.001$ ,  $I^2 = 60.9\%$ ; and 2.57, 95% CI 1.09–4.05;  $p = 0.001$ ;  $I^2 = 94.7\%$ , respectively) (Fig. 3, Appendix S8 and S10). Meta-regressions demonstrated that oxidative stress markers were higher in KC of North America than Asia (coefficient 2.07; 95% CI 0.53–3.61;  $p = 0.010$ ). We did not retrieve any significant results on the other parameters (Fig. 4).

*Meta-analysis of antioxidants in keratoconus*

*Stratified by type of antioxidants.* We included 28 studies [1,2,4–7,9,11,13–17,19–24,27–33,35,36], with seven studies for superoxide dismutase(SOD) [5,7,11,22,23,28,29], eight studies for glutathione (GSH)/glutathione peroxidase (GPx)/glutathione reductase (GR/

glutathione-S-transferase (GST) [1,2,5,11,15,20,30,31], three studies for catalase [1,13,21], five studies for total antioxidant capacity/status (TAC/TAS) [2,14,22,24,33], three studies for aldehyde (ALDH)/NADPH dehydrogenase [15,16,19], two studies for cysteine/tyrosine [30,31], three studies for lactoferrin/transferrin/albumin [4,19,27], two studies for ascorbic acid [30,31], three studies for acid uric [30–32], four studies for selenium/zinc [6,23,28,36] and four studies for other antioxidants (heme-oxidase 2 enzyme (HO-2) [9], native thiol/total thiol [17], vitamin B12 [35] and folic acid [35]). The overall SMD was -0.63 (95% CI -0.89 to -0.36,  $p < 0.001$ ) with an important heterogeneity ( $I^2 = 93.4\%$ ), involving a significant decrease of antioxidants in keratoconus compared with healthy controls (Fig. 5, and Appendix S8 and S11). Total antioxidant capacity/status (TAC/TAS) (-1.65, 95% CI -2.88 to -0.43,  $p = 0.008$ ,  $I^2 = 94.7\%$ ), aldehyde (ALDH)/NADPH dehydrogenase (-0.77, 95% CI -1.38 to -0.17,  $p = 0.013$ ,  $I^2 = 68.8\%$ ), lactoferrin/transferrin/albumin (-1.92, 95% CI

-2.96 to -0.89,  $p < 0.001$ ,  $I^2 = 86.0\%$ ), selenium/zinc (-1.42, 95% CI -2.23 to -0.61,  $p = 0.001$ ,  $I^2 = 96.2\%$ ) and other antioxidants were decreased in keratoconus compared with healthy controls (-0.66, 95% CI -1.04 to -0.27,  $p = 0.001$ ,  $I^2 = 74.4\%$ ) (Fig. 5, and Appendix S8 and S11). Meta-regressions demonstrated that lactoferrin/transferrin/albumin were more decreased in keratoconus than catalase (coefficient -2.70; 95% CI -5.38 to -0.02;  $p = 0.048$ ), than cysteine/tyrosine (-2.25; 95% CI -4.48 to -0.03;  $p = 0.047$ ) and than ascorbic acid (-2.47; 95% CI -4.02 to -0.92;  $p = 0.048$ ). Meta-regressions did not demonstrate any other significant results on the socio-demographical parameters, corneal topography measures and between other type of oxidative stress markers (Appendix S12).

*Stratified by type of samples.* We included 28 studies [1,2,4–7,9,11,13–17,19–24,27–33,35,36], with six studies for tears [4,5,20,22,27,30], 10 studies for cornea [2,7,9,13,15,16,19,21,29,31], two studies for aqueous humour [31,32] and 11 studies for blood samples

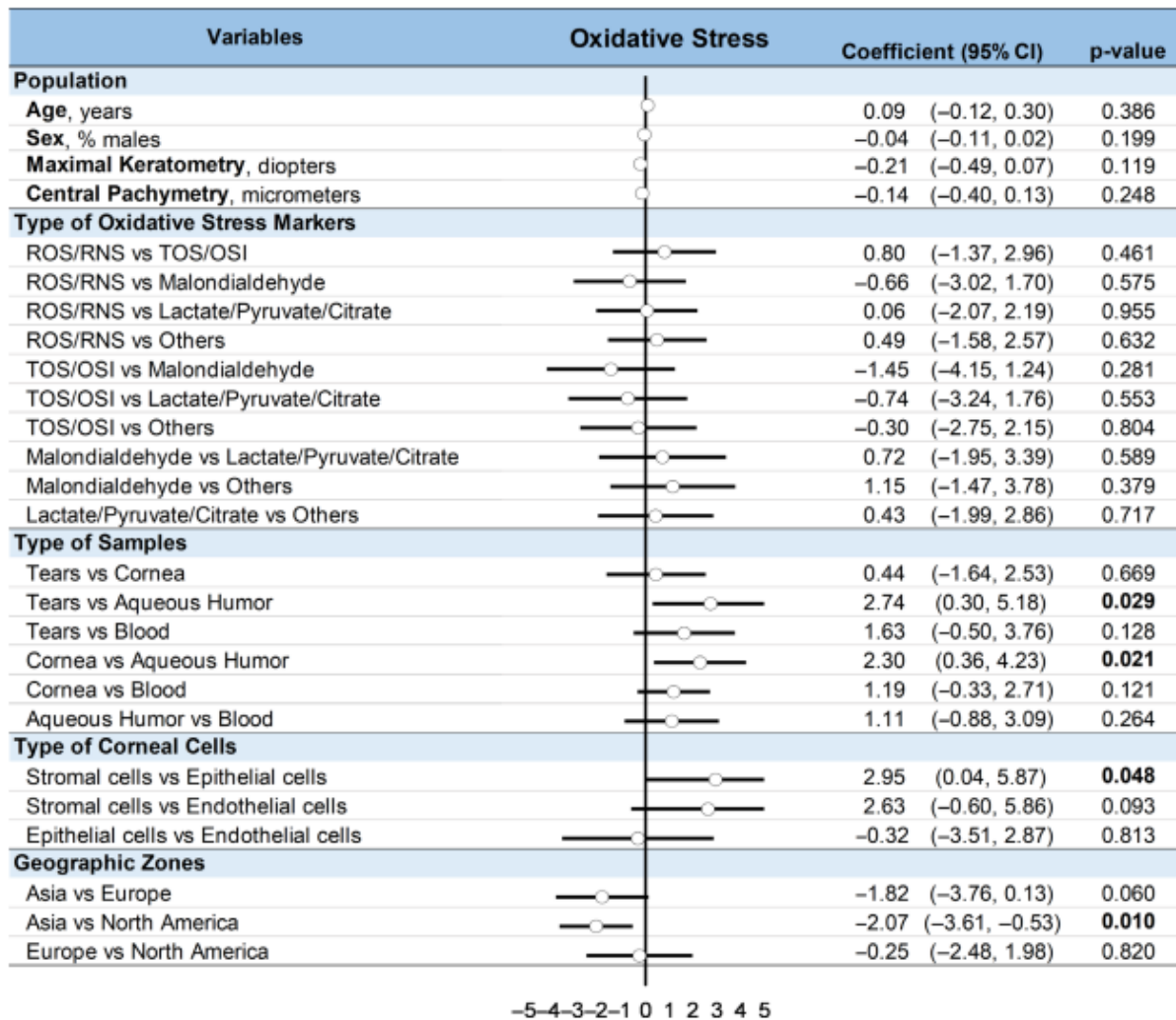


Fig. 4. Meta-regressions on oxidative stress markers in keratoconus comparing with healthy controls.

[1,6,11,14,17,23,24,28,33,35,36]. We highlighted a decrease of antioxidants in tears (SMD = -0.65, 95% CI -1.15 to -0.16, p = 0.01, I<sup>2</sup> = 93.4%), in aqueous humour (-0.72, 95% CI -1.39 to -0.06, p = 0.034, I<sup>2</sup> = 73.7%) and in blood samples of keratoconus compared with healthy controls (-0.89, 95% CI -1.37 to -0.40, p < 0.001, I<sup>2</sup> = 96.1%) (Fig. 5, Appendix S8 and S13). Meta-regressions did not demonstrate any significant results (Appendix S12). *Stratified by type of corneal cells.* We included six studies [9,13,16,19,29], with two studies for stromal cells [13,29], two studies for epithelial cells [9,19] and one study for endothelial cells [16]. Only antioxidants in endothelial cells were decreased in keratoconus compared with healthy

controls (SMD = -0.82, 95% CI -1.59 to -0.05, p = 0.036) (Fig. 5, Appendix S8 and S14). Meta-regressions did not demonstrate any significant results (Appendix S12). *Stratified by geographic zone.* We included 28 studies [1,2,4-7,9,11,13-17, 19-24,27-33,35,36], with 16 studies for Asia [6,9,14-17,22-24,28-31,33,35,36], six studies for Europe [2,5,7,11,27,32], four studies for North America [13,19-21], 1 study for Africa [1] and one study for Oceania [4]. Studies taking place in Asia (SMD = -0.38, 95% CI -0.64 to -0.12, p = 0.005, I<sup>2</sup> = 91.3%), Europe (-1.01, 95% CI -1.76 to -0.27, p = 0.008, I<sup>2</sup> = 92.7%), Africa (-2.20, 95% CI -2.43 to -1.97, p < 0.001, I<sup>2</sup> = 0.0%) and Oceania showed a decrease of

antioxidants in keratoconus compared with healthy controls (-1.09, 95% CI -2.14 to -0.03, p = 0.044, I<sup>2</sup> = 80.6%) (Fig. 5, Appendix S8 and S15). Meta-regressions did not demonstrate any significant results (Appendix S12).

## Discussion

Keratoconus is classically defined as a non-inflammatory corneal disease, involving corneal conical shape because of thinning and protrusion (Krachmer et al. 1984; Rabinowitz 1998). However, oxidative stress is a complex homeostasis pathway implicated in several diseases, that could be dysregulated in keratoconus. In this systematic review and meta-analysis, we underlined that imbalance between

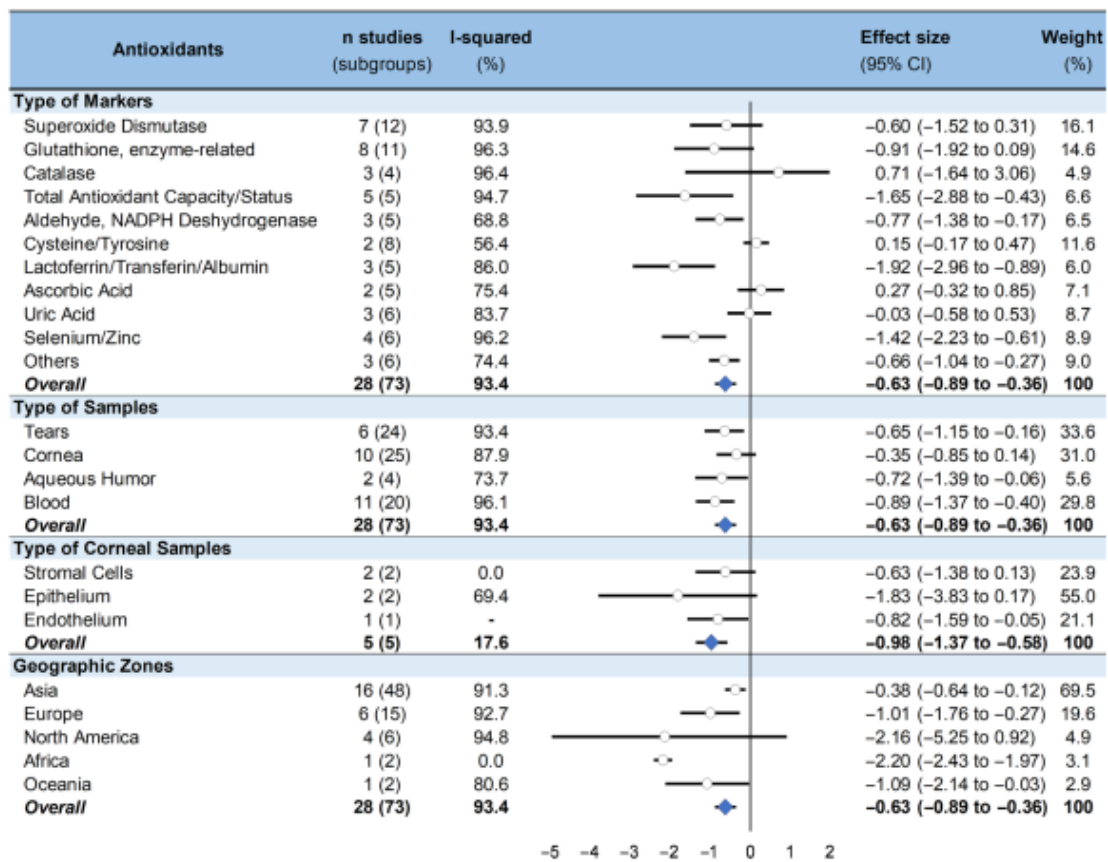


Fig. 5. Summary of meta-analysis on antioxidants in keratoconus comparing with healthy controls, stratified by type of markers, by type of samples (tears, cornea, aqueous humour and blood), by type of corneal samples (stromal cells, epithelium and endothelium) and by geographic zones (Asia, Europe, North America, Africa and Oceania).

oxidative stress and antioxidants seems to be a central process in the pathophysiology of keratoconus. We highlighted that oxidative stress markers were increased and antioxidants decreased in several samples of keratoconus compared to healthy subjects. Interestingly, we identified a geographic tropism of the imbalance of redox homeostasis in keratoconus. Considering these results, we could better understand the complexity of pathological process in keratoconus, and may consider innovative therapies related with inflammatory hypothesis of keratoconus.

**Keratoconus: an imbalance of redox homeostasis**

We showed that oxidative stress markers were increased and antioxidants decreased in keratoconus compared with healthy controls. Interestingly, we described two components of the

ocular surface (i.e. tears and cornea) more affected by the imbalance of redox homeostasis in keratoconus. Furthermore, antioxidants were significantly decreased in aqueous humour of keratoconus. The ocular surface and aqueous humour are daily exposed to the intense burden of extracellular oxygen free radicals generated by high concentration of atmospheric oxygen, UV-radiation of the sun, sulphur dioxide produced by fossil fuel, or eye rubbing (Shoham et al. 2008; Chen et al. 2009; Poljšak & Fink 2014; Najmi et al. 2019). The majority of intracellular reactive oxygen species (ROS) are produced during the mitochondrial respiratory cycle, in the electron transport chain of complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome c reductase), involving the metabolic rate of cells (Finkel & Holbrook 2000). ROS is a general term to commonly describe the superoxide anion radical (O<sub>2</sub><sup>-</sup>),

hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and the hydroxyl radical (OH) that are extremely unstable, free diffusible and long-lived (Finkel & Holbrook 2000; Shoham et al. 2008; Chen et al. 2009). Fenton reaction can generate the highly ROS implicating metals as iron or copper increased in samples of keratoconus (iron ring at the basis of cone entitled Fleischer sign) (Rabinowitz 1998; Kiliç et al. 2016; Bamdad et al. 2018; Zarei-Ghanavati et al. 2019). Interestingly, we highlighted that iron-carrier protein as lactoferrin/transferin/albumin were significantly decreased in keratoconus, involving a susceptibility to generate oxidative stress in keratoconus tissues. Furthermore, the imbalance between ROS and the redox capacity of detoxifying systems involved some damage of cellular DNA, lipid peroxidation of membrane, inactivation of receptor protein and enzymes, and finally cells apoptosis and autophagy mediated by

autophagosome (Chen et al. 2009; Čejka & Čejkova 2015; Čejková & Čejka 2015; Shetty et al. 2017; Yin et al. 2018). Considering the imbalance of redox homeostasis in keratoconus, we could hypothesize that corneal tissues were more at-risk to develop ectasia in relation with a susceptibility of extracellular oxygen free radicals and dysfunction of the mitochondrial respiratory cycle. The oxidative stress is already highlighted as crucial part of some ocular disease like glaucoma (Benoist d'Azy et al. 2016; Kimura et al. 2017; McMonnies 2018), dry eye disease (Dogru et al. 2018), pinguecula and pterygia (Tenkate 1999; Kilic-Toprak et al. 2019), diabetes retinopathy or age-related macular degeneration (Tangvarasittichai & Tangvarasittichai 2019). Fortunately, the ocular surface is rich in enzymatic and nonenzymatic antioxidant molecules to scavenge, neutralize and repair the oxidative aggressions of ROS (Shoham et al. 2008; Chen et al. 2009; Čejková & Čejka 2015). Evidence data showed that ocular surface and aqueous humour of diurnal animals contained a lot of antioxidant, that is mucins, glycoproteins, detoxifying enzymes derived from goblets cells, lacrimal and meibomian glands including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), ascorbic acid, glutathione, uric acid, cysteine, tyrosine and transferrin (Chen et al. 2009; Lema et al. 2010; Balasubramanian et al. 2012; Saijyothi et al. 2012; Karamichos et al. 2015; Willcox et al. 2017; Balmus et al. 2020). Thus, the complex antioxidant pathways could counterbalance the daily ROS generations produced by cells metabolism and environmental aggressions in tears, corneal tissues and endocular fluids (Reiss et al. 1986; Gondhowiardjo & van Haeringen 1993; Gondhowiardjo et al. 1993; Behndig et al. 2001; Buddi et al. 2002; Kenney et al. 2005; Chwa et al. 2006; Arnal et al. 2011; Joseph et al. 2011; Saeed-Rad et al. 2013; Uzunoğlu et al. 2013; Lackner et al. 2014; Hao et al. 2016; Stachon et al. 2017; Atilano et al. 2019; Caglayan et al. 2019). However, we showed that antioxidants (and particularly aldehyde (ALDH)/NADPH dehydrogenase, lactoferrin/transferrin/albumin, selenium/zinc, TAC/TAS, HO-2, native thiol/total thiol, vitamin B<sub>12</sub> and folic acid) were significantly

decreased in keratoconus, reducing the upper-mentioned defence mechanisms against oxidative stress. In the light of these evidences, we could propose that keratoconus is a corneal disease involving corneal ectasia, promoting by an imbalance of redox homeostasis in tears, corneal tissues, aqueous humour and blood. Considering that oxidative stress markers and antioxidants were significantly increased and decreased in blood of keratoconus, this corneal disease seems to be also associated with systemic redox imbalance. Thus, it seems to be interesting to hypothesize that keratoconic ectasia is a local or systemic expression of an inflammatory process.

#### **From enigmatic disease to inflammatory vicious circle?**

The classical definition of keratoconus is a non-inflammatory condition where the cornea is progressively warping in a conical shape because of thinning and protrusion of its layers (Krachmer et al. 1984; Rabinowitz 1998). However, the triggering factors and causes of corneal thinning remain an enigma. Thus, many theories have been postulated regarding the aetiology of keratoconus: ectodermal or mesodermal disease (Galin & Berger 1958; Teng 1963), eye rubbing (McMonnies 2016; Perone et al. 2017; Najmi et al. 2019), hormonal dysregulation (Tachibana et al. 2002; Goldich et al. 2011; Magalhaes et al. 2018), genetic disorder (Pearson et al. 2000; Rabinowitz 2003; Wheeler et al. 2012; Gordon-Shaag et al. 2015; Rong et al. 2017; Valgaeren et al. 2018), collagenous disease or corneal manifestations of systemic disease (Dudakova & Jirsova 2013; Woodward et al. 2016; Bak-Nielsen et al. 2019a,b). However, all of them could be exact according to a multifactorial disease models where environmental stress factors seem to play a triggering role on an inherited predispositions. Interestingly, we showed that oxidative stress markers were increased in European and North American samples of keratoconus, without significative relation with Asiatic samples. Thus, oxidative stress markers were more increased in North America than Asia. Similarly, all geographic origins of keratoconus samples except North American were associated with a significant decrease of antioxidants. Thus,

the imbalance of redox homeostasis in keratoconus may include a geographic tropism or involve ethnic predispositions. Indeed, it is well known that genetic heritage and ethnicity could promote the clinical severity of keratoconus, involving the progression of corneal ectasia (Bak-Nielsen et al. 2019a,b; Volatier et al. 2020), need of invasive therapeutics as corneal cross-linking or corneal graft, and risks of cecity with corneal melting and perforation (Zhang et al. 2019). Considering the intense burden of air pollution in past decades, the association between geographic origins and redox imbalance in tissues of keratoconus could be explained by the free radical aggressions related to biomass burning-derived, particulate matters, and fossil fuel exhausts (Daiber et al. 2020; Milani et al. 2020; Mudway et al. 2020; Vehof et al. 2020). As explained above, keratoconus is an imbalance of redox condition where enzymatic and nonenzymatic antioxidant molecules are exceeded by the oxidizing charge. Surprisingly, we showed that oxidative stress markers were increased and antioxidants decreased in blood of keratoconus, involving a systemic imbalance of redox homeostasis. Without evidence of other organ failure than cornea, we could postulate that genetic predispositions expose keratoconus to oxidative stress damages only in corneal tissues. Focusing the analysis into the different layers of cornea, we showed an increase of oxidative stress markers in stromal cells and a decrease of antioxidants in endothelium. Thus, most of corneal layers seem to be affected by the pathological process, probably excluding an exclusive stromal disease. Similarly, lysyl oxidase (LOX), collagenases, matrix metalloproteinases (MMP-1, MMP-3 and MMP-9) and inflammatory cytokines (IL-1, IL-6, TNF- $\alpha$  and TGF- $\beta$ ) seem to be overexpressed and deregulated in tears and corneas of keratoconus compared with healthy subjects (Collier 2001; Dudakova & Jirsova 2013; Wisse et al. 2015). Therefore, we can stipulate that chronic environmental aggressions, like eye rubbing or atopy (Gordon-Shaag et al. 2012; Gordon-Shaag et al. 2015), in predisposed subjects (Rong et al. 2017), could trigger the chronic liberation of inflammatory cytokines by aggressed cells and deregulate the delicate balance of cellular

redox homeostasis (Collier 2001; Wisse et al. 2015). The chronicity of inflammatory environment, notably with TGF- $\beta$  and IL-1 (Girard et al. 1991), could promote the degradation of extracellular matrix by upregulated collagenases and metalloproteases, the acceleration of cells renewal and the apoptosis mediated by autophagy (Collier 2001; Shoham et al. 2008; Shetty et al. 2017; Yin et al. 2018). Considering that cytokines and degrading enzymes should be activated to trigger the cascade of inflammatory activation, we could hypothesize that imbalance of redox homeostasis could be a key factor at the beginning of ectasia process (Cristina Kenney & Brown 2003). Hence, experimental models based on knockout mouse for oxidative stress markers or antioxidants could be effective to better understand their role in the pathophysiological process of keratoconus. Furthermore, identifying biomarkers of keratoconus which can easily collect in the ocular surface seems to be very salient to diagnose early stage of disease in concordance with corneal topography patterns, and to exclude the preclinical subjects of refractive surgery (Nishtala et al. 2016; Dupps 2020). Further studies are necessary to broaden the knowledge of inflammatory pathways in keratoconus, allowing elaboration of specific treatments in asymptomatic, advanced or progressive stage of the disease. Well-knowing these activation pathways in the ocular surface will allow to elaborate aetiological treatments acting at the beginning of pathological process.

#### Limitations

Our study has some limitations. Our meta-analysis inherited the limitations of the 36 individual studies of which it was composed and therefore was subjected to the bias of included studies. However, this is the first meta-analysis to systematically quantify the strength of associations between keratoconus, antioxidants, and oxidative stress markers in eye tissues and physiological fluids. Despite our stratification by type of markers (antioxidants and oxidative stress markers), type of samples (tears, cornea, aqueous humour and blood), type of corneal samples (stromal cells, epithelium and endothelium) and geographic zones (Asia, Europe, North America, Africa and

Oceania), there is heterogeneity within each subgroup regarding sample methods, methods of biomarker quantification and study population. Moreover, although the diagnostic of keratoconus is well described using slight lamp examination, corneal optic coherence tomography (OCT) and corneal topography, it was not clearly defined in some published studies, which might have affected precision of the results. In addition, twenty-four studies did not report clinical or paraclinical severity scales of keratoconus (Gondhowiardjo & van Haeringen 1993; Gondhowiardjo et al. 1993; Behndig et al. 2001; Buddi et al. 2002; Kenney et al. 2005; Chwa et al. 2006; Chwa et al. 2008; Arnal et al. 2011; Joseph et al. 2011; Ortak et al. 2012; Saeed-Rad et al. 2013; Uzunoğlu et al. 2013; Lackner et al. 2014; Karamichos et al. 2015; Hao et al. 2016; Cantemir et al. 2016a, 2016b; Gulpamuk et al. 2017; Snytnikova et al. 2017; Stachon et al. 2017; Bamdad et al. 2018; Atilano et al. 2019; Yilmaz et al. 2020), precluding stratified analysis by degree of keratoconus severity. Most studies also described case-control study designs, with only one cohort study. However, keratoconus is a progressive and chronic corneal disease which can diagnose thanks to evolution pattern of corneal ectasia during several months or years. Therefore, the associations between keratoconus and oxidative stress might be biased by the potential misdiagnosis or underdiagnosis of keratoconus. There is also heterogeneity in reporting oxidative stress markers and antioxidants between studies, describing different enzymatic pathways and cellular interplay mechanisms. Considering that included studies used several methods of biomarkers quantification with different cut-off and laboratory protocols, the methodologic variability between studies could preclude robust conclusions. Even if methodological quality of the included studies was evaluated by subjective assessments (STROBE and NOS), overall methodological quality was reasonably good without major comparability and selection bias between keratoconus and control groups, with a large volume of data available (1328 keratoconus versus 1208 healthy controls) and consistency for the main outcomes of interest. Generalizability of our results may also suffer from the limited

number of studies from Africa, Oceania and South America, particularly for the oxidative stress markers. For the future, it would be interesting to investigate more prospective studies to explore the underlying mechanisms and putative therapeutics.

## Conclusion

We showed that oxidative stress markers and antioxidants were dysregulated in keratoconus, involving an imbalance of redox homeostasis in tears, cornea, aqueous humour and blood. Further studies are needed to better understand the complex relation between corneal ectasia, redox homeostasis and inflammatory pathway to promote innovant and aetiological therapeutics that could slow down (or arrest) disease progression.

## Data sharing statement

No additional data are available.

## References

- Abalain JH, Dossou H, Colin J & Floch HH (2000): Levels of collagen degradation products (telopeptides) in the tear film of patients with keratoconus. *Cornea* **19**: 474–476.
- Abdul-Maksoud RS, Fouad RA, Elsayed TG, Ibrahim RA & Badawi AE (2020): The impact of catalase and glutathione peroxidase-1 genetic polymorphisms on their enzyme activities among Egyptian patients with keratoconus. *J Gene Med* **22**: e3192.
- Allard K & Zetterberg M (2018): Toric IOL implantation in a patient with keratoconus and previous penetrating keratoplasty: a case report and review of literature. *BMC Ophthalmology* **18**.
- Andreassen TT, Hjorth Simonsen A & Oxlund H (1980): Biomechanical properties of keratoconus and normal corneas. *Exp Eye Res* **31**: 435–441.
- Arnal E, Peris-Martinez C, Menezes JL, Johnsen-Soriano S & Romero FJ (2011): Oxidative stress in keratoconus? *Invest Ophthalmol Vis Sci* **52**: 8592.
- Atilano S, Lee D, Fukuhara P, Chwa M, Nesburn A, Udari N & Kenney C (2019): Corneal oxidative damage in keratoconus cells due to decreased oxidant elimination from modified expression levels of SOD enzymes, PRDX6, SCARA3, CPSF3, and FOXM1. *J Ophthalmic Vis Res* **14**: 62.
- Bak-Nielsen S, Ramlau-Hansen CH, Ivarsen A, Plana-Ripoll O & Hjortdal J (2019a): Incidence and prevalence of keratoconus in Denmark – an update. *Acta Ophthalmol* **97**: 752–755.

- Bak-Nielsen S, Ramlau-Hansen CH, Ivarsen A, Plana-Ripoll O & Hjortdal J (2019b): A nationwide population-based study of social demographic factors, associated diseases and mortality of keratoconus patients in Denmark from 1977 to 2015. *Acta Ophthalmol* **97**: 497–504.
- Balasubramanian SA, Pye DC & Willcox MDP (2012): Levels of lactoferrin, secretory IgA and serum albumin in the tear film of people with keratoconus. *Exp Eye Res* **96**: 132–137.
- Balasubramanian SA, Pye DC & Willcox MD (2013): Effects of eye rubbing on the levels of protease, protease activity and cytokines in tears: relevance in keratoconus: eye rubbing in keratoconus. *Clin Exp Optom* **96**: 214–218.
- Balmus I-M, Alexa AI, Ciuntu R-E, Danilescu C, Stoica B, Cojocaru SI, Ciobica A & Cantemir A (2020): Oxidative stress markers dynamics in keratoconus patients' tears before and after corneal collagen crosslinking procedure. *Exp Eye Res* **190**: 107897.
- Bamdad S, Owji N & Bolkheir A (2018): Association between advanced keratoconus and serum levels of zinc, calcium, magnesium, iron, copper, and selenium. *Cornea* **37**: 1306–1310.
- Behndig A, Karlsson K, Johansson BO, Brännström T & Marklund SL (2001): Superoxide dismutase isoenzymes in the normal and diseased human cornea. *Invest Ophthalmol Vis Sci* **42**: 2293–2296.
- Benoist d'Azy C, Pereira B, Chiambaretta F & Duthheil F (2016a): Oxidative and anti-oxidative stress markers in chronic glaucoma: a systematic review and meta-analysis. *PLoS One* **11**: e0166915.
- Benoist d'Azy C, Pereira B, Chiambaretta F & Duthheil F (2019): Efficacy of different procedures of intra-corneal ring segment implantation in keratoconus: a systematic review and meta-analysis. *Transl Vis Sci Technol* **8**: 38.
- Benoist d'Azy C, Pereira B, Naughton G, Chiambaretta F & Duthheil F (2016b): Antibiotrophylaxis in prevention of endophthalmitis in intravitreal injection: a systematic review and meta-analysis. *PLoS One* **11**: e0156431.
- Bron AJ (1988): Keratoconus. *Cornea* **7**: 163–169.
- Buddi R, Lin B, Atilano SR, Zorapapel NC, Kenney MC & Brown DJ (2002): Evidence of oxidative stress in human corneal diseases. *J Histochem Cytochem* **50**: 341–351.
- Caglayan M, Kocamus SI, Sarac O, Tatli Dogan H, Kosekahya P, Ayan M & Cagil N (2019): Investigation of heme oxygenase 2 enzyme protein expression in keratoconus and normal human corneal epithelium: an immunohistochemical study. *Curr Eye Res* **44**: 25–29.
- Cantemir A, Alexa AI, Ciobica A, Balmus IM, Antioch I, Stoica B, Chiselita D & Costin D (2016b): Evaluation of antioxidant enzymes in keratoconus. *Rev Chim* **67**: 1538–1541.
- Cantemir A, Alexa A, Ciobica A, Toader E, Balmus I, Cojocaru S, Chiselita D & Costin D (2016a): The pathological relevance of some biochemical changes in keratoconus: focusing on serum lipid peroxidation products. *Rev Chim* **67**: 1725–1727.
- Cejka C & Cejkova J (2015): Oxidative stress to the cornea, changes in corneal optical properties, and advances in treatment of corneal oxidative injuries. *Oxid Med Cell Longev* **2015**: 591530.
- Čejková J & Čejka Č (2015): The role of oxidative stress in corneal diseases and injuries. *Histol Histopathol* **30**: 893–900.
- Chen Y, Mehta G & Vasiliou V (2009): Antioxidant defenses in the ocular surface. *Ocul Surf* **7**: 176–185.
- Chwa M, Atilano SR, Hertzog D, Zheng H, Langberg J, Kim DW & Kenney MC (2008): Hypersensitive response to oxidative stress in keratoconus corneal fibroblasts. *Invest Ophthalmol Vis Sci* **49**: 4361.
- Chwa M, Atilano SR, Reddy V, Jordan N, Kim DW & Kenney MC (2006): Increased stress-induced generation of reactive oxygen species and apoptosis in human keratoconus fibroblasts. *Invest Ophthalmol Vis Sci* **47**: 1902.
- Clark I, Swingler T, Sampieri C & Edwards D (2008): The regulation of matrix metalloproteinases and their inhibitors. *Int J Biochem Cell Biol* **40**: 1362–1378.
- Collier SA (2001): Is the corneal degradation in keratoconus caused by matrix-metalloproteinases? *Clin Exp Ophthalmol* **29**: 340–344.
- da Costa BR, Cevallos M, Altman DG, Rutjes AWS & Egger M (2011): Uses and misuses of the STROBE statement: bibliographic study. *BMJ Open* **1**: e000048.
- Courtin R, Pereira B, Naughton G, Chamoux A, Chiambaretta F, Lanhers C & Duthheil F (2016): Prevalence of dry eye disease in visual display terminal workers: a systematic review and meta-analysis. *BMJ Open* **6**: e009675.
- Cristina Kenney M & Brown DJ (2003): The cascade hypothesis of keratoconus. *Cont Lens Anterior Eye* **26**: 139–146.
- Daiber A, Kröller-Schön S, Oelze M, Hahad O, Li H, Schulz R, Steven S & Münzel T (2020): Oxidative stress and inflammation contribute to traffic noise-induced vascular and cerebral dysfunction via uncoupling of nitric oxide synthases. *Redox Biol* **34**: 101506.
- DerSimonian R & Laird N (1986): Meta-analysis in clinical trials. *Control Clin Trials* **7**: 177–188.
- Dogru M, Kojima T, Simsek C & Tsubota K (2018): Potential role of oxidative stress in ocular surface inflammation and dry eye disease. *Invest Ophthalmol Vis Sci* **59**: DES163.
- Dragnea DC, Birbal RS, Ham L, Dapena I, Oellerich S, van Dijk K & Melles GRJ (2018): Bowman layer transplantation in the treatment of keratoconus. *Eye and Vis* **5**: 24.
- Dudakova L & Jirsova K (2013): The impairment of lysyl oxidase in keratoconus and in keratoconus-associated disorders. *J Neural Transm* **120**: 977–982.
- Dupps WJ (2020): Corneal refractive surgery in keratoconus. *J Cataract Refr Surg* **46**: 495–496.
- von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC & Vandenbroucke JP (2014): The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Int J Surg* **12**: 1495–1499.
- Finkel T & Holbrook NJ (2000): Oxidants, oxidative stress and the biology of ageing. *Nature* **408**: 239–247.
- Galin MA & Berger R (1958): Atopy and keratoconus. *Am J Ophthalmol* **45**: 904–906.
- Galvis V, Sherwin T, Tello A, Merayo J, Barrera R & Acera A (2015): Keratoconus: an inflammatory disorder? *Eye* **29**: 843–859.
- Girard MT, Matsuura M & Fini ME (1991): Transforming growth factor-beta and interleukin-1 modulate metalloproteinase expression by corneal stromal cells. *Invest Ophthalmol Vis Sci* **32**: 2441–2454.
- Godefrooij DA, de Wit GA, Uitterwaal CS, Imhof SM & Wisse RPL (2017): Age-specific incidence and prevalence of keratoconus: a nationwide registration study. *Am J Ophthalmol* **175**: 169–172.
- Goldich Y, Barkana Y, Pras E et al. (2011): Variations in corneal biomechanical parameters and central corneal thickness during the menstrual cycle. *J Cataract Refr Surg* **37**: 1507–1511.
- Göncü T, Akal A, Adbelli FM, Çakmak S, Sezen H & Yılmaz ÖF (2015): Tear film and serum prolidase activity and oxidative stress in patients with keratoconus. *Cornea* **34**: 1019–1023.
- Gondhowiardjo TD & van Haeringen NJ (1993): Corneal aldehyde dehydrogenase, glutathione reductase, and glutathione S-transferase in pathologic corneas. *Cornea* **12**: 310–314.
- Gondhowiardjo TD, van Haeringen NJ, Völker-Dieben HJ, Beekhuis HW, Kok JHC, van Rij G, Pels L & Kijlstra A (1993): Analysis of corneal aldehyde dehydrogenase patterns in pathologic corneas. *Cornea* **12**: 146–154.
- Gordon-Shaag A, Millodot M & Shneor E (2012): The epidemiology and etiology of keratoconus. *Int J Keratoconus Ectatic Corneal Dis* **1**: 7–15.
- Gordon-Shaag A, Millodot M, Shneor E & Liu Y (2015): The genetic and environmental factors for keratoconus. *BioMed Res Int* **2015**: 1–19.
- Gulpamuk B, Koç M, Karatepe MS, Yıldız A, Erel O, Neselioglu S & Yilmazbas P (2017): Novel assay assessment of oxidative stress biomarkers in patients with keratoconus: Thiol-Disulfide homeostasis. *Curr Eye Res* **42**: 1215–1219.
- Hao X-D, Chen Z-L, Qu M-L, Zhao X-W, Li S-X & Chen P (2016): Decreased integrity,

- content, and increased transcript level of mitochondrial DNA are associated with keratoconus. *PLoS One* **11**: e0165580.
- Ionescu IC, Corbu CG, Tanase C et al. (2018): Overexpression of tear inflammatory cytokines as additional finding in keratoconus patients and their first degree family members. *Mediators Inflamm* **2018**: 1–9.
- Jiang Y, Yang S, Li Y, Cui G & Lu TC (2019): Accelerated versus conventional corneal collagen cross-linking in the treatment of keratoconus: a meta-analysis and review of the literature. *Interdiscip Sci Comput Life Sci* **11**: 282–286.
- Jinabhai AN (2020): Customised aberration-controlling corrections for keratoconic patients using contact lenses. *Clin Exp Optom* **103**: 31–43.
- Joseph R, Srivastava OP & Pfister RR (2011): Differential epithelial and stromal protein profiles in keratoconus and normal human corneas. *Exp Eye Res* **92**: 282–298.
- Kabza M, Karolak JA, Rydzanicz M, Udziela M, Gasperowicz P, Ploski R, Szaflik JP & Gajdecka M (2019): Multiple differentially methylated regions specific to keratoconus explain known keratoconus linkage loci. *Invest Ophthalmol Vis Sci* **60**: 1501.
- Karamichos D, Zieske JD, Sejersen H, Sarker-Nag A, Asara JM & Hjortdal J (2015): Tear metabolite changes in keratoconus. *Exp Eye Res* **132**: 1–8.
- Kenney MC, Chwa M, Atilano SR et al. (2005): Increased levels of catalase and cathepsin V/L2 but decreased TIMP-1 in keratoconus corneas: evidence that oxidative stress plays a role in this disorder. *Invest Ophthalmol Vis Sci* **46**: 823.
- Khaled ML, Helwa I, Drewry M, Seremwe M, Estes A & Liu Y (2017): Molecular and histopathological changes associated with keratoconus. *BioMed Res Int* **2017**: 1–16.
- Khalimov AR, Khalimova LI & Usubov EL (2019): Local antioxidant status in patients with keratoconus after ultraviolet corneal crosslinking. *Ophthalmol Russia* **16**: 74–78.
- Kiliç R, Bayraktar AC, Bayraktar S, Kurt A & Kavutçu M (2016): Evaluation of Serum superoxide dismutase activity, malondialdehyde, and zinc and copper levels in patients with keratoconus. *Cornea* **35**: 1512–1515.
- Kiliç R, Cumurcu T, Sancaktar E, Evliyaoğlu O & Sezer H (2016b): Proper evaluation of factors affecting oxidative status. *Curr Eye Res* **41**: 1129.
- Kiliç R, Cumurcu T, Sancaktar E, Evliyaoğlu O & Sezer H (2016a): Systemic prolidase activity and oxidative stress in keratoconus. *Curr Eye Res* **41**: 28–33.
- Kiliç-Toprak E, Toprak I, Caliskan S, Ozdemir Y, Demirtas O, Altintas F & Kucukatay V (2019): Oxidative stress and genotoxicity in pterygium: a systemic investigation. *Eye Contact Lens* **45**: 399–404.
- Kimura A, Namekata K, Guo X, Noro T, Harada C & Harada T (2017): Targeting oxidative stress for treatment of glaucoma and optic neuritis. *Oxid Med Cell Longev* **2017**: 1–8.
- Kracher JH, Feder RS & Belin MW (1984): Keratoconus and related noninflammatory corneal thinning disorders. *Surv Ophthalmol* **28**: 293–322.
- Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, Kamoi K & Suda T (1998): Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption\*. *Endocrinology* **139**: 1338–1345.
- Lackner E-M, Matthaei M, Meng H, Ardjomand N, Eberhart CG & Jun AS (2014): Design and analysis of keratoconus tissue microarrays. *Cornea* **33**: 49–55.
- Lanthers C, Pereira B, Naughton G, Troussellard M, Lesage F-X & Duthheil F (2015): Creatine supplementation and lower limb strength performance: a systematic review and meta-analyses. *Sports Med* **45**: 1285–1294.
- Lanthers C, Pereira B, Naughton G, Troussellard M, Lesage F-X & Duthheil F (2017): Creatine supplementation and upper limb strength performance: a systematic review and meta-analysis. *Sports Med* **47**: 163–173.
- Lema I, Brea D, Rodríguez-González R, Díez-Feijoo E & Sobrino T (2010): Proteomic analysis of the tear film in patients with keratoconus. *Mol Vis* **16**: 2055–2061.
- Lema I & Duran J (2005): Inflammatory molecules in the tears of patients with keratoconus. *Ophthalmology* **112**: 654–659.
- Lema I, Sobrino T, Duran JA, Brea D & Díez-Feijoo E (2009): Subclinical keratoconus and inflammatory molecules from tears. *Br J Ophthalmol* **93**: 820–824.
- Magalhaes OA, Marafon SB & Ferreira RC (2018): Gender differences in keratoconus keratoplasty: a 25-year study in Southern Brazil and global perspective. *Int Ophthalmol* **38**: 1627–1633.
- di Martino E, Ali M & Inglehearn CF (2019): Matrix metalloproteinases in keratoconus – too much of a good thing? *Exp Eye Res* **182**: 137–143.
- McMonnies CW (2005): The biomechanics of keratoconus and rigid contact lenses. *Eye Contact Lens* **31**: 80–92.
- McMonnies CW (2016): Eye rubbing type and prevalence including contact lens ‘removal-relief’ rubbing: Eye rubbing prevalence *McMonnies*. *Clin Exp Optom* **99**: 366–372.
- McMonnies C (2018): Reactive oxygen species, oxidative stress, glaucoma and hyperbaric oxygen therapy. *J Optom* **11**: 3–9.
- McMonnies CW & Boneham GC (2010): Corneal responses to intraocular pressure elevations in keratoconus. *Cornea* **29**: 764–770.
- Milani C, Farina F, Botto L et al. (2020): Systemic exposure to air pollution induces oxidative stress and inflammation in mouse brain, contributing to neurodegeneration onset. *Int J Mol Sci* **21**: 3699.
- Mudway IS, Kelly FJ & Holgate ST (2020): Oxidative stress in air pollution research. *Free Radic Biol Med* **151**: 2–6.
- Naderan M, Rajabi MT, Zarrinbakhsh P & Bakhshi A (2017): Effect of allergic diseases on keratoconus severity. *Ocul Immunol Inflamm* **25**: 418–423.
- Najmi H, Mobarki Y, Mania K, Altowairqi B, Basehi M, Mahfouz MS & Elmahdy M (2019): The correlation between keratoconus and eye rubbing: a review. *Int J Ophthalmol* **12**: 1775–1781.
- Navel V, Mulliez A, Benoist d’Azy C, Baker JS, Malecaze J, Chiambaretta F & Duthheil F (2019): Efficacy of treatments for Demodex blepharitis: a systematic review and meta-analysis. *Ocul Surf* **17**: 655–669.
- Nishtala K, Pahuja N, Shetty R, Nuijts RMM & Ghosh A (2016): Tear biomarkers for keratoconus. *Eye Vis (Lond)* **3**: 19.
- Ollier M, Chamoux A, Naughton G, Pereira B & Duthheil F (2014): Chest CT scan screening for lung cancer in asbestos occupational exposure: a systematic review and meta-analysis. *Chest* **145**: 1339–1346.
- Ortak H, Söğüt E, Taş U, Mesci C & Mendil D (2012): The relation between keratoconus and plasma levels of MMP-2, zinc, and SOD. *Cornea* **31**: 1048–1051.
- Pearson AR, Soneji B, Sarvananthan N & Sandford-Smith JH (2000): Does ethnic origin influence the incidence or severity of keratoconus? *Eye* **14**: 625–628.
- Perone JM, Conart JB, Bertaux P-J, Sujet-Perone N, Ouamara N, Sot M & Henry JJ (2017): Mechanical modeling of a keratoconic cornea. *Cornea* **36**: 1263–1266.
- Poljšak B & Fink R (2014): The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxid Med Cell Longev* **2014**: 1–22.
- Pouliquen Y (1987): Keratoconus. *Eye* **1**: 1–14.
- Rabinowitz YS (1998): Keratoconus. *Surv Ophthalmol* **42**: 297–319.
- Rabinowitz YS (2003): The genetics of keratoconus. *Ophthalmol Clin North Am* **16**: 607–620, vii.
- Reiss GR, Werness PG, Zollman PE & Brubaker RF (1986): Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. *Arch Ophthalmol* **104**: 753–755.
- Rico-Del-Viejo L, Garcia-Montero M, Hernández-Verdejo JL, García-Lázaro S, Gómez-Sanz FJ & Lorente-Velázquez A (2017): Nonsurgical procedures for keratoconus management. *J Ophthalmol* **2017**: 1–17.
- Rong SS, Ma STU, Yu XT et al. (2017): Genetic associations for keratoconus: a systematic review and meta-analysis. *Sci Rep* **7**: 4620.
- Saeed-Rad S, Raoofian R, Mahbod M, Mir-aftab M, Mojarrad M, Asgari S, Rezvan F & Hashemi H (2013): Analysis of superoxide dismutase 1, dual-specificity phosphatase 1, and transforming growth factor, beta 1 genes expression in keratoconic and non-keratoconic corneas. *Mol Vis* **19**: 2501–2507.
- Saijyothi AV, Fowjana J, Madhumathi S, Rajeshwari M, Thennarasu M, Prema P & Angayarkanni N (2012): Tear fluid small

- molecular antioxidants profiling shows lowered glutathione in keratoconus. *Exp Eye Res* **103**: 41–46.
- Shajari M, Eberhardt E, Müller M, Al Khaateb G, Friderich S, Remy M & Kohnen T (2016): Effects of atopic syndrome on keratoconus. *Cornea* **35**: 1416–1420.
- Shetty R, Sharma A, Pahuja N et al. (2017): Oxidative stress induces dysregulated autophagy in corneal epithelium of keratoconus patients. *PLoS One* **12**: e0184628.
- Shoham A, Hadziahmetovic M, Dunaief JL, Mydlarski MB & Schipper HM (2008): Oxidative stress in diseases of the human cornea. *Free Radic Biol Med* **45**: 1047–1055.
- Snytnikova OA, Yanshole LV, Iskakov IA, Yanshole VV, Chernykh VV, Stepanov DA, Novoselov VP & Tsentelovich YP (2017): Quantitative metabolomic analysis of the human cornea and aqueous humor. *Metabolomics* **13**: 152.
- Song Y, Zhang J & Pan Z (2019): Systematic review and meta-analysis of clinical outcomes of penetrating keratoplasty versus deep anterior lamellar keratoplasty for keratoconus. *Exp Clin Transplant* **18**: 417–428.
- Stachon T, Stachon A, Hartmann U, Seitz B, Langenbacher A & Szentmáry N (2017): Urea, uric acid, prolactin and fT4 concentrations in aqueous humor of keratoconus patients. *Curr Eye Res* **42**: 842–846.
- Sterne JAC, Sutton AJ, Ioannidis JPA et al. (2011): Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. *BMJ* **343**: d4002.
- Tachibana M, Adachi W, Kinoshita S, Kobayashi Y, Honma Y, Hiai H & Matsushima Y (2002): Androgen-dependent hereditary mouse keratoconus: linkage to an MHC region. *Invest Ophthalmol Vis Sci* **43**: 51–57.
- Tangvarasittichai O & Tangvarasittichai S (2019): Oxidative stress, ocular disease and diabetes retinopathy. *Curr Pharm Des* **24**: 4726–4741.
- Teng CC (1963): Electron microscope study of the pathology of keratoconus: part I\*. *Am J Ophthalmol* **55**: 18–47.
- Tenkate TD (1999): Occupational exposure to ultraviolet radiation: a health risk assessment. *Rev Environ Health* **14**: 187–209.
- Toprak I, Kucukatay V, Yildirim C, Kilic-Toprak E & Kilic-Erkek O (2014): Increased systemic oxidative stress in patients with keratoconus. *Eye* **28**: 285–289.
- Uzunoglu E, Lortlar Unlu N, Erdogan D, Erdamar H, Akyol E & Akata F (2013): Immunohistochemical and ultrastructural presentation of apoptosis and aqueous humor's nitric oxide levels in keratoconus. *Nobel Med* **9**: 5–9.
- Valgaeren H, Koppen C & Van Camp G (2018): A new perspective on the genetics of keratoconus: why have we not been more successful? *Ophthalmic Genet* **39**: 158–174.
- Vehof J, Snieder H, Jansonius N & Hammond CJ (2020): Prevalence and risk factors of dry eye in 79,866 participants of the population-based Lifelines cohort study in the Netherlands. *Ocul Surf*.
- Volatier TLA, Figueiredo FC & Connon CJ (2020): Keratoconus at a molecular level: a review. *Anat Rec* **303**: 1680–1688.
- Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M & Tugwell P (2014): The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-Analyses. Ottawa, ON: Ottawa Hospital Research Institute.
- Wen D, Li Q, Song B, Tu R, Wang Q, O'Brart DPS, McAlinden C & Huang J (2018a): Comparison of standard versus accelerated corneal collagen cross-linking for keratoconus: a meta-analysis. *Invest Ophthalmol Vis Sci* **59**: 3920.
- Wen D, Song B, Li Q et al. (2018b): Comparison of epithelium-off versus transepithelial corneal collagen cross-linking for keratoconus: a systematic review and meta-analysis. *Cornea* **37**: 1018–1024.
- Wheeler J, Hauser MA, Afshari NA, Allingham RR & Liu Y (2012): The genetics of keratoconus: a review. *Reprod Syst Sex Disord Suppl* **6**: 001.
- Willcox MDP, Argüeso P, Georgiev GA et al. (2017): TFOS DEWS II tear film report. *Ocul Surf* **15**: 366–403.
- Wisse RPL, Kuiper JJW, Gans R, Imhof S, Radstake TRDJ & Van der Lelij A (2015): Cytokine expression in keratoconus and its corneal microenvironment: a systematic review. *Ocul Surf* **13**: 272–283.
- Woodward MA, Blachley TS & Stein JD (2016): The association between sociodemographic factors, common systemic diseases, and keratoconus. *Ophthalmology* **123**: 457–465.e2.
- Yilmaz M, Arıkan S & Türkön H (2020): Plasma homocysteine levels in patients with keratoconus. *Clin Exp Optom* **103**: 804–807.
- Yin Y, Zong R, Bao X, Zheng X, Cui H, Liu Z & Zhou Y (2018): Oxidative stress suppresses cellular autophagy in corneal epithelium. *Invest Ophthalmol Vis Sci* **59**: 3286.
- Zarei-Ghanavati S, Yahaghi B, Hassanzadeh S, Ghayour-Mobarhan M, Hakimi HR & Eghbali P (2019): Serum 25-hydroxyvitamin D, selenium, zinc and copper in patients with keratoconus. *J Curr Ophthalmol* **32**: 26–31.
- Zhang W, Margines JB, Jacobs DS et al. (2019): Corneal perforation after corneal cross-linking in keratoconus associated with potentially pathogenic ZNF469 mutations. *Cornea* **38**: 1033–1039.

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VN and FD were responsible for the design and conception of the study. VN and FD searched and collected studies and data. VN, JM, FC, FD and BP analysed and interpreted data. VN, FD and BP were in charge of statistical analyses. VN, FD, JSB and FM wrote the manuscript. All authors made critical revisions to the article. All authors gave their final approval of the article.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Details of keywords and search strategies used within each database.

**Appendix S2.** PRISMA checklist.

**Appendix S3.** Detailed characteristics of included studies.

**Appendix S4.** Details on quality of articles (Figs 1 and 2), population (sample size, gender, age, body mass index, smoking status, atopy, contact lenses users, best-corrected visual acuity, maximal keratometry and central pachymetry), keratoconus diagnosis and severity scales (diagnostic methods and severity scales of keratoconus), samples (type of samples, origins of samples and sampling methods), oxidative stress markers/antioxidants (levels and methods of quantification), study designs, geographic zones, follow-up, aims and outcomes of included articles.

**Appendix S5.** Funnel plot (meta-funnel) based on data from oxidative stress markers and antioxidants in keratoconus comparing with healthy controls, stratified by type of markers. Each dot represents a single study, with its corresponding effect size (*x*-axis) and its associated standard error of the effect estimate (*y*-axis). Large high-powered studies are placed towards the top and smaller low-powered studies towards the bottom. The plot should ideally resemble a pyramid or inverted funnel, with scatter due to sampling variation. Studies outside funnel plot are likely to present bias (Sterne et al. 2011).

**Appendix S6.** Meta-analysis on oxidative stress markers in keratoconus comparing with healthy controls, stratified by type of markers.

**Appendix S7.** Meta-analysis on oxidative stress markers in keratoconus

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comparing with healthy controls, stratified by type of samples.

**Appendix S8.** Funnel plot (meta-funnel) based on data from oxidative stress markers and antioxidants in keratoconus comparing with healthy controls, stratified by type of samples (tears, cornea, aqueous humour and blood), by type of corneal samples (stromal cells, epithelium and endothelium) and by geographic zones (Asia, Europe, North America, Africa and Oceania). Each dot represents a single study, with its corresponding effect size ( $x$ -axis) and its associated standard error of the effect estimate ( $y$ -axis). Large high-powered studies are placed towards

the top and smaller low-powered studies towards the bottom. The plot should ideally resemble a pyramid or inverted funnel, with scatter due to sampling variation. Studies outside funnel plot are likely to present bias (Sterne et al. 2011).

**Appendix S9.** Meta-analysis on oxidative stress markers in keratoconus comparing with healthy controls, stratified by type of corneal samples.

**Appendix S10.** Meta-analysis on oxidative stress markers in keratoconus comparing with healthy controls, stratified by geographic zones.

**Appendix S11.** Meta-analysis on antioxidants in keratoconus comparing

with healthy controls, stratified by type of markers.

**Appendix S12.** Meta-regressions on antioxidants in keratoconus comparing with healthy controls.

**Appendix S13.** Meta-analysis on antioxidants in keratoconus comparing with healthy controls, stratified by type of samples.

**Appendix S14.** Meta-analysis on antioxidants in keratoconus comparing with healthy controls, stratified by type of corneal samples.

**Appendix S15.** Meta-analysis on antioxidants in keratoconus comparing with healthy controls, stratified by geographic zones.

## **Publication N°3**

### **Oxidative and antioxidative stress markers in dry eye disease: a systematic review and meta-analysis**

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# Oxidative and antioxidative stress markers in dry eye disease: A systematic review and meta-analysis

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## ABSTRACT.

**Purpose:** To conduct a systematic review and meta-analysis on the levels of oxidative stress markers and antioxidants in dry eye disease (DED) compared with healthy subject.

**Method:** The PubMed, Cochrane Library, Embase, Science Direct and Google Scholar databases were searched on 10 January 2021 for studies reporting oxidative and antioxidative stress markers in DED and healthy controls. Main meta-analysis was stratified by type of biomarkers, type of samples (tears, conjunctival cells or biopsies), Sjögren's syndrome (SS) (patients with or without SS) and by geographical zones (Asia or Europe).

**Results:** We included nine articles, for a total of 333 patients (628 eye samples) with DED and 165 healthy controls (451 eye samples). There is an overall increase in oxidative stress markers in DED compared with healthy controls (standard mean deviation = 2.39, 95% confidence interval 1.85–2.94), with a significant increase in lipid peroxide (1.90, 0.69–3.11), myeloperoxidase (2.17, 1.06–3.28), nitric oxide synthase 3 (2.52, 0.95–4.08), xanthine oxidase/oxidoreductase (2.41, 1.40–5.43), 4-hydroxy-2-nonenal (4HNE) (4.75, 1.67–7.84), malondialdehyde (3.00, 2.55–3.45) and reactive oxygen species (1.31, 0.94–1.68). Oxidative stress markers were higher in tears, conjunctival cells and conjunctival biopsies of DED than controls. Even if small number of studies were included for antioxidants, catalase seemed to be decreased in DED compared with healthy controls (–2.17, –3.00 to –1.34), with an increase of antioxidants in tears of DED patients without SS (1.13, 0.76–1.49).

**Conclusion:** Oxidative stress markers, and probably antioxidants, were dysregulated in DED, establishing a local oxidative environment in tears, conjunctival cells and tissues.

**Key words:** biomarkers – dry eye disease – inflammation – ocular surface – oxidative stress

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## Highlights

- In this systematic review and meta-analysis, we aimed to compare the levels of oxidative stress markers and antioxidants in DED patients versus healthy controls.
- We stratified meta-analyses on the type of biomarkers, type of samples (tears, conjunctival cells or biopsies), geographical zones and diagnosis or not of Sjögren's syndrome.
- We highlighted that oxidative stress markers (in tears and conjunctiva) were increased in DED compared with healthy individuals.
- Oxidative stress imbalance seems to be a central process in DED, involving a better comprehension of pathological pathways and innovative therapeutic approach.

## Introduction

Dry eye disease (DED) is one of the most common ocular surface diseases, affecting from 5 to 50% of worldwide people (Stapleton et al. 2017). Dry eye disease (DED) induced a large panel of ocular symptoms in relation with the stage of tissue damages, such as superficial pain, foreign body sensation, ocular discomfort and visual

disturbance. Moreover, DED is a multifactorial diseases associated with age, gender, hormonal status, leisure and occupational activities (i.e. air conditioning and working at visual display terminal), geographic zones (i.e. climate and humidity rate), duration of preserved-topical treatment, and several others ocular and systemic conditions (e.g. dopamine dysregulation of Parkinson disease, palpebral abnormalities or glaucoma) (Craig et al. 2017; Pflugfelder & de Paiva 2017; Hanyuda et al. 2020). Definition of DED has been recently remodelled as an inflammatory disease involving all components of the ocular surface with an increase of the tear film osmolarity, a disturbance of lacrimal functional unit with corneal neurogenic abnormalities, and a tear film instability (Craig et al. 2017; Pflugfelder & de Paiva 2017). Tear film hyperosmolarity seems to be the core mechanism inducing the vicious circle of DED: epithelial damage inducing cell death by apoptosis, dysregulated autophagy, loss of goblet cells with lack of mucin expression, release of inflammatory mediators (i.e. NF $\kappa$ B, TNF $\alpha$ , IL-1 $\beta$ , IL-6 and MMP-9) and finally the exacerbation of tear film instability (Bron et al. 2017; Pflugfelder & de Paiva 2017; Baudouin et al. 2019; Martin et al. 2019). Thus, ocular surface damages in DED caused by osmotic, inflammatory or mechanical stresses (desiccation and loss of lubrication) are causative factors to improve fragility of eye against environmental aggressions. Indeed, the integrity of tears fluid and ocular tissues are needed to express abundantly antioxidants and protective enzymes, maintaining the redox homeostasis of the ocular surface (Chen, Mehta & Vasiliou 2009). Considering the intense burden of free-radical stress in the first-line barrier tissues during life (e.g. particular matter, fossil fuels exhausts, or solar ultraviolet radiations) (Ivanov et al. 2018; Kim et al. 2020), it has been proposed that oxidative/antioxidative imbalance was a central pathological process in the pathophysiology of DED (Shoham et al. 2008; Dogru et al. 2018; Ivanov et al. 2018). Evidence data tended to describe a dysregulation of redox homeostasis in ocular tissues of DED, but the variability of studies in this field needed to rigorously summarize the literature to avoid conflicting

conclusions. Thus, it seemed to be necessary to clarify the role of oxidative stress involved in the pathological process of DED. Therefore, we aimed to compute a systematic review and meta-analysis on the levels of oxidative stress markers and antioxidants in DED compared with healthy controls.

## Methods

### Literature search

We searched all articles in PubMed, Cochrane Library, Embase, ScienceDirect and Google Scholar databases to 10<sup>th</sup> January 2021 with the following keywords: ("Dry Eye Syndromes" OR "dry eye\*" OR "DED") AND ("reactive oxygen species" OR "oxidative stress" OR "oxidative stresses" OR "nitrate stress" OR "nitrate damage" OR "oxidant stress" OR "oxidative damage" OR "antioxidant effect" OR "antioxidant effects" OR "antioxidants" OR "antioxidative stress" OR "antioxidant stress") (details of search strategy are presented in Appendix S1). The search was not limited to specific years, language restrictions and no minimal sample size was applied. To be included, articles needed to evaluate a level of oxidative stress and/or antioxidative markers in sample of DED and healthy controls. We excluded animal studies. We imposed no limitation of regional origin. In addition, references list of all relevant publications (e.g. studies on oxidative stress in DED or literature review) was manually searched to identify any other ones not found with electronic search. The search strategy is presented in Fig. 1. One author conducted all literature searches (Valentin Navel) and collated the abstracts. Two authors (Valentin Navel and Frédéric Dutheil) separately reviewed the abstracts and based on the selection criteria, decided the suitability of the articles for inclusion. A third author (Frédéric Chiambaretta) was asked to review the articles where consensus on suitability was debated. Finally, all authors reviewed eligible articles. We followed the PRISMA guidelines (Appendix S2) (Moher et al. 2009).

### Data collection

The data collected included first author's name, publication year, study

design (case-control or prospective randomized controlled study), type of study (laboratory or field study), aims and outcomes of included articles, inclusion and exclusion criteria, sample size, mean age, percentage of male, type of sample (conjunctival impression cytology, conjunctival biopsy, or tears), origin of samples (during clinical examination, cataract surgery or others ocular interventions), sampling methods (glass microcapillary tubes, surgical sponges, impression cytology, or surgical procedure for biopsy), follow-up, best-corrected visual acuity (logMar), Schirmer-I test (mm), tear break-up time (TBUT) test (seconds), rose Bengal staining test, type of biomarkers (oxidants or antioxidants), method of determination (fluorometry, spectrophotometry, histochemistry, immunohistochemistry, enzyme-linked immunosorbent assays (ELISA) and others enzymatic tests and assays) and putative adjustment/explaining factors (such as geographical zone, or systemic diseases as Sjögren's syndrome).

### Outcomes

We studied the levels of oxidative stress markers and antioxidants in DED comparing with healthy controls as primary outcome. Secondary outcomes were the difference of oxidative stress markers or antioxidants levels according to the type of biomarkers, type of samples, geographic zones, Sjögren's syndrome and their association with covariates.

### Methodological quality assessment

The quality assessment only involved the nine included studies and did not affect the selection process. We used the Newcastle-Ottawa Quality Assessment Scale model to judge observational studies with the dedicated evaluation grids. The following nine items were assessed in all case-control studies: four items on selection bias, two items on comparability bias and three items on outcome bias (Wells et al. 2014). To evaluate randomized controlled studies, we used the Scottish Intercollegiate Guidelines Network (SIGN) with the dedicated evaluation grids. The following 11 items were assessed in randomized controlled studies: 10 items on internal validity and one item on overall assessment of the study (Harbour & Miller 2001).

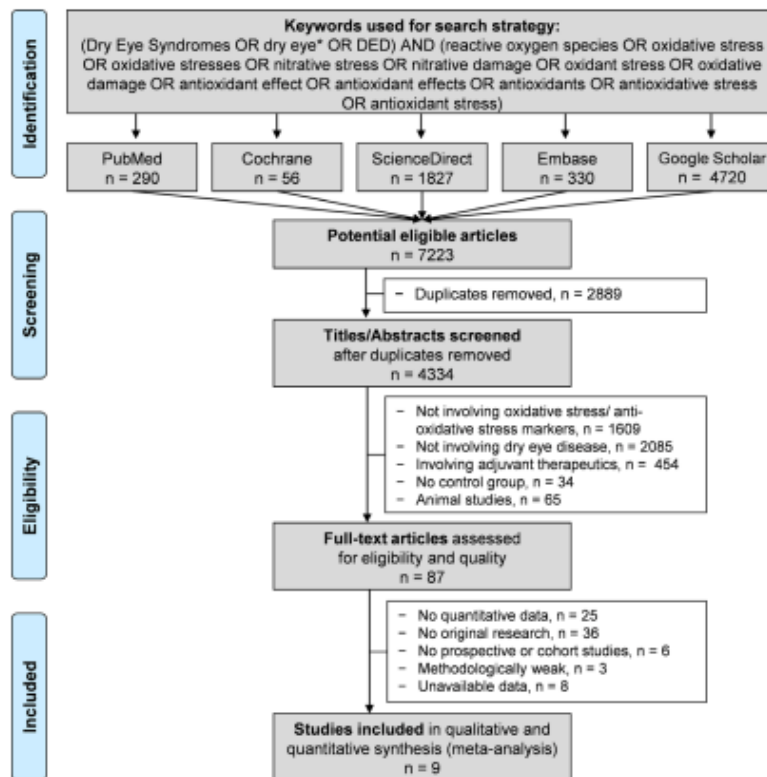


Fig. 1. Flow diagram according with PRISMA Guidelines.

Each item was assigned a judgement of 'Yes', 'No', 'Can't say' or 'Not applicable'. The methodological quality of the studies was evaluated by two authors (Valentin Navel and Frédéric Dutheil). Disagreements were addressed by obtaining a consensus with a third author (Frédéric Chimbaretta) (Fig. 2).

#### Statistical considerations

Statistical analyses were conducted using Comprehensive Meta-analysis software (version 2, Biostat Corporation, Englewood, NJ, USA) and Stata software (version 13, StataCorp, College Station, TX, USA) (Ollier et al. 2014; Lanhers et al. 2015; Benoist d'Azy et al. 2016a, b; Courtin et al. 2016; Lanhers et al. 2017; Benoist d'Azy et al. 2019; Navel et al. 2019). Characteristics of the biomarkers and subjects (dry eye and controls) were summarized for each study sample and reported as mean  $\pm$  standard-deviation (SD) and number (%) for continuous and categorical variables respectively. For rigour, effect sizes were defined as

standard mean differences (SMD) of oxidants/antioxidants levels. First, we conducted meta-analyses on oxidants/antioxidants levels. Standard difference in means between groups (SMD, difference between means divided by the pooled standard deviation, with a correction for small sample bias) and 95% confidence interval (95% CI) were estimated using DerSimonian and Laird random-effects models (assuming that the true effect estimates varied among studies) (DerSimonian & Laird 1986). A SMD is defined as a unitless measure of the concentration of oxidants/antioxidants centred at zero if oxidants/antioxidants levels in DED are not different from healthy controls. A positive SMD denoted higher oxidants/antioxidants levels in DED than healthy controls. A scale for SMD has been suggested with 0.8 reflecting a large effect, 0.5 a moderate effect and 0.2 a small effect (DerSimonian & Laird 1986). p Values less than 0.05 were considered statistically significant. Then, we stratified these meta-analyses on the type of biomarkers, the type of samples (tears, conjunctival cells or

biopsies), geographical zones and diagnosis or not of Sjögren's syndrome. Statistical heterogeneity between results was assessed by examining forest plots, confidence intervals and using  $I^2$ , which is the most common metric for measuring the magnitude of between-study heterogeneity and is easily interpretable.  $I^2$  values range between 0% and 100% and are typically considered low for heterogeneity scores of <25%, modest for 25–50% and high for >50%. For example, a significant heterogeneity may be due to the variability between the characteristics of the studies, such as those of the subjects (age, sex, etc.), the type of samples or other covariables. Publication bias was taken into consideration and funnel plots were compiled, with sensitivity analysis excluding studies not evenly distributed around the base of the metafunnels. When possible (sufficient sample size), meta-regressions were proposed to study the relation between oxidants/antioxidants levels and characteristics of subjects (e.g. age, sex, country, Schirmer-I test, TBUT, rose Bengal staining test) and other samples characteristics (e.g. type of samples). Results were expressed as regression coefficients and 95% CI.

## Results

An initial search produced a possible for 7223 articles (Fig. 1). We identified nine eligible articles including eight case-control studies (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Cejková et al. 2008; Soria et al. 2013; Wakamatsu et al. 2013; Zheng et al. 2015; Choi et al. 2016) and one prospective, randomized-controlled trial (Macri et al. 2015). Overall, these studies included a total of 333 patients with DED and 165 controls, that is 628 eye samples with DED and 451 control eye samples (Table 1). All articles were written in English.

More detailed information on quality of articles (Figs 1 and 2), population (sample size, gender, age, Schirmer-I test, TBUT, rose Bengal staining test and others clinical explorations), DED diagnosis and severity scales (diagnostic methods and severity scales of DED), samples (type of samples, sampling methods and tissues conservation), oxidative stress markers/antioxidants (methods of quantification), study designs, geographic

	Selection Bias			Comparability Bias		Exposure Bias		
	Adequacy of case definition	Representativeness of the cases	Selection of controls	Definition of controls	Comparability of groups *	Assessment of exposure	Method of ascertainment / follow-up	
<b>Observational studies</b>								
Augustin et al.	1	1	1	?	0	1	1	1
Cejková et al.	1	1	1	1	1	1	1	?
Cejková et al.	1	1	1	1	1	1	1	1
Cejková et al.	1	1	1	1	1	1	1	1
Choi et al.	1	1	1	?	2	1	1	1
Soria et al.	1	1	?	1	1	1	1	1
Wakamatsu et al.	1	1	?	1	1	1	1	1
Zheng et al.	1	1	?	1	2	1	1	?
<b>Randomized-Controlled Trial</b>								
Macri et al.	1	1	1	0	1	?	1	0
	Appropriate and clearly focused question	Assignment of subjects is randomised	Adequate concealment method is used	Subjects and investigators blind about treatment	Treatment and control groups are similar	Only difference between group is treatment	Standard and valid outcomes	Number of dropped outs (%)
								Analyses in randomly allocated groups
								Results are comparable for all sites
								<b>General level of evidence</b>

Fig 2. Methodological quality of included articles using the Newcastle—Ottawa Quality Assessment Scale (NOS) for observational studies and the Scottish Intercollegiate Guidelines Network (SIGN) for controlled trials studies. For each item, criteria fulfilled: No: 0, Yes: 1 (\*: 2 subitems), Can't say:?, Not applicable: NA.

zones, follow-up, aims and outcomes of included articles are given in Table 1 and Appendix S3.

**Oxidative stress markers/antioxidants**

*Oxidative stress markers*

Seven studies reported oxidative stress markers in DED comparing with healthy controls (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), including two studies for lipid peroxide (LPO) (Augustin et al. 1995; Macri et al. 2015), two studies for hexanoyl-lysine (HEL) (Wakamatsu

et al. 2013; Choi et al. 2016), two studies for 4-hydroxy-2-nonenal (4HNE) (Wakamatsu et al. 2013; Choi et al. 2016), one study for myeloperoxidase (MPO) (Augustin et al. 1995), one study for nitric oxide synthase 3 (NOS3) (Cejková et al. 2007), one study for xanthine oxidase/oxidoreductase (Cejková et al. 2007), one study for malondialdehyde (MDA) (Choi et al. 2016) and one study for reactive oxygen species (ROS) (Zheng et al. 2015).

*Antioxidants*

Two studies (Cejková et al. 2008; Soria et al. 2013) reported antioxidants levels in DED comparing with healthy

controls, including superoxide dismutase (SOD), glutathione-dependent enzymes [i.e. glutathione peroxidase (GPX) and glutathione S-transferase P-1 (GSTP1)] and one study (Cejková et al. 2008) for catalase (CAT).

**Meta-analysis**

*Meta-analysis of oxidative stress in DED Stratified by type of oxidative stress markers.* The overall SMD was 2.39 (95 CI 1.85–2.94,  $p < 0.001$ ) with an important heterogeneity ( $I^2 = 96.1\%$ ), involving a significant increase of oxidative stress markers in DED compared with healthy controls (Fig. 3, and Appendices S4 and S5). Lipid peroxide (LPO) (1.90, 0.69–3.11,  $p = 0.002$ ,  $I^2 = 97.7\%$ ), MPO (2.17, 1.06–3.28;  $p < 0.001$ ;  $I^2 = 96.8\%$ ), NOS3 (2.52, 0.95–4.08,  $p = 0.002$ ,  $I^2 = 90.4\%$ ), xanthine oxidase/oxidoreductase (2.41, 1.40–5.43;  $p < 0.001$ ;  $I^2 = 75.0\%$ ), 4HNE (4.75, 1.67–7.84;  $p = 0.003$ ;  $I^2 = 95.2\%$ ), MDA (3.00, 2.55–3.45,  $p < 0.001$ ,  $I^2 = 0.0\%$ ) and ROS (1.31, 0.94–1.68;  $p < 0.001$ ) were increased in DED compared with healthy controls (Fig. 3 and Appendices S4 and S5).

*Stratified by type of samples.* We included seven studies (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), with three studies for tears (Augustin et al. 1995; Wakamatsu et al. 2013; Choi et al. 2016) and six studies for conjunctival samples (Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016). We highlighted an increase of oxidative stress markers in tears (SMD = 2.36, 95CI 1.49–3.24,  $p < 0.001$ ,  $I^2 = 98.2\%$ ) and in conjunctival samples of DED compared with healthy controls (2.40, 1.78–3.01,  $p < 0.001$ ,  $I^2 = 85.8\%$ ) (Fig. 3, Appendices S6 and S7).

*Stratified by type of ocular surface samples.* We included seven studies (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), with three studies for tears (Augustin et al. 1995; Wakamatsu et al. 2013; Choi et al. 2016), four studies for conjunctival cells (Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013;

**Table 1.** Detailed characteristics of included studies.

Study	Methodology				Dry eye disease				Controls				Oxidants/Antioxidants			
	Country	Design	Material (tissues)	n eyes	Age mean±SD	Sex %men	Sjögren Yes/No	Schirmer-I (mm) Mean ± SD	TBUT (s) Mean ± SD	Rose Bengal Mean ± SD	n eyes	Age mean±SD	Sex %men	Biomarkers	Status	Methods
Augstein 1995	Germany	Case-control	Tears	78	-	-	No	-	-	3.66 ± 1.9	132	-	-	Lipid peroxide (LPO)	Oxidant	Fluorometry
				109	-	-	No	-	-	2.96 ± 2.2	132	-	-	Lipid peroxide (LPO)	Oxidant	Fluorometry
				78	-	-	No	-	-	1.91 ± 2.3	132	-	-	Lipid peroxide (LPO)	Oxidant	Fluorometry
				78	-	-	No	-	-	3.66 ± 1.9	132	-	-	Myeloperoxidase (MPO)	Oxidant	Spectrophotometry
Cejkova 2007a	Czech Republic	Case-control	Conjunctiva (C cells)	78	-	-	No	-	-	2.96 ± 2.2	132	-	-	Myeloperoxidase (MPO)	Oxidant	Spectrophotometry
				16	49.2 ± 5.4	25	Yes	1.36 ± 0.4	5.4 ± 1.2	6.61 ± 0.7	22	48.5 ± 5.2	18.2	Nitric oxide synthase 3 (NOS3)	Oxidant	Immunohistochemistry
				6	49.2 ± 5.4	0	Yes	1.36 ± 0.4	5.4 ± 1.2	6.61 ± 0.7	22	48.5 ± 5.2	18.2	Nitric oxide synthase 3 (NOS3)	Oxidant	Immunohistochemistry
				16	49.2 ± 5.4	25	Yes	1.36 ± 0.4	5.4 ± 1.2	6.61 ± 0.7	22	48.5 ± 5.2	18.2	Nitric oxide synthase 3 (NOS3)	Oxidant	Immunohistochemistry
Cejkova 2007b	Czech Republic	Case-control	Conjunctiva (C cells)	6	49.2 ± 5.4	0	Yes	1.36 ± 0.4	5.4 ± 1.2	6.61 ± 0.7	22	48.5 ± 5.2	18.2	Nitric oxide synthase 3 (NOS3)	Oxidant	Immunohistochemistry
				18	43.8 ± 5.4	11.1	Yes	1.42 ± 0.4	5.4 ± 1.2	6.58 ± 0.72	18	43.2 ± 4.8	11.1	Xanthine oxidase	Oxidant	Histochemistry
Cejkova 2008	Czech Republic	Case-control	Conjunctiva (C cells)	18	43.8 ± 5.4	11.1	Yes	1.42 ± 0.4	5.4 ± 1.2	6.58 ± 0.72	18	43.2 ± 4.8	11.1	Xanthine oxidase	Oxidant	Immunohistochemistry
				14	41.5 ± 5.8	22.2	Yes	3.40 ± 0.3	5.3 ± 1.2	6.50 ± 0.3	18	46.2 ± 3.1	22.2	Superoxide dismutase (SOD)	Antioxidant	Immunohistochemistry
Choi 2016	South Korea	Case-control	Tears	14	41.5 ± 5.8	22.2	Yes	3.40 ± 0.3	5.3 ± 1.2	6.50 ± 0.3	18	46.2 ± 3.1	22.2	Glutathione peroxidase (GPX)	Antioxidant	Immunohistochemistry
				88	50.5 ± 11.7	38.1	No	4.90 ± 1.6	3.7 ± 1.4	-	66	48.0 ± 10.3	39.4	Catalase (CAT) Hexanoyl-lysine (HEL)	Antioxidant Oxidant	ELISA
Muri 2015	Italy	Randomized controlled	Conjunctiva (biopsy)	88	50.5 ± 11.7	38.1	No	4.90 ± 1.6	3.7 ± 1.4	-	66	48.0 ± 10.3	39.4	4-hydroxy-2-nonenal (4HNE)	Oxidant	ELISA
				5	-	-	No	-	-	-	5	-	-	Malondialdehyde (MDA) Hexanoyl-lysine (HEL)	Oxidant Oxidant	ELISA Immunohistochemistry
Soria 2013	Spain	Case-control	Tears	5	-	-	No	-	-	-	5	-	-	4-hydroxy-2-nonenal (4HNE)	Oxidant	Immunohistochemistry
				58	74.0 ± 6.0	31.0	No	6.0 ± 2.4	6.0 ± 2.0	-	84	75.0 ± 7.0	28.6	Malondialdehyde (MDA) Lipid peroxide (LPO)	Oxidant Oxidant	Immunohistochemistry LP-CHOLOX test
Wakamatsu 2013	Japan	Case-control	Conjunctiva (C cells)	47	55.3 ± 14.1	38.3	No	-	-	-	31	42.7 ± 14.0	45.2	Glutathione S-transferase P (GSTP1)	Antioxidant	MALDITOF/TOF mass spectrometry
				31	62.8 ± 12.9	0	Yes	3.60 ± 2.0	2.8 ± 0.7	4.0 ± 2.1	31	42.7 ± 14.0	45.2	Superoxide dismutase (SOD2)	Antioxidant	MALDITOF/TOF mass spectrometry
Zheng 2015	China	Case-control	Conjunctiva (C cells)	31	62.8 ± 12.9	0	Yes	3.60 ± 2.0	2.8 ± 0.7	4.0 ± 2.1	15	65.3 ± 18.3	20.0	Hexanoyl-lysine (HEL)	Oxidant	ELISA
				80	32.3 ± 6.5	35.0	No	7.13 ± 3.6	3.1 ± 1.2	-	60	33.7 ± 5.8	33.3	4-hydroxy-2-nonenal (4HNE) Hexanoyl-lysine (HEL) Reactive oxygen species (ROS)	Oxidant Oxidant Oxidant	Immunohistochemistry ROS Fluorescence Assay Kit

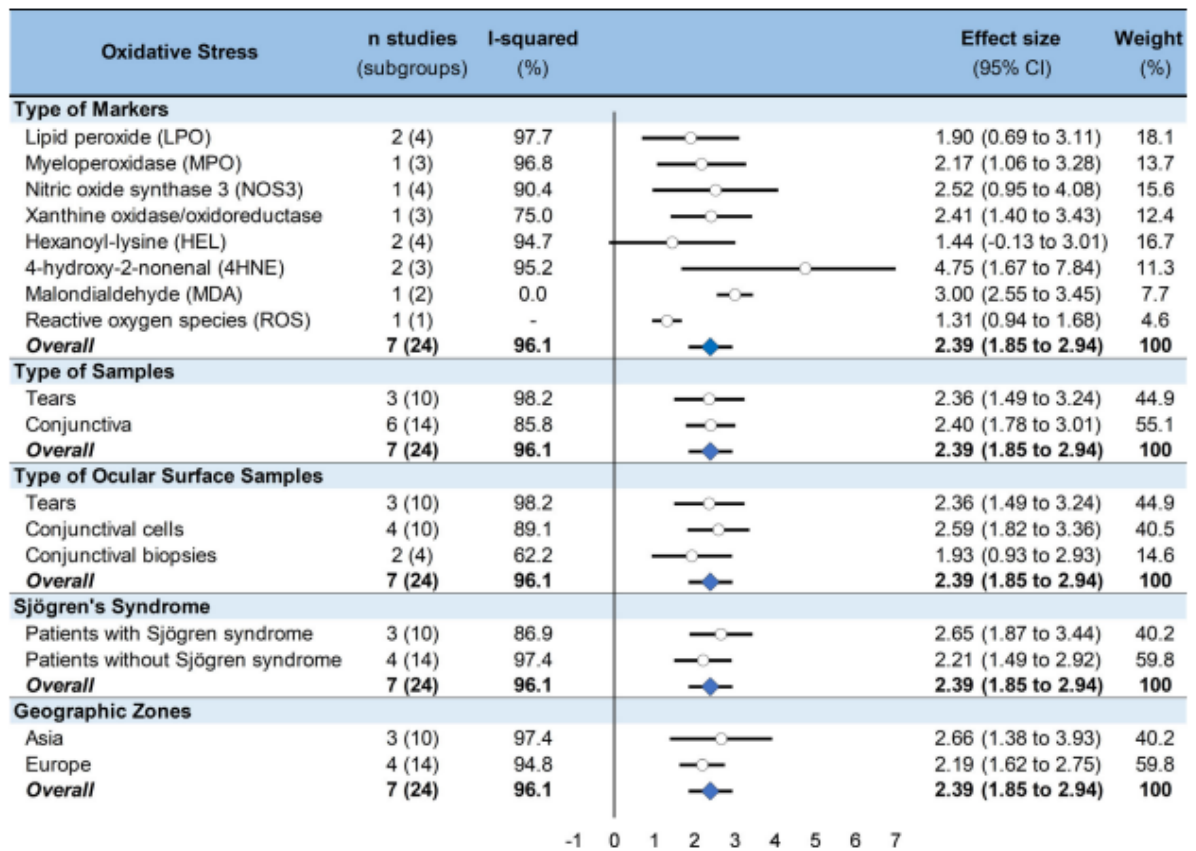


Fig 3. Summary of meta-analysis on oxidative stress markers in dry eye disease (DED) comparing with healthy controls, stratified by type of markers, by type of samples (tears or conjunctiva), by type of ocular surface samples (tears, conjunctival cells or conjunctival tissues), by Sjögren's syndrome (DED patients with or without Sjögren's syndrome) and by geographic zones (Asia or Europe). The column Weight is the weight of each study/stratification within the meta-analysis; weight is the inverse of the estimates' variance, so that larger studies tend to contribute more than smaller studies.

Zheng et al. 2015) and two studies for conjunctival biopsies (Macri et al. 2015; Choi et al. 2016). We highlighted an increase of oxidative stress markers in tears (SMD = 2.36, 95CI 1.49–3.24,  $p < 0.001$ ,  $I^2 = 98.2\%$ ), in conjunctival cells (2.59, 1.82–3.36,  $p < 0.001$ ,  $I^2 = 89.1\%$ ) and in conjunctival biopsies of DED (1.93, 0.93–2.93,  $p < 0.001$ ,  $I^2 = 62.2\%$ ) compared with healthy controls (Fig. 3, Appendices S7 and S8).

**Stratified by Sjögren's syndrome.** We included seven studies (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), with 3 studies including DED patients with Sjögren's syndrome (Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013) and four studies without Sjögren's syndrome (Augustin et al. 1995; Macri et al. 2015; Zheng et al.

2015; Choi et al. 2016). We highlighted an increase of oxidative stress markers in DED patients with Sjögren's syndrome (SMD = 2.65, 95 CI 1.87–3.44,  $p < 0.001$ ,  $I^2 = 86.9\%$ ) and in DED patients without Sjögren's syndrome (2.21, 1.49–2.92,  $p < 0.001$ ,  $I^2 = 97.4\%$ ) (Fig. 3, Appendices S7 and S9).

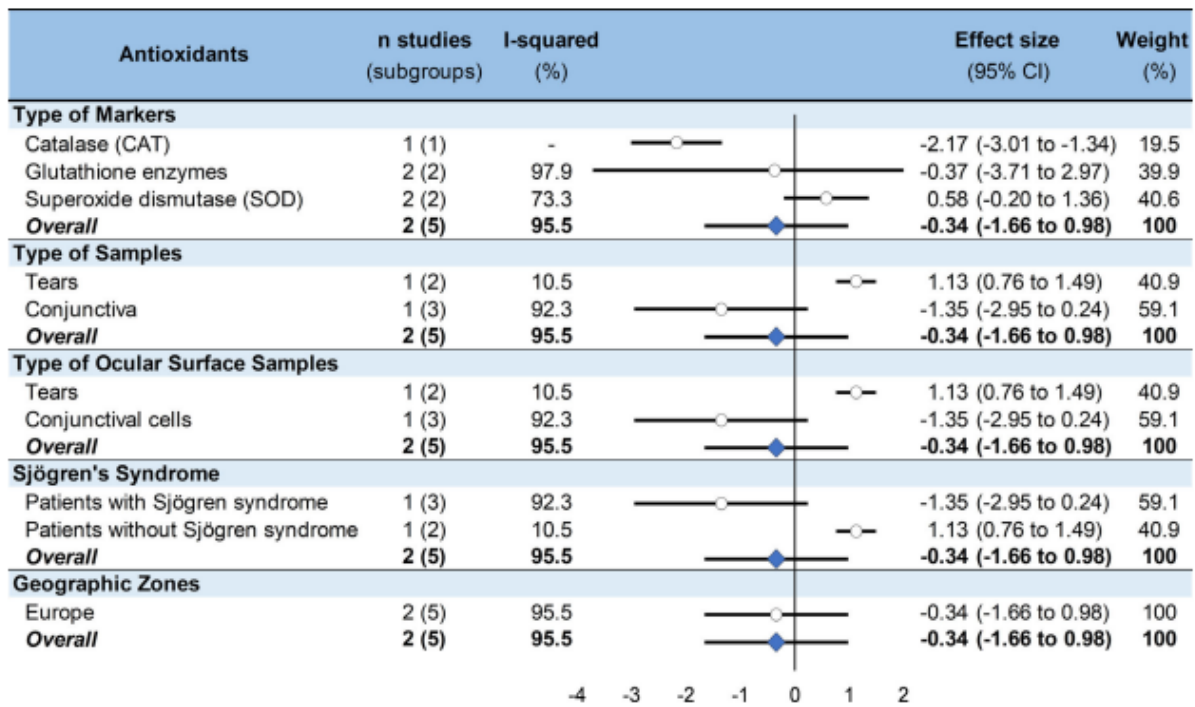
**Stratified by geographic zone.** We included seven studies (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), with three studies for Asia (Wakamatsu et al. 2013; Zheng et al. 2015; Choi et al. 2016) and four studies for Europe (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Macri et al. 2015). Both geographical zones showed an increase of oxidative stress markers in DED compared with healthy controls (SMD = 2.66, 95 CI 1.38–3.93,  $p < 0.001$ ,  $I^2 = 97.4\%$ ;

and 2.19, 1.62–2.75;  $p < 0.001$ ;  $I^2 = 94.8\%$ , respectively) (Fig. 3, Appendices S7 and S10).

**Meta-analysis of antioxidants in DED**

**Stratified by type of antioxidants.** The overall SMD was  $-0.34$  (95 CI  $-1.66$ – $0.98$ ,  $p = 0.618$ ) with an important heterogeneity ( $I^2 = 95.5\%$ ) (Fig. 4, and Appendices S7 and S11). Even if only one study was included, CAT was decreased in DED compared with healthy controls ( $-2.17$ ,  $-3.00$  to  $-1.34$ ,  $p < 0.001$ ). Glutathione-dependent enzymes were showed to be increased in one study (Soria et al. 2013) and decreased in other one study (Cejková et al. 2008), involving non-significant overall result ( $-0.37$ ,  $-3.71$ – $2.97$ ,  $p = 0.829$ ). In the same way, SOD was increased in only one study (Soria et al. 2013), without any significant overall result (0.58,  $-0.20$ ,





**Fig 4.** Summary of meta-analysis on antioxidants in dry eye disease (DED) comparing with healthy controls, stratified by type of markers, by type of samples (tears or conjunctiva), by type of ocular surface samples (tears or conjunctival cells), by Sjögren's syndrome (DED patients with or without Sjögren's syndrome) and by geographic zones (Europe). The column Weight is the weight of each study/stratification within the meta-analysis; weight is the inverse of the estimates' variance, so that larger studies tend to contribute more than smaller studies.

1.36,  $p = 0.146$ ) (Fig. 4, and Appendices S7 and S11).

*Stratified by type of samples.* We included two studies (Cejková et al. 2008; Soria et al. 2013), with one study for tears (Soria et al. 2013) and one study for conjunctival samples (i.e. only conjunctival cells) (Cejková et al. 2008). We highlighted an increase of antioxidants in tears (SMD = 1.13, 95 CI 0.76–1.49,  $p < 0.001$ ,  $I^2 = 10.5\%$ ) (Fig. 4, Appendices S7, S12 and S13).

*Stratified by Sjögren's syndrome.* We included two studies (Cejková et al. 2008; Soria et al. 2013), with one study including DED patients with Sjögren's syndrome (Cejková et al. 2008) and one study without Sjögren's syndrome (Soria et al. 2013). We highlighted an increase of antioxidants in DED patients without Sjögren's syndrome (SMD = 1.13, 95 CI 0.76–1.49,  $p < 0.001$ ,  $I^2 = 10.5\%$ ) (Fig. 4, Appendices S7 and S14).

*Stratified by geographic zone.* We included two studies (Cejková et al. 2008; Soria et al. 2013), with only studies for Europe (Augustin et al. 1995; Cejková et al. 2007; Cejková et al. 2007; Macri et al. 2015). Meta-

analysis and meta-regressions did not retrieve any significant results (Fig. 4, Appendices S7, S12 and S15).

*Meta-regressions and sensitivity analysis*

Meta-regressions demonstrated that 4HNE were more increased in DED than LPO (Coefficient 2.95; 95 CI 0.34–5.55;  $p = 0.029$ ) and than HEL (3.41; 0.78–6.04;  $p = 0.014$ ) (Fig. 5). For both oxidants and antioxidants, meta-regressions did not demonstrate any influence of sociodemographic (age and gender), ocular surface testing (Schirmer-I test, TBUT and Rose Bengal staining test), nor between types of antioxidants or types of samples, nor depending on Sjögren's syndrome (Fig. 5 and Appendix S16). Heterogeneity between studies precluded further sensitivity analyses.

**Discussion**

Dry eye disease (DED) is classically defined as a common ocular surface disease with loss of tear film homeostasis, involving tear film hyperosmolarity and instability, corneal neurosensory abnormalities and chronic vicious circle

of local inflammation (Baudouin et al. 2013; Craig et al. 2017). As the first-line barrier of the eye towards environment, ocular surface is daily exposed to the intense burden of free radical stress through environmental pollution, diesel exhausts, solar ultraviolet radiations, indoor pollution and mitochondrial sources of radical species derived from oxygen (Vallabh, Romano & Wiloughby 2017; Ivanov et al. 2018; Kim et al. 2020; Yan et al. 2020). However, dysregulation of oxidative stress homeostasis has not been described in the classical definition of DED. In this systematic review and meta-analysis, we underlined that imbalance between oxidative stress and antioxidants seems to be a central process in the pathophysiology of DED. We highlighted that oxidative stress markers (in tears and conjunctiva) were increased in DED compared with healthy individuals. Even if antioxidative defences (in tears) seemed to be increased in DED, the small number of included studies precluded any robust conclusions on antioxidants. Considering these results, we could better understand the complexity of pathological process in DED

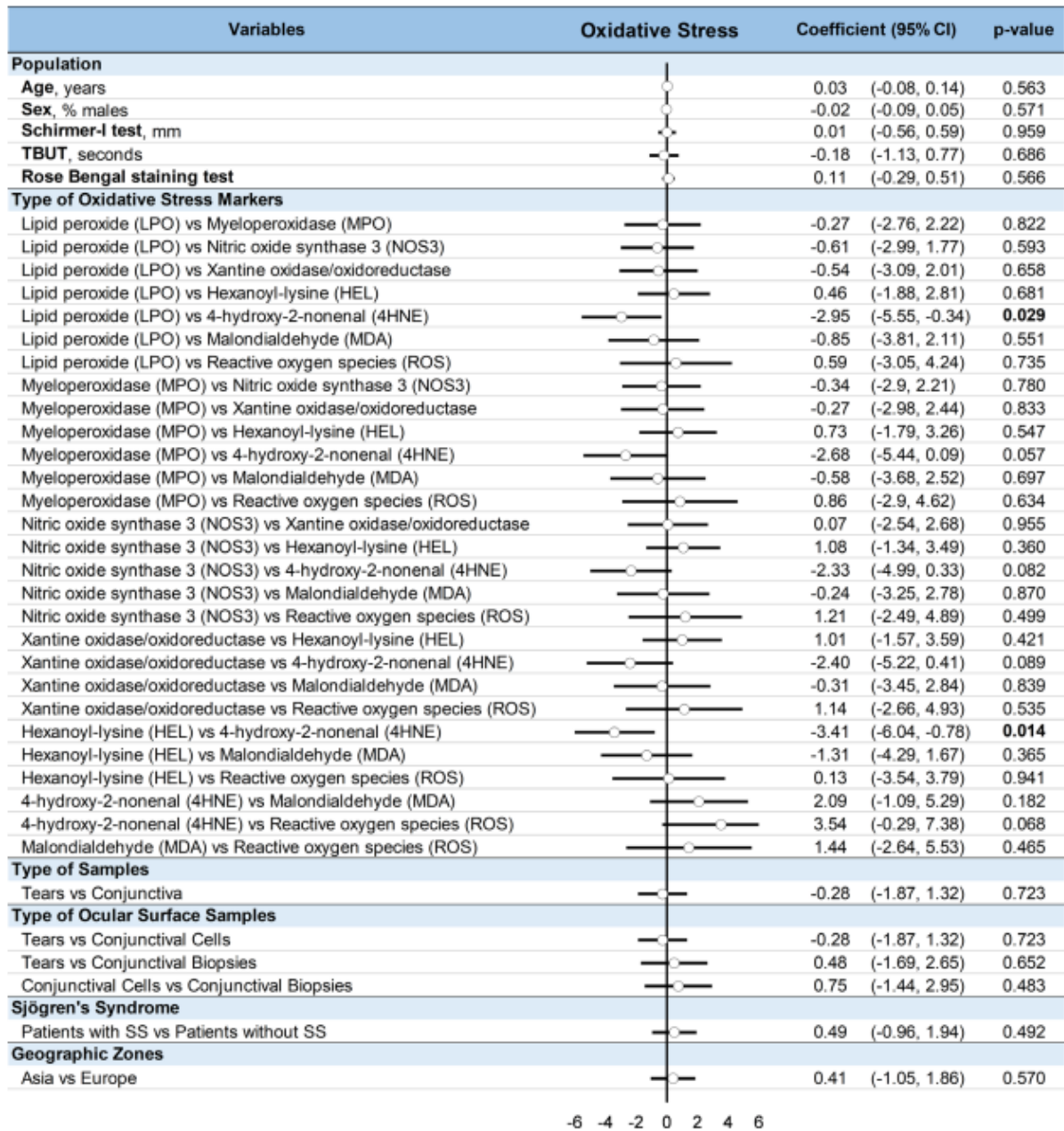


Fig 5. Meta-regressions on oxidative stress markers in dry eye disease (DED) comparing with healthy controls.

and may consider systemic, topical, or non-pharmacological innovative therapies.

**Dry eye disease: a disruption of ocular surface homeostasis**

Causing discomfort and visual disturbance, DED is a significant growing health problem around the world. Indeed, the prevalence of DED varied

from 5 to 50% according to the populations, and DED was diagnosed in more than 16 million people in the US (Stapleton et al. 2017). Defined as a multifactorial disease with tear film instability, DED is mediated by hyperosmolarity of the tear film and chronic inflammation of the ocular surface (Bron et al. 2017). Caused by desiccating stress (e.g. loss of tear film volume or excessive evaporation) and/or

alteration of the tear film quality (e.g. goblet cells and mucins, or meibomian glands and tear film lipid layer) (Argüeso & Gipson 2001; Arciniega et al. 2011), DED is particularly exposed to the deregulation of tear film composition. Indeed, hyperosmolarity in DED is underlined by dysfunction of the lacrimal functional unit (LFU) (Stern et al. 2004). Lacrimal functional unit (LFU) could regulate the

production of aqueous tears and blinking reflex, maintaining homeostasis of the tear film (Stern et al. 2004). Thus, a loss of regulatory loop of LFU or secretory tear reflex in DED induced low blink rate and high desiccating stress of ocular surface (Collins et al. 1989; Tsubota 1998). Considering cell apoptosis (i.e. epithelial cells of cornea, goblet cells and acini of secretory glands) and tissue damage in conditions of tear film hyperosmolarity (Pflugfelder & Stern 2020), it is established that a vicious circle of inflammation affected the ocular surface of DED, causing sustained symptoms, aggravation of clinical severity and neurogenic pain (Baudouin et al. 2013). In this way, dysregulated autophagy in DED is driven by cascade of inflammatory factors such as NF $\kappa$ B, TNF $\alpha$ , IL-1 $\beta$ , IL-6 and MMP-9 (Massingale et al. 2009), sustaining cellular and tissular dysfunctions (Martin et al. 2019). Cornea is majorly affected during the evolution of DED with several pathological attempts as sicca keratoconjunctivitis, superficial punctate keratitis, or filamentous keratitis (Pflugfelder & Stern 2020). However, integrity of ocular surface in diurnal mammals is crucial to eliminate oxidative stress markers accumulated during the daily environmental exposures (i.e. fossil fuel exhaust, tobacco smokes, mitochondrial metabolism, UV-light radiation) (Reiss et al. 1986; Tenkate 1999; Poljšak & Fink 2014). In healthy subjects, antioxidants are particularly abundant in tear film, aqueous humour and corneal tissues, avoiding a dysregulation of redox homeostasis (Reiss et al. 1986; Módis Jr., Marshall & Lee 1998). Being avascular, cornea is particularly depending on detoxifying systems contained in ocular surface and is predisposed to mitochondrial dysfunctions (Vallabh, Romano & Willoughby 2017). Causing mutations in DNA of mitochondria (mtDNA), oxidative stress though by environmental aggressions could alter repair mechanisms, increase the transcription rates of mtDNA, decrease the protective levels of histone proteins and finally generate a greatest amount of ROS in corneal tissues (Ballinger et al. 1999; Vallabh, Romano & Willoughby 2017). Furthermore, leisure or occupational environmental exposures are intimately linked with urban areas, oxidative stress and the prevalence of

DED: working at visual display terminal, using smartphone, physical inactivity, environmental pollutions mediated by fossil fuel and particulate matter, tobacco smokes, or confined spaces with air conditioning (Choi et al. 2018; Dogru et al. 2018; Hanyuda et al. 2020; Kim et al. 2020). Thus, we could postulate that oxidative stress may be a leading factor in the pathophysiology of DED.

#### **Dry eye disease or 'oxidative stress' disease?**

We highlighted that oxidative stress markers (and particularly LPO, \*\*\*\*MPO, NOS3, xanthine oxidase/oxidoreductase, 4HNE, MDA and ROS) were significantly increased in DED compared with healthy controls. In addition, we showed that two major components of the ocular surface were attempted by loss of redox homeostasis in DED: tear film and conjunctiva (cells and biopsies). The diagnosis of Sjögren's syndrome and geographical origins seemed not to affect the imbalance of redox condition in our included population, except for antioxidants in tears of DED patients without Sjögren's syndrome. Affecting exocrine glands, Sjögren's syndrome is known to induce autoimmune destruction of salivary and lacrimal functions, inducing severe aqueous-deficient DED and xerostomia (Vehof et al. 2020). However, evidence data showed that systemic auto-immune disease as Sjögren syndrome increase the levels of oxidative stress markers with severe repercussions on organs and organism (i.e. lipid metabolism, cardiovascular system, osteoarticular dysfunctions and exocrine functions) (Smallwood et al. 2018; Charras et al. 2019; Hu et al. 2020; Martínez-Rosales et al. 2020). Even if ageing decreases the antioxidative defences on ocular surface (Choy, Cho & Benzie 2011), we did not show any influence of age in oxidative stress homeostasis. Furthermore, the prevalence of DED increases in the elderly population with several ocular diseases (e.g. glaucoma or age-related macular degeneration), necessitating long-term multidose eye drops with antimicrobial preservative solutions (Baudouin et al. 2010; Stapleton et al. 2017). Using quaternary ammonium benzalkonium chloride (BAK), preservative eye drops induce cells toxicity and mitochondrial

alterations, involving a decrease in antioxidants as glutathione, and a ROS production in tissues of ocular surface (Baudouin et al. 2010). Use of BAK in ocular treatments could explain the incidence of iatrogenic dry eye in ocular chronic diseases or elderly people (Gomes et al. 2017). In animal models for age-related DED, using superoxide dismutase-1 (SOD1) knockout mice, Kojima et al. showed that loss of SOD1 could accelerate the peroxidation of lipids in ocular surface, increase DNA oxidative damage in nuclei and mitochondria and decrease goblet cells in conjunctiva (Kojima et al. 2012; Kojima et al. 2015). Similarly SOD1 knockout mice model showed a decrease of BUT associated with tear film instability, meibomian gland dysfunctions and elevation of oxidative stress markers (Ibrahim et al. 2014). In the light of these results, we could hypothesize that loss of oxidative stress homeostasis seems to be a key factor in the pathophysiology of DED, starting the vicious circle in addition of desiccating stress and tear film hyperosmolarity, or aggravating the inflammatory cascade in ocular surface tissues. Interestingly, all these news findings in pathophysiology of DED could revolutionize future systemic or topical therapies.

#### **From oxidative stress pathways to antioxidative treatments in DED?**

Dry eye disease (DED) is a chronic ocular condition, often requiring long-term treatments. However, oxidative stress was already highlighted as crucial part of some other chronic ocular diseases like keratoconus (Navel et al. 2020), glaucoma (Benoist d'Azy et al. 2016), pinguecula and pterygia (Kilic-Toprak et al. 2019), diabetic retinopathy or age-related macular degeneration (Tangvarasittichai & Tangvarasittichai 2019). Furthermore, older people seem to have a low rate of antioxidants in tears than younger subjects, predisposing this fragile population to have a severe form of DED without specified treatments (Choy, Cho & Benzie 2011). Thus, there is a need for innovative management in DED, exploring new therapeutic possibilities. In past decades, several studies described antioxidant effects of trehalose, a non-reducing disaccharide naturally absent in mammals or other vertebrates (Cejková et al.

2011; Cejková, Cejka & Luyckx 2012; Cejka et al. 2019; Laihia & Kaarniranta 2020). With similar functions as molecular chaperone, trehalose could promote the expression of nuclear factor erythroid 2-related factor 2 (NFE2L2), protecting mitochondria from oxidative damage (Cejková et al. 2011; Laihia & Kaarniranta 2020). In the same way, high levels of lactoferrin (physiologically produced by lacrimal gland) in tears seemed to be a protective factor against UV-light radiations and oxidative stress. Indeed, Dogru et al. treated dry eye patients with Sjögren's syndrome with 270 mg/day of oral enteric lactoferrin capsules for 1 month, increasing ocular surface test, central tear film lipid layer thickness, squamous metaplasia grades and goblet cell densities (Dogru et al. 2007). Kawashima et al. described similar results in mice with lactoferrin diet during 6 months, attenuating oxidative damage and inflammation in lacrimal gland (Kawashima et al. 2012). Recent *in vitro* and *in vivo* study showed similar efficacy of topical application of antioxidant (manganese porphyrin) than 0.5% ophthalmic cyclosporine emulsion, opening perspectives for future clinical trials (Ziniauskaitė et al. 2019). Others studies also highlighted an improvement of objective and subjective dry eye symptoms with anti-ageing and lifestyle-intervention approach, involving physical activity and diet therapy (Kawashima & Tsubota 2011; Kawashima et al. 2014; Kawashima et al. 2018). The basis of these non-pharmacological therapies in DED was similar to that applied in diabetes and metabolic syndrome to reduce oxidative stress (Bruemmer & Nissen 2020). Among many others, all these molecules and innovative treatments show that dry eye management is currently changing to include the correction of oxidative stress imbalance, as central process in pathophysiology of DED.

### Limitations

Our study has some limitations. Our meta-analysis inherited the limitations of the 9 individual studies of which it was composed and therefore was subjected to the bias of included studies. The meta-analyses were only conducted on published articles so there was the potential for publication bias (Rosenthal 1991). Unfortunately, some

studies were also excluded because of a lack of data (Galbis-Estrada et al. 2015; Ribelles et al. 2015; Nezzar et al. 2017; Soria et al. 2018; Ambaw et al. 2020). Despite similar diagnosis methods to recruit DED patients, the included studies described number of inclusion criteria with large heterogeneity concerning ocular surface testing and severity scales, which might have affected precision of the results. Only one study (Zheng et al. 2015) followed the criteria of the Dry Eye Work Shop (DEWS) (Craig et al. 2017) to diagnose DED patients. In addition, five studies did not report clinical or paraclinical severity scales of DED (Soria et al. 2013; Wakamatsu et al. 2013; Macri et al. 2015; Zheng et al. 2015; Choi et al. 2016), precluding stratified analysis by degree of DED severity. In addition, the few number of included studies, and particularly for antioxidants, limited the statistical strength of our results. However, this is the first meta-analysis to systematically quantify the strength of associations between DED, antioxidants and oxidative stress markers in ocular tissues and tear film. Despite our stratifications, we failed to explain the origin of heterogeneity between studies. Most studies had a case-control design, traditionally associated with a low-level of evidence – even if methodological quality of included studies were good (Burns, Rohrich & Chung 2011). There is also heterogeneity in reporting oxidative stress markers and antioxidants between studies, describing different enzymatic pathways and cellular interplay mechanisms. Furthermore, levels of biomarkers could vary among times of analysis, depending on levels of inflammation, duration of redox imbalance and characteristics of biomarkers. To our knowledge, no study describes kinetic changes of oxidative stress markers/ antioxidants in DED compared with healthy subjects. Considering that included studies used several methods of biomarkers quantification (i.e. large heterogeneity between analytical performance, technical features, cut-off and laboratory protocols), the methodological variability between studies could preclude robust conclusions. Generalizability of our results may also suffer from the absence of studies from North America, Africa, Oceania and South America, particularly for the antioxidants. For the

future, it would be interesting to investigate more prospective studies to explore the underlying mechanisms and putative therapeutics.

## Conclusion

We showed that oxidative stress markers, and probably antioxidants, were dysregulated in DED, involving an imbalance of redox homeostasis in tears, conjunctival cells and biopsies. Further studies are needed to better understand the complex relation between environmental exposure, tear film hyperosmolarity, redox homeostasis and inflammatory pathway to promote novel and mechanism-based therapies in DED.

### Data Availability Statement

No additional data are available.

## References

- Ambaw YA, Fuchs D, Raida M, Mazengia NT, Torta F, Wheelock CE, Wenk MR & Tong L (2020): Changes of tear lipid mediators after eyelid warming or thermopulsation treatment for meibomian gland dysfunction. *Prostaglandins Other Lipid Mediat* **151**: 106474.
- Arciniega JC, Wojtowicz JC, Mohamed EM & McCulley JP (2011): Changes in the evaporation rate of tear film after digital expression of meibomian glands in patients with and without dry eye. *Cornea* **30**: 843–847.
- Argüeso P & Gipson IK (2001): Epithelial mucins of the ocular surface: structure, biosynthesis and function. *Exp Eye Res* **73**: 281–289.
- Augustin AJ, Spitznas M, Kaviani N, Meller D, Koch FHJ, Grus F & Göbbels MJ (1995): Oxidative reactions in the tear fluid of patients suffering from dry eyes. *Graefes Arch Clin Exp Ophthalmol* **233**: 694–698.
- Ballinger SW, Van Houten B, Conklin CA, Jin G-F & Godley BF (1999): Hydrogen peroxide causes significant mitochondrial DNA damage in human RPE cells. *Exp Eye Res* **68**: 765–772.
- Baudouin C, Aragona P, Messmer EM et al. (2013): Role of hyperosmolarity in the pathogenesis and management of dry eye disease: Proceedings of the OCEAN Group Meeting. *Ocul Surf* **11**: 246–258.
- Baudouin C, Labbé A, Liang H, Pauly A & Brignole-Baudouin F (2010): Preservatives in eyedrops: the good, the bad and the ugly. *Prog Retin Eye Res* **29**: 312–334.
- Baudouin C, Rolando M, Benitez Del Castillo JM, Messmer EM, Figueiredo FC, Irkec M, Van Setten G & Labetoulle M (2019):

- Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Prog Retin Eye Res* **71**: 68–87.
- Benoist d'Azy C, Pereira B, Chiambaretta F & Duthheil F (2016a): Oxidative and anti-oxidative stress markers in chronic glaucoma: a systematic review and meta-analysis. *PLoS One* **11**: e0166915.
- Benoist d'Azy C, Pereira B, Chiambaretta F & Duthheil F (2019): Efficacy of different procedures of intra-corneal ring segment implantation in Keratoconus: a systematic review and meta-analysis. *Transl Vis Sci Technol* **8**: 38.
- Benoist d'Azy C, Pereira B, Naughton G, Chiambaretta F & Duthheil F (2016b): Antibioophthalmitis in prevention of endophthalmitis in intravitreal injection: a systematic review and meta-analysis. *PLoS One* **11**: e0156431.
- Bron AJ, de Paiva CS, Chauhan SK et al. (2017): TFOS DEWS II pathophysiology report. *Ocul Surf* **15**: 438–510.
- Bruemmer D & Nissen SE (2020): Prevention and management of cardiovascular disease in patients with diabetes: current challenges and opportunities. *Cardiovasc Endocrinol Metab* **9**: 81–89.
- Burns PB, Rohrich RJ & Chung KC (2011): The levels of evidence and their role in evidence-based medicine. *Plast Reconstr Surg* **128**: 305–310.
- Cejka C, Koss J, Hermankova B, Holan V, Kubinova S, Olmiere C & Cejkova J (2019): The healing of oxidative injuries with trehalose in UVB-irradiated rabbit corneas. *Oxid Med Cell Longev* **2019**: 1–10.
- Cejková J, Ardan T, Cejka C & Luyckx J (2011): Favorable effects of trehalose on the development of UVB-mediated antioxidant/pro-oxidant imbalance in the corneal epithelium, proinflammatory cytokine and matrix metalloproteinase induction, and heat shock protein 70 expression. *Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Klin Exp Ophthalmol* **249**: 1185–1194.
- Cejková J, Ardan T, Jirsová K et al. (2007a): The role of conjunctival epithelial cell xanthine oxidoreductase/xanthine oxidase in oxidative reactions on the ocular surface of dry eye patients with Sjögren's syndrome. *Histol Histopathol* **22**: 997–1003.
- Cejková J, Ardan T, Šimonová Z et al. (2007b): Nitric oxide synthase induction and cytotoxic nitrogen-related oxidant formation in conjunctival epithelium of dry eye (Sjögren's syndrome). *Nitric Oxide* **17**: 10–17.
- Cejková J, Ardan T, Šimonová Z, Cejka C, Malec J, Dotrelová D & Brunová B (2008): Decreased expression of antioxidant enzymes in the conjunctival epithelium of dry eye (Sjögren's syndrome) and its possible contribution to the development of ocular surface oxidative injuries. *Histol Histopathol* **23**: 1477–1483.
- Cejková J, Cejka C & Luyckx J (2012): Trehalose treatment accelerates the healing of UVB-irradiated corneas. Comparative immunohistochemical studies on corneal cryostat sections and corneal impression cytology. *Histol Histopathol* **27**: 1029–1040.
- Charras A, Arvaniti P, Le Dantec C, Dalekos GN, Zachou K, Bordron A & Renaudineau Y (2019): JAK inhibitors and oxidative stress control. *Front Immunol* **10**: 2814.
- Chen Y, Mehta G & Vasilou V (2009): Antioxidant defenses in the ocular surface. *Ocul Surf* **7**: 176–185.
- Choi JH, Li Y, Kim SH, Jin R, Kim YH, Choi W, You IC & Yoon KC (2018): The influences of smartphone use on the status of the tear film and ocular surface. *PLoS One* **13**: e0206541.
- Choi W, Lian C, Ying L, Kim GE, You IC, Park SH & Yoon KC (2016): Expression of lipid peroxidation markers in the tear film and ocular surface of patients with non-Sjogren syndrome: potential biomarkers for dry eye disease. *Curr Eye Res* **41**: 1143–1149.
- Choy CKM, Cho P & Benzie IFF (2011): Antioxidant content and ultraviolet absorption characteristics of human tears. *Optom Vis Sci Off Publ Am Acad Optom* **88**: 507–511.
- Collins M, Seeto R, Campbell L & Ross M (1989): Blinking and corneal sensitivity. *Acta Ophthalmol (Copenh)* **67**: 525–531.
- Courtin R, Pereira B, Naughton G, Chamoux A, Chiambaretta F, Lanhers C & Duthheil F (2016): Prevalence of dry eye disease in visual display terminal workers: a systematic review and meta-analysis. *BMJ Open* **6**: e009675.
- Craig JP, Nichols KK, Akpek EK et al. (2017): TFOS DEWS II definition and classification report. *Ocul Surf* **15**: 276–283.
- DerSimonian R & Laird N (1986): Meta-analysis in clinical trials. *Control Clin Trials* **7**: 177–188.
- Dogru M, Kojima T, Simsek C & Tsubota K (2018): Potential role of oxidative stress in ocular surface inflammation and dry eye disease. *Investig Ophthalmology Vis Sci* **59**: DES163.
- Dogru M, Matsumoto Y, Yamamoto Y, Goto E, Saiki M, Shimazaki J, Takebayashi T & Tsubota K (2007): Lactoferrin in Sjögren's Syndrome. *Ophthalmology* **114**: 2366–2367.e4.
- Galbis-Estrada C, Pinazo-Durán MD, Martínez-Castillo S, Morales JM, Monleón D & Zanon-Moreno V (2015): A metabolomic approach to dry eye disorders. The role of oral supplements with antioxidants and omega 3 fatty acids. *Mol Vis* **21**: 555–567.
- Gomes JAP, Azar DT, Baudouin C et al. (2017): TFOS DEWS II iatrogenic report. *Ocul Surf* **15**: 511–538.
- Hanyuda A, Sawada N, Uchino M et al. (2020): Physical inactivity, prolonged sedentary behaviors, and use of visual display terminals as potential risk factors for dry eye disease: JPHC-NEXT study. *Ocul Surf* **18**: 56–63.
- Harbour R & Miller J (2001): A new system for grading recommendations in evidence based guidelines. *BMJ* **323**: 334–336.
- Hu C, Zhang J, Hong S et al. (2020): Oxidative stress-induced aberrant lipid metabolism is an important causal factor for dysfunction of immunocytes from patients with systemic lupus erythematosus. *Free Radic Biol Med* **163**: 210–219.
- Ibrahim OMA, Dogru M, Matsumoto Y et al. (2014): Oxidative stress induced age dependent meibomian gland dysfunction in Cu, Zn-superoxide dismutase-1 (Sod1) knockout mice. *PLoS One* **9**: e99328.
- Ivanov IV, Mappes T, Schapp P, Lappe C & Wahl S (2018): Ultraviolet radiation oxidative stress affects eye health. *J Biophotonics* **11**: e201700377.
- Kawashima M, Kawakita T, Inaba T et al. (2012): Dietary lactoferrin alleviates age-related lacrimal gland dysfunction in mice. *PLoS One* **7**: e33148.
- Kawashima M, Sano K, Takechi S & Tsubota K (2018): Impact of lifestyle intervention on dry eye disease in office workers: a randomized controlled trial. *J Occup Health* **60**: 281–288.
- Kawashima M & Tsubota K (2011): Effect of calorie restriction on change in lacrimal gland with age. *Cornea* **30**(Suppl 1): S29–33.
- Kawashima M, Uchino M, Yokoi N et al. (2014): The association between dry eye disease and physical activity as well as sedentary behavior: results from the Osaka study. *J Ophthalmol* **2014**: 1–6.
- Kilic-Toprak E, Toprak I, Caliskan S, Ozdemir Y, Demirtas O, Altintas F & Kucukatay V (2019): Oxidative stress and genotoxicity in pterygium: a systemic investigation. *Eye Contact Lens Sci Clin Pract* **45**: 399–404.
- Kim Y, Choi Y-H, Kim MK, Paik HJ & Kim DH (2020): Different adverse effects of air pollutants on dry eye disease: Ozone, PM2.5, and PM10. *Environ Pollut* **265**: 115039.
- Kojima T, Dogru M, Ibrahim OMA et al. (2015): Effects of oxidative stress on the conjunctiva in Cu, Zn-superoxide dismutase-1 (Sod1)-knockout mice. *Investig Ophthalmology Vis Sci* **56**: 8382.
- Kojima T, Wakamatsu TH, Dogru M et al. (2012): Age-related dysfunction of the lacrimal gland and oxidative stress. *Am J Pathol* **180**: 1879–1896.
- Laihia J & Kaamiranta K (2020): Trehalose for ocular surface health. *Biomolecules* **10**: 809.
- Lanhers C, Pereira B, Naughton G, Troussellard M, Lesage F-X & Duthheil F (2015): Creatine supplementation and lower limb strength performance: a systematic review and meta-analyses. *Sports Med Auckl NZ* **45**: 1285–1294.
- Lanhers C, Pereira B, Naughton G, Troussellard M, Lesage F-X & Duthheil F (2017): Creatine supplementation and upper limb strength performance: a systematic review and meta-analysis. *Sports Med Auckl NZ* **47**: 163–173.
- Macri A, Scanarotti C, Bassi AM, Giuffrida S, Sangalli G, Traverso CE & Iester M (2015): Evaluation of oxidative stress levels in the

- conjunctival epithelium of patients with or without dry eye, and dry eye patients treated with preservative-free hyaluronic acid 0.15 % and vitamin B12 eye drops. *Graefes Arch Clin Exp Ophthalmol* **253**: 425–430.
- Martin LM, Jeyabalan N, Tripathi R, Panigrahi T, Johnson PJ, Ghosh A & Mohan RR (2019): Autophagy in corneal health and disease: A concise review. *Ocul Surf* **17**: 186–197.
- Martínez-Rosales E, Sola-Rodríguez S, Vargas-Hitos JA et al. (2020): Heart rate variability in women with systemic lupus erythematosus: association with health-related parameters and effects of aerobic exercise. *Int J Environ Res Public Health* **17**: 9501.
- Massingale ML, Li X, Vallabhajosyula M, Chen D, Wei Y & Asbell PA (2009): Analysis of inflammatory cytokines in the tears of dry eye patients. *Cornea* **28**: 1023–1027.
- Módis L Jr, Marshall GE & Lee WR (1998): Distribution of antioxidant enzymes in the normal aged human conjunctiva: an immunocytochemical study. *Graefes Arch Clin Exp Ophthalmol* **236**: 86–90.
- Moher D, Liberati A, Tetzlaff J & Altman DG & The PRISMA Group (2009): Preferred reporting items for systematic reviews and meta-analyses: The PRISMA Statement. *PLoS Med* **6**: e1000097.
- Navel V, Malecaze J, Pereira B, Baker JS, Malecaze F, Sapin V, Chiambaretta F & Duthel F (2020): Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis. *Acta Ophthalmol (Copenh)*. <https://doi.org/10.1111/aos.14714>
- Navel V, Mulliez A, Benoist d’Azy C, Baker JS, Malecaze J, Chiambaretta F & Duthel F (2019): Efficacy of treatments for *Demodex blepharitis*: A systematic review and meta-analysis. *Ocul Surf* **17**: 655–669.
- Nezzar H, Mbekeani JN, Noblanc A, Chiambaretta F, Drevet JR & Kocer A (2017): Investigation of antioxidant systems in human meibomian gland and conjunctival tissues. *Exp Eye Res* **165**: 99–104.
- Ollier M, Chamoux A, Naughton G, Pereira B & Duthel F (2014): Chest CT scan screening for lung cancer in asbestos occupational exposure: a systematic review and meta-analysis. *Chest* **145**: 1339–1346.
- Pflugfelder SC & de Paiva CS (2017): The pathophysiology of dry eye disease. *Ophthalmology* **124**: S4–S13.
- Pflugfelder SC & Stern ME (2020): The cornea in keratoconjunctivitis sicca. *Exp Eye Res* **201**: 108295.
- Poljšak B & Fink R (2014): The protective role of antioxidants in the defence against ROS/RNS-mediated environmental pollution. *Oxid Med Cell Longev* **2014**: 1–22.
- Reiss GR, Werness PG, Zollman PE & Brubaker RF (1986): Ascorbic acid levels in the aqueous humor of nocturnal and diurnal mammals. *Arch Ophthalmol* **104**: 753–755.
- Ribelles A, Galbis-Estrada C, Parras MA, Vivar-Llopis B, Marco-Ramírez C & Diaz-Llopis M (2015): Ocular surface and tear film changes in older women working with computers. *BioMed Res Int* **2015**: 467039.
- Rosenthal R (1991): Meta-analytic procedures for social research. Thousand Oaks, CA: SAGE Publications Inc.
- Shoham A, Hadziahmetovic M, Dunaief JL, Mydlarski MB & Schipper HM (2008): Oxidative stress in diseases of the human cornea. *Free Radic Biol Med* **45**: 1047–1055.
- Smallwood MJ, Nissim A, Knight AR, Whiteman M, Haigh R & Winyard PG (2018): Oxidative stress in autoimmune rheumatic diseases. *Free Radic Biol Med* **125**: 3–14.
- Soria J, Acera A, Durán JA, Boto-de-los-Bueis A, Del-Hierro-Zaruelo A, González N, Reigada R & Suárez T (2018): The analysis of human conjunctival epithelium proteome in ocular surface diseases using impression cytology and 2D-DIGE. *Exp Eye Res* **167**: 31–43.
- Soria J, Durán JA, Etxebarria J et al. (2013): Tear proteome and protein network analyses reveal a novel pentamer panel for tear film characterization in dry eye and meibomian gland dysfunction. *J Proteomics* **78**: 94–112.
- Stapleton F, Alves M, Bunya VY et al. (2017): TFOS DEWS II Epidemiology Report. *Ocul Surf* **15**: 334–365.
- Stern ME, Gao J, Siemasko KF, Beuerman RW & Pflugfelder SC (2004): The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res* **78**: 409–416.
- Tangvarasittichai O & Tangvarasittichai S (2019): Oxidative stress, ocular disease and diabetes retinopathy. *Curr Pharm Des* **24**: 4726–4741.
- Tenkate TD (1999): Occupational Exposure To Ultraviolet Radiation: A Health Risk Assessment. *Rev Environ Health* **14**.
- Tsubota K (1998): Tear dynamics and dry eye. *Prog Retin Eye Res* **17**: 565–596.
- Vallabh NA, Romano V & Willoughby CE (2017): Mitochondrial dysfunction and oxidative stress in corneal disease. *Mitochondrion* **36**: 103–113.
- Vehof J, Utheim TP, Bootsma H & Hammond CJ (2020): Advances, limitations and future perspectives in the diagnosis and management of dry eye in Sjögren’s syndrome. *Clin Exp Rheumatol* **38**(Suppl 126): 301–309.
- Wakamatsu TH, Dogru M, Matsumoto Y et al. (2013): Evaluation of lipid oxidative stress status in Sjögren syndrome patients. *Investig Ophthalmology Vis Sci* **54**: 201.
- Wells GA, Shea B, O’Connell D, Peterson J, Welch V, Losos M & Tugwell P (2014): The Newcastle-Ottawa Scale (NOS) for Assessing The Quality Of Nonrandomised Studies In Meta-Analyses. Ottawa: Ottawa Hospital Research Institute.
- Yan C, Zhang L, Lu B et al. (2020): Trans, trans-2,4-decadienal (tt-DDE), a composition of cooking oil fumes, induces oxidative stress and endoplasmic reticulum stress in human corneal epithelial cells. *Toxicol Vitro Int J Publ Assoc BIBRA* **68**: 104933.
- Zheng Q, Ren Y, Reinach PS, Xiao B, Lu H, Zhu Y, Qu J & Chen W (2015): Reactive oxygen species activated NLRP3 inflammasomes initiate inflammation in hyperosmolarity stressed human corneal epithelial cells and environment-induced dry eye patients. *Exp Eye Res* **134**: 133–140.
- Žiniauskaitė A, Ragauskas S, Ghosh AK et al. (2019): Manganese(III) tetrakis(1-methyl-4-pyridyl) porphyrin, a superoxide dismutase mimetic, reduces disease severity in vitro and in vivo models for dry-eye disease. *Ocul Surf* **17**: 257–264.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Details of keywords and search strategies used within each database.

**Appendix S2.** PRISMA checklist.

**Appendix S3.** Details on quality of articles (Figs 1 and 2), population (sample size, gender, age, Schirmer-I test, TBUT, rose Bengal staining test and others clinical explorations), DED diagnosis and severity scales (diagnostic methods and severity scales of DED), samples (type of samples, sampling methods and tissues conservation), oxidative stress markers/

antioxidants (methods of quantification), study designs, geographic zones, follow-up, aims and outcomes of included articles.

**Appendix S4.** Meta-analysis on oxidative stress markers in DED comparing with healthy controls, stratified by type of markers.

**Appendix S5.** Funnel plot (meta-funnel) based on data from oxidative stress markers and antioxidants in DED comparing with healthy controls, stratified by type of markers.

**Appendix S6.** Meta-analysis on oxidative stress markers in DED comparing with healthy controls, stratified by type of Samples.

**Appendix S7.** Funnel plot (meta-funnel) based on data from oxidative stress markers and antioxidants in DED comparing with healthy controls,

stratified by type of samples (tears or conjunctiva), by type of ocular surface samples (tears, conjunctival cells or conjunctival tissues), by Sjögren's syndrome (DED patients with or without Sjögren's syndrome) and by geographic zones (Asia or Europe).

**Appendix S8.** Meta-analysis on oxidative stress markers in DED comparing with healthy controls, stratified by type of ocular surface samples.

**Appendix S9.** Meta-analysis on oxidative stress markers in DED comparing with healthy controls, stratified by Sjögren's syndrome.

**Appendix S10.** Meta-analysis on oxidative stress markers in DED comparing with healthy controls, stratified by geographic zones.

**Appendix S11.** Meta-analysis on antioxidants in DED comparing with

healthy controls, stratified by type of markers.

**Appendix S12.** Meta-analysis on antioxidants in DED comparing with healthy controls, stratified by type of samples.

**Appendix S13.** Meta-analysis on antioxidants in DED comparing with healthy controls, stratified by type of ocular surface samples.

**Appendix S14.** Meta-analysis on antioxidants in DED comparing with healthy controls, stratified by Sjögren's syndrome.

**Appendix S15.** Meta-analysis on antioxidants in DED comparing with healthy controls, stratified by geographic zones.

**Appendix S16.** Metaregressions on antioxidants in DED comparing with healthy controls.

## **Travaux en cours**

### **Expérimentation sur modèle animal (souris C57 Black 6) :**

#### **Expression de RAGE dans le syndrome sec oculaire.**

#### **Objectif du projet (issue de la demande ministérielle d'autorisation APAFIS)**

L'oeil sec est une pathologie de la surface oculaire d'étiologie multifactorielle, très fréquente, touchant environ 50 à 60 % des personnes de plus de 50 ans. Ses retentissements oculaires peuvent aller de la simple gêne oculaire (prurit, brûlure oculaire superficielle, sensation de grain de sable) à une baisse de vision fluctuante, et dans de rares cas à des complications infectieuses cornéennes. Sa physiopathologie est complexe : anomalies quantitatives (anomalies des glandes lacrymales principales et accessoires) et qualitatives (contenu du film lacrymal, composition ionique et protéique, anomalies osmolaires) du film lacrymal. Des travaux récents ont démontré que des anomalies de l'innervation cornéenne, ainsi que la mise en place d'un cercle vicieux inflammatoire au niveau de la surface oculaire, pouvaient être impliqués dans la survenue et l'aggravation du syndrome sec oculaire. Il s'agit donc d'une pathologie multifactorielle dont on comprend peu à peu les mécanismes complexes et intriqués. Notre étude porte sur l'implication du récepteur RAGE (Receptor for Advanced Glycation Endproducts) qui est un récepteur transmembranaire de la famille des immunoglobulines, dont les ligands appartiennent à la famille des alarmines, impliquées dans des voies de signalisation pro-inflammatoires en réponse à des stimuli/ agressions cellulaires aseptiques. Très peu de travaux ont exploré cette voie de signalisation tant au niveau moléculaire, cellulaire, qu'au sein de modèles animaux dans l'oeil sec. L'utilisation d'un modèle animal, en parallèle de l'étude menée sur modèle cellulaire (cellules primaires et lignées cellulaires cornéennes et conjonctivales), permettra de compléter la compréhension de l'implication du récepteur RAGE dans la physiopathologie de l'oeil sec, et notamment les mécanismes inflammatoires impliqués dans sa survenue et chronicisation. Notre objectif par ce travail « in-vivo » consistera donc, chez des souris, à caractériser RAGE et ses voies cellulaires en provoquant la survenue d'un syndrome sec oculaire dans plusieurs tissus et fluides de l'oeil (cornée, conjonctive, cellules à mucus, larmes). Pour ce faire, nous utiliserons des souris KO RAGE -/- que nous comparerons à des souris "sauvages" RAGE +/+ soumises à des conditions environnementales connues pour être à risque de syndrome sec oculaire (attention et concentration visuelle soutenue, condition venteuse et hygrométrie de l'air faible). Afin



d'inscrire cette expérimentation animale dans le cadre de la Règle des 3R (Réduire, Raffiner, Remplacer), nous réduirons notre étude à 4 groupes distincts (pour tenir compte de la variabilité expérimentale): chaque groupe sera constitué de 10 souris pour un effectif total de 40 souris. Dans l'optique de raffiner au mieux notre protocole, nous veillerons au bien-être animal (pas de surpopulation dans les cages, éviter les actes douloureux inutiles, enrichissement des cages avec des roues de course, des tubes en plastique, boîtes en carton et des matériaux de nidification). Provoquer un syndrome sec oculaire peut être considéré comme un acte de classe légère puisque provoque une gêne / inconfort oculaire chez l'Homme. Il ne semble pas envisageable de remplacer l'animal dans ce type d'étude puisqu'un organisme vivant entier est nécessaire. En effet, notre hypothèse sur ce travail fait appel à une collaboration entre les différents types cellulaires de la conjonctive et de la cornée rendant impossible une modélisation par d'autres systèmes (comme la culture cellulaire même en utilisant des co-cultures) afin d'être le plus proche de la physiopathologie suspectée.

### **Descriptif du projet (issue de la demande ministérielle APAFIS)**

**- Matériel et méthodes** – Des souris KO RAGE -/- et des souris « sauvages » RAGE +/+ de la lignée C57 Black 6 (âgées de 4 à 6 semaines) seront soumises à des stimuli environnementaux connus pour être à risque de survenue de sécheresse oculaire (comme une attention soutenue sur une tâche nécessitant de la concentration, et/ou une condition venteuse avec hygrométrie faible). En parallèle, des souris témoins des 2 fonds génétiques seront utilisés comme contrôle de l'expérimentation. Dans un premier temps, l'ensemble des souris seront placées dans leur cage en condition standard pendant 8 jours : température 23 +/- 2°C, humidité 50 à 75%, alternance 12 heures lumière – obscurité (8h to 20h), et eau/alimentation ad libitum. Pour les groupes « œil sec », l'induction du syndrome se fera par l'injection sous-cutanée 4 fois par jour (8h00, 12h00, 14h00 et 17h00) de scopolamine (0.5 mg/0.2 ml ; Sigma-Aldrich) en alternant le flanc gauche et droit. Les souris seront de plus exposées à un courant d'air continu à partir d'un ventilateur placé à 15 cm devant la cage (courant d'air de 2-4 m/secondes) dans une pièce à environnement contrôlé (30-35 % humidité, 23°C, alternance jour/nuit 12h) et ce pendant 10 heures par jour durant 12 jours consécutifs. Le modèle de maladie de l'œil sec proposé dans ce projet a déjà largement été utilisé sans effet nocif perceptible lié au traitement à la scopolamine ni à une faible humidité [Luo, L. et al., 2004 ; Yeh, S. et al., 2003]. Pour les groupes témoins, les souris seront placées dans un environnement non stressant contenant 50 à 75% d'humidité

à la température de 23 +/- 2°C, sans exposition à l'air forcé et eau/alimentation ad libitum. Lors de l'expérimentation, des tests « non invasifs » seront réalisés afin d'évaluer au jour le jour l'avancée du syndrome. Tout d'abord, la perte de production de larmes sera testée en utilisant des fils de coton imprégnés de rouge de phénol qui seront placés dans le ménisque des larmes du canthus latéral pendant 30 secondes (1 fois par jour pendant la durée de l'expérimentation). Ce test est considéré comme équivalent au test de Schirmer mené chez les patients souffrant d'un syndrome de l'œil sec (bandelette buvard graduée permettant de quantifier la production lacrymale sur 5 minutes). En parallèle, l'étude de l'intégrité des épithéliums sera réalisée en utilisant l'instillation de fluorescéine (1%) et l'utilisation d'une lampe à fente (1 fois par jour pendant la durée de l'expérimentation), technique déjà utilisée dans l'équipe de recherche [Joubert et al., 2017]. Durant tout le protocole d'étude, l'absence de souffrance animale (en application de la règle Raffiner des 3R) sera contrôlée de manière très régulière (3 fois par jour en systématique, et plus fréquemment durant chaque intervention du manipulateur). De plus, toujours par soucis de raffinement, nous veillerons à enrichir les cages d'expérimentations avec des roues de course, des tubes en plastique, boîtes en carton et des matériaux de nidification. A la fin de l'expérimentation (12 jours), les souris seront sacrifiées sous anesthésie générale et ce de manière éthique, en accord avec le décret 2013-118, afin de prélever les yeux et annexes pour des études moléculaires. Ces prélèvements seront utilisés pour des analyses histologiques (profondeur des lésions cornéennes, analyse microscopique de la surface cornéenne), immunohistochimiques et/ou moléculaires (expression et localisation de RAGE et de ses principaux ligands (familles des alarmines)).

- **Résultats attendus** – Si l'on considère l'hypothèse d'un mécanisme d'activation de la voie inflammatoire aseptique médiée par RAGE dans le syndrome sec oculaire, les anomalies de la surface oculaire et les altérations cornéennes devraient être plus importantes, ou a minima différentes dans leur gravité et leur expression phénotypique :

- chez les souris appartenant aux groupes « œil sec » par rapport aux groupes contrôles.
- chez les souris « sauvages » par rapport aux souris KO RAGE -/-.

- **Points Limites** – La provocation expérimentale d'un œil sec n'est pas anticipée comme étant une condition risquant de provoquer une douleur majeure ou une angoisse animale. Cependant,

en cas de constatation d'un comportement douloureux (intolérance majeure à la lumière, animal prostré, ne s'alimentant plus) ou d'un ulcère cornéen profond (stromal profond de la cornée) et/ou à risque de perforation cornéenne, l'animal sera anesthésié et sacrifié par surdosage en produits anesthésiques (Pentobarbital 150 mg/kg en injection intrapéritonéale), aboutissant à une apnée conduisant au décès. Ce type de symptômes ou constatation clinique est exceptionnellement lié à un syndrome sec oculaire chez l'Homme. Ils sont le plus souvent dus à des comorbidités oculaires sous-jacentes (pathologies inflammatoires, troubles liés à l'innervation cornéenne, anomalies des cellules souches limbiques, pathologies infectieuses cornéennes).

### **Bénéfices attendus du projet (issue de la demande ministérielle APAFIS)**

Le récepteur des produits avancés de glycation (RAGE) est un récepteur ubiquitaire dans l'organisme, connu pour être pro-inflammatoire, pro-thrombogène, ou encore pro-apoptotique au niveau cellulaire. Notre objectif est de caractériser le récepteur RAGE, ses voies cellulaires, et l'expression phénotypique du syndrome sec oculaire dans plusieurs tissus et fluides de l'œil (cornée, conjonctive, cellules à mucus, larmes) ainsi qu'au niveau physiologique (développement de la maladie) chez des souris KO RAGE  $-/-$  et chez des souris "sauvages" soumises à des conditions environnementales connues pour être à risque de syndrome sec oculaire. Ainsi, nous essayons par ce projet de mieux connaître les voies d'activation cellulaire de RAGE dans la physiopathologie du syndrome sec oculaire. Un des bénéfices majeurs de ce travail pourrait être une meilleure compréhension des mécanismes physiopathologiques complexes du syndrome sec oculaire, notamment les voies d'activation de l'inflammation au niveau de la surface oculaire.

### **Nuisances ou effets indésirables attendus sur les animaux (issue de la demande ministérielle APAFIS)**

La provocation expérimentale d'un œil sec n'est pas anticipée comme étant une condition risquant de provoquer une douleur majeure ou une angoisse animale. Les signes cliniques et fonctionnels du syndrome sec oculaire sont minimes à modérés : une gêne oculaire à type de grains de sable, des sensations de brûlures oculaires superficielles, un prurit, une vision trouble fluctuante. Cependant, en cas de constatation d'un comportement douloureux (intolérance

majeure à la lumière, animal prostré, ne s'alimentant plus) ou d'un ulcère cornéen profond (stromal profond de la cornée) et/ou à risque de perforation cornéenne, l'animal sera anesthésié et sacrifié par surdosage en produits anesthésiques (Pentobarbital 150 mg/kg en injection intrapéritonéale), aboutissant à une apnée conduisant au décès. Ce type de symptômes ou constatation clinique est exceptionnellement lié à un syndrome sec oculaire chez l'Homme. Ils sont le plus souvent dus à des comorbidités oculaires sous-jacentes (pathologies inflammatoires, troubles liés à l'innervation cornéenne, anomalies des cellules souches limbiques, pathologies infectieuses cornéennes).

### **Stratégie d'expérimentation (issue de la demande ministérielle APAFIS)**

Ce protocole expérimental utilisant la souris nécessitera la constitution de 4 groupes distincts dont les effectifs doivent permettre un traitement statistique robuste. En tenant compte de la variabilité expérimentale concomitante à la conduite de tels projets chaque groupe sera constitué de 10 souris pour un effectif total de 40 :

- Groupe 1 : Souris Wild-Type \*Groupe contrôle (effectif :10)
- Groupe 2 : Souris RAGE -/- \*Groupe contrôle (effectif :10)
- Groupe 3 : Souris Wild-Type \*Groupe Œil-sec (effectif :10)
- Groupe 4 : Souris RAGE -/- \*Groupe Œil-sec (effectif :10)

Ces effectifs nous permettront d'obtenir des tests statistiques non paramétriques (Kruskal-Wallis et Mann-Whitney U test).

Cette étude sur modèle animal est en cours au moment de l'écriture de ce manuscrit et va consister à quantifier l'expression de RAGE et des alarmines (ARNm et protéines) au niveau de la surface oculaire des différentes souris. Il va aussi conduire à la réalisation d'expériences d'immunohistochimie permettant de caractériser et de localiser ces mêmes acteurs. Enfin, des colorations de coupes permettront de caractériser la présence de cellules inflammatoires au sein des tissus agressés et d'étudier la présence d'un infiltrat de ce type de cellules.



## 5. Conclusion & Perspectives

### 5.1 Déséquilibre du stress oxydatif dans le syndrome sec oculaire : un avenir antioxydants ?

En synthétisant de façon exhaustive la littérature, nous avons montré qu'il existait un déséquilibre de la balance oxydative dans la surface oculaire des patients DED.<sup>87</sup> Ce résultat est cohérent avec la physiopathologie du DED: instabilité et hyperosmolarité du film lacrymal, entraînant un cercle vicieux inflammatoire et des lésions tissulaires de la surface oculaire par apoptose cellulaire précoce.<sup>43</sup> Ainsi, le *primum movens* dans le DED est l'éviction des facteurs de risque modifiables (pollution atmosphérique, allergènes, conservateurs des collyres, hygrométrie basse, travail prolongé sur écran), associé à des suppléments par larmes artificielles et anti-inflammatoires topiques (en cures courtes).<sup>88,124</sup> Dans les formes sévères de DED, le plus souvent secondaire à des pathologies systémiques tel que le syndrome de Gougerot-Sjögren ou la réaction de greffon contre l'hôte oculaire,<sup>125,126</sup> il est commun de recourir aux inhibiteurs de la calcineurine en topique (i.e. ciclosporine, tacrolimus) et/ou aux collyres de sérum autologue.<sup>127</sup> De plus, il existe un faisceau d'arguments pour favoriser une alimentation de type méditerranéen dans le DED.<sup>128</sup> D'ailleurs, une étude récente montre qu'un régime riche en vitamine C, thiamine, acides gras polyinsaturés et calcium diminue le risque de développer un DED.<sup>129</sup> Le groupe des acides gras polyinsaturés essentiels de type omega-3 et omega-6 est particulièrement intéressant dans le DED puisqu'ayant des propriétés anti-inflammatoires et immunorégulatrices. Un régime alimentaire riche en huile de poissons gras (e.g. morue, harengs, sardines) et huile de lin semble être profitable pour diminuer l'inflammation locale du DED, sans argument significatif pour une supplémentation nutritionnelle systématique.<sup>130</sup> Des collyres à base d'acides gras polyinsaturés de type omega-3 sont en cours d'évaluation, avec des résultats intéressants sur les scores cliniques de DED.<sup>131</sup> Par ailleurs, sur modèle animal, une déficience en superoxyde dismutase (SOD) dans le DED entraînerait une sur-péroxydation lipidique au niveau de la surface oculaire, retentissant sur la trophicité des glandes de Meibomius et des cellules à mucus.<sup>132,133</sup> Ainsi, des collyres lubrifiants enrichis en facteurs antioxydants et/ou osmoprotecteurs, comme le tréhalose, font déjà partis de l'arsenal thérapeutique du DED. En maintenant l'intégrité des phospholipides membranaires, et en évitant la dénaturation protéique, ce type de molécules antioxydantes jouent un rôle barrière face aux agressions radicalaires de la surface oculaire.<sup>134,135</sup>

Connaissant les liens d'activation entre radicaux libres et RAGE, la question est de savoir quelle place pourrait avoir la voie RAGE dans la physiopathologie du DED. Cette problématique fait l'objet d'un travail de recherche sur modèle cellulaire dans le cadre d'un Doctorat au sein de notre équipe (données non publiées à ce jour). Pour étoffer cette réflexion, nous avons élaboré un protocole de recherche sur modèle animal de DED (décrit plus haut) afin de mieux comprendre les voies d'activation cellulaire de RAGE dans le DED. Cette étude sur modèle animal est en cours d'expérimentation au moment de l'écriture de ce manuscrit : certaines souris ont été sacrifiées avec prélèvement des cornées, les analyses en RT-qPCR et Western Blot vont pouvoir débiter.

## 5.2 L'hypothèse inflammatoire dans le kératocône

Le KC est défini historiquement comme une pathologie cornéenne ectasiente, non inflammatoire, et n'impliquant pas d'infiltration cellulaire ou de néovascularisation.<sup>48,49</sup> Cependant, des travaux récents mettent en évidence une surexpression de biomarqueurs de l'inflammation dans la surface oculaire de patients KC. C'est le cas pour IL-1, IL-6, TNF- $\alpha$ , et MMP-9.<sup>64,65</sup> Des travaux plus anciens avaient déjà identifié une surexpression d'enzymes matrix-métalloprotéinases (MMPs) dans les tissus cornéens de KC par rapport à des sujets sains.<sup>136</sup> Par ailleurs, les protéines S100 sont connues pour être des biomarqueurs de l'inflammation de la surface oculaire. Il s'agit également de ligands pour le récepteur RAGE. Elles sont impliquées dans les dommages cellulaires oxydatifs, et retrouvées élevées en cas de lésions cellulaires aseptiques. Elles participent ainsi aux voies d'activations pro-inflammatoires, via le relargage de cytokines et autres facteurs de croissance.<sup>116</sup> Une propriété intéressante dans le KC a ainsi été démontré : l'élévation de la protéine S100-A4 peut promouvoir la surexpression des MMPs. C'est également le cas avec l'IL-1 $\beta$  et TNF- $\alpha$ .<sup>137</sup> Or, c'est précisément ces enzymes de remodelage tissulaire qui sont en ligne de mire dans l'amincissement cornéen, et la diminution de la biomécanique cornéenne dans le KC.<sup>138</sup>

Il existe également un déséquilibre oxydatif dans la surface oculaire des patients KC.<sup>67</sup> On peut citer l'exemple de la lactoferrine (LTF), de concentration élevée dans les larmes (1 à 2mg/ml), qui joue un rôle antioxydant au niveau de la surface oculaire via son groupement de liaison Fe<sup>3+</sup>.<sup>139</sup> La LTF est retrouvée à des concentrations plus basses chez le KC par rapport à des sujets sains, notamment chez des KC en progression.<sup>140</sup> Plus globalement, nous avons montré que les protéines antioxydantes porteuses de groupement Fe<sup>3+</sup> (LTF, transferrine,

albumine) étaient significativement diminuée dans le KC.<sup>67</sup> Cette concentration de LTF basse dans le KC est associée à des niveaux plus élevés de biomarqueurs de l'inflammation (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , MMP-9).<sup>141</sup> Par ailleurs, des niveaux de kératines élevés ont été montrés dans les larmes de KC, mis en relation avec une apoptose cellulaire épithéliale précoce et l'amincissement des tissus cornéens. Des niveaux de kératines élevées pourraient être également le fait d'un traumatisme oculaire chronique, comme les frottements oculaires qui sont bien connus dans le KC.<sup>142</sup> De plus, des enzymes impliquées dans la régulation de l'apoptose cellulaire médiée par le rayonnement UV (PPIases, protéines aldéhyde oxydo-réactions (ALDHases)) semblent surexprimés dans le KC. De façon surprenante, nous avons montré qu'il existait un déséquilibre rédox systémique (i.e. prélèvements sanguins) dans le KC, impliquant une diminution de micronutriments (e.g. sélénium, cuivre) et d'enzymes antioxydantes (e.g. superoxyde dismutase, catalase).<sup>67</sup> Considérant qu'il ne s'agisse qu'une pathologie cornéenne, la question se pose d'une atteinte subclinique d'autres organes (foie, rein, cerveau, système cardiovasculaire) en lien avec ce déséquilibre rédox systémique. Ainsi, le déséquilibre global de la balance oxydative dans le KC, associé à une diminution des protéines transporteuses de fer (i.e. LTF, transferrine, albumine),<sup>143</sup> semblent provoquer un désordre inflammatoire chronique commun à de nombreuses pathologies dégénératives.<sup>144</sup>

Ces constatations sont particulièrement intéressantes dans les formes frustes de KC, uniquement détectable grâce aux technologies de topographie cornéenne d'élévation. L'étude biomoléculaire de ces formes subcliniques de KC sont sans doute la clé de la compréhension physiopathologique de la maladie. Ces formes frustes de KC sont également le siège d'une inflammation chronique via une élévation d'IL-6 et TNF- $\alpha$ , mais sans une élévation franche de MMP-9.<sup>65</sup> Il se pourrait donc que l'activation des MMPs dans le KC soit dépendante d'une cascade d'activation inflammatoire primitive, sans doute médiée/déclenchée par un déséquilibre du stress oxydant dans la surface oculaire. C'est à ce niveau que les facteurs de susceptibilités génétiques, interagissant avec l'exposome (radiations UV, pollutions atmosphériques, allergènes, frottements oculaires) jouent sans doute leur rôle de rupture d'homéostasie tissulaire. Considérant que la majorité des patients KC ont une importante asymétrie d'atteinte entre les deux yeux (très fréquemment un œil plus atteint que l'autre), le frottement préférentiel d'un œil pourrait concourir comme élément favorisant de cette cascade d'activation inflammatoire.<sup>57,145</sup>

Comme nous avons montré qu'il existait une dérégulation des isoformes de RAGE dans la surface oculaire du KC, on peut se poser la question des voies d'activation de RAGE dans



cette pathologie cornéenne. Il est intéressant d'ajouter que fl-RAGE est surexprimé dans les cellules épithéliales de KC, avec une diminution des formes solubles s-RAGE (leurres) dans les larmes.<sup>8</sup> Nous avons par ailleurs montré qu'il existait un déséquilibre de la balance oxydative dans le KC.<sup>67</sup> Sachant, encore une fois, que les médiateurs du stress oxydatif interagissent avec la voie d'activation RAGE, on peut supposer ici que les médiateurs pro-oxydants soient partie prenante de l'activation de RAGE dans le KC, et réciproquement. Ainsi, en concordance avec la littérature existante sur le sujet, notre travail sous-tend l'hypothèse d'un cercle vicieux pro-inflammatoire à l'œuvre dans le KC.

### 5.3 Perspectives thérapeutiques futures dans le kératocône

Comme explicité plus haut dans le paragraphe 2.2.3, le seul traitement actuellement validé pour réduire la progression du KC est le crosslinking cornéen (CXL). Il semblerait que le CXL aurait un effet anti-inflammatoire sur la surface oculaire du KC, ou en tout cas diminuerait les niveaux de biomarqueurs de l'inflammation (IL-6, TNF- $\alpha$ , CXCL8, MMPs).<sup>146,147</sup> Après un CXL, il y aurait un pic inflammatoire dans les larmes et les tissus cornéens entre les 4<sup>ème</sup> et 8<sup>ème</sup> jour post-opératoires, suivi d'une lente décroissance des biomarqueurs de l'inflammation jusqu'au 6<sup>ème</sup> mois<sup>146,147</sup>. Ces constatations sont cohérentes avec les mécanismes connus de cicatrisation cornéenne, et recrutement cellulaire inflammatoire à l'œuvre dans les jours suivants une intervention cornéenne. Par ailleurs, on peut rappeler que l'activation de la cicatrisation cornéenne semble être médiée par le couple AGEs – fl-RAGE, de manière dose dépendante, sans implication de HMGB1.<sup>119</sup> Ainsi, certains auteurs avancent que la stabilisation du KC dans les suites d'un CXL provient, au moins en partie, de cet effet anti-inflammatoire sur la surface oculaire.<sup>71</sup>

Par ailleurs, il existerait un lien entre des désordres métaboliques ou nutritionnels, en rapports avec une carence vitaminique ou en micronutriments, et une prévalence augmentée de KC.<sup>148</sup> Une déficience en vitamine A (Vit A) ou un taux de cuivre sérique bas serait retrouvé chez les patients KC.<sup>149,150</sup> Ainsi, en cas de carence en Vit A, une supplémentation vitaminique réduirait la concentration plasmatique de MMP-9, augmenterait celle de TIMP-1 (principal inhibiteur de MMP-9), et améliorerait la biodisponibilité du cuivre dans l'organisme (i.e. enzymes antioxydantes dépendantes du cuivre type LOX). De plus, les auteurs ont montré une stabilisation du KC chez 60% des patients après supplémentation en Vit A (critère de progression : Kmax < 1D sur 1 an).<sup>150</sup> Même s'il s'agit d'études non randomisées contrôlées,

ces données récentes sur l'intérêt d'une thérapeutique systémique dans le kératocône doit nous faire repenser notre conception du KC. Comment une pathologie cornéenne, le plus souvent isolée à l'œil sans autre atteinte d'organe, peut-elle se stabiliser par un traitement systémique ou une supplémentation vitaminique ? La question reste ouverte au moment de l'écriture de ce manuscrit, mais on pourrait imaginer une supplémentation antioxydante et/ou vitaminique dans le KC, à l'instar de la supplémentation dans la DMLA.<sup>151</sup>

Comme évoqué plus haut, les larmes et la surface oculaire des animaux diurnes (i.e. exposés au rayonnement UV) sont riches en facteurs antioxydants (vitamine C, LTF, enzymes type superoxyde dismutase (SOD), catalases, glutathionne peroxydases).<sup>152</sup> Ainsi, des modèles de cultures cellulaires ont évalué l'intérêt de lentilles souples hydrophiles (en silicone-hydrogels) imprégnées de molécules antioxydantes, comme la LTF.<sup>153</sup> Il semblerait que ces dispositifs pourraient diminuer le stress oxydant de la surface oculaire des patients KC, réduisant ainsi l'apoptose cellulaire épithéliale.<sup>154</sup> L'efficacité sur la stabilisation du KC de tels dispositifs reste à prouver en conditions réelles de port, mais on peut également imaginer des collyres enrichis en antioxydants. Par ailleurs, il n'existe pas de données de la littérature sur l'intérêt de collyres anti-inflammatoires dans le KC. Les corticostéroïdes topiques seraient à proscrire sur une longue durée du fait de leurs trop nombreux effets secondaires sur l'œil (hypertension intraoculaire, opacification capsulaire précoce),<sup>155</sup> mais on pourrait imaginer l'utilisation des inhibiteurs de la calcineurine en topique (i.e. ciclosporine, tacrolimus). De fait, ce sont de puissants anti-inflammatoires en formulations topiques, avec très peu d'effets secondaires oculaires et systémiques (exceptionnel risque de néoplasie de la surface oculaire).<sup>127</sup> Dans ce cadre, on pourrait également imaginer la pose d'anneaux intracornéens imprégnés de substances antioxydantes et/ou régulatrices de l'inflammation, en association avec le CXL et/ou les autres procédures chirurgicales du KC.<sup>156</sup>

Nous avons montré une dérégulation de l'expression de RAGE dans la surface oculaire du KC.<sup>8</sup> Or, il existe des molécules "bloqueurs" du récepteur transmembranaire de RAGE (fl-RAGE) ou encore les isoformes solubles de RAGE (s-RAGE) qui agissent comme des "leurre" pour inactiver les ligands de fl-RAGE (AGEs, HMGB1, protéines S100, calgranulines).<sup>1</sup> L'utilisation, sur modèles cellulaires ou animales, de protéines recombinantes s-RAGE ou de peptides antagonistes de RAGE (RAGE antagonist peptide, RAP) semble une piste thérapeutique prometteuse pour les pathologies liées à la voie d'activation de RAGE.<sup>157-162</sup> Ainsi, l'utilisation de recombinants s-RAGE ou RAP dans un modèle animal de détresse respiratoire aigüe semble améliorer la clairance du fluide alvéolaire, l'oxygénation, et atténuer

les lésions histologiques pulmonaires.<sup>161</sup> De nombreux autres travaux ont montré un bénéfice dans l'utilisation d'anticorps anti-RAGE dans des modèles de chocs septiques,<sup>163</sup> d'infections pulmonaires ou intrapéritonéales à *Streptococcus Pneumoniae* ou *Escherichia coli*,<sup>162,164</sup> ou encore l'insuffisance rénale chronique en lien avec la drépanocytose.<sup>157</sup> Il semblerait donc intéressant d'étudier ces molécules antagonistes de la voie RAGE dans des modèles cellulaires, puis animaux, de KC. Une perspective pertinente dans le traitement du KC serait donc d'imaginer des thérapeutiques bloquant la voie RAGE, sans doute associés à des antioxydants et/ou anti-inflammatoires topiques.

## 6. Annexes

### Participation à des congrès

Ce travail de thèse a été présenté sous forme de poster au congrès international de l'ARVO à Baltimore en 2020 (eCongrès pour cause de pandémie à COVID-19) :

“Dysregulation of Receptor for Advanced Glycation End Products (RAGE) in Keratoconus”

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Autres publications signées en tant que co-auteurs sans rapport direct avec le travail présenté.

1. Béal L, Navel V, Pereira B, et al. Efficacy of Thin and Ultrathin Descemet Stripping Automated Endothelial Keratoplasty and Influence of Graft Thickness on Postoperative Outcomes: Systematic Review and Meta-analysis. *Am J Ophthalmol* 2022;240:170–186.

2. Besson A, Tarpin A, Flaudias V, et al. Smoking Prevalence among Physicians: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2021;18:13328.

3. Bouillon-Minois J-B, Roux V, Pereira B, et al. Stress among Emergency Health Care Workers on Nuclear or Radiation Disaster: A Preliminary State Survey. *Int J Environ Res Public Health* 2021;18:8384.

4. Brusseau V, Tauveron I, Bagheri R, et al. Heart rate variability in hypothyroid patients: A systematic review and meta-analysis. *PloS One* 2022;17:e0269277.

5. Brusseau V, Tauveron I, Bagheri R, et al. Heart Rate Variability in Hyperthyroidism: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2022;19:3606.

6. Brusseau V, Tauveron I, Bagheri R, et al. Effect of Hyperthyroidism Treatments on Heart Rate Variability: A Systematic Review and Meta-Analysis. *Biomedicines* 2022;10:1982.

7. Buisson M, Navel V, Labbé A, et al. Deep learning versus ophthalmologists for screening for glaucoma on fundus examination: A systematic review and meta-analysis. *Clin Experiment Ophthalmol* 2021.
8. Dutheil F, Baker JS, Navel V. Firearms or SARS-Cov-2: what is the most lethal? *Public Health* 2020;183:44–45.
9. Dutheil F, Aubert C, Pereira B, et al. Suicide among physicians and health-care workers: A systematic review and meta-analysis. *PloS One* 2019;14:e0226361.
10. Dutheil F, Baker JS, Mermillod M, et al. Shift work, and particularly permanent night shifts, promote dyslipidaemia: A systematic review and meta-analysis. *Atherosclerosis* 2020;313:156–169.
11. Dutheil F, Baker JS, Navel V. To fight SARS-CoV-2: putting your guns down. *Can J Public Health Rev Can Sante Publique* 2020;111:411–412.
12. Dutheil F, Baker JS, Navel V. COVID-19 as a factor influencing air pollution? *Environ Pollut Barking Essex 1987* 2020;263:114466.
13. Dutheil F, Baker JS, Navel V. The SARS-Cov-2 Pandemic: A Good Time for Stem Cell Transplantation? *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant* 2020.
14. Dutheil F, Baker JS, Navel V. COVID-19 and cardiovascular risk: Flying toward a silent world? *J Clin Hypertens Greenwich Conn* 2020.
15. Dutheil F, Baker JS, Navel V. COVID-19 and air pollution: the worst is yet to come. *Environ Sci Pollut Res Int* 2020;27:44647–44649.
16. Dutheil F, Baker JS, Navel V. Air pollution in post-COVID-19 world: the final countdown of modern civilization? : Comment on: “COVID-19 and air pollution: the worst is yet to come.” *Environ Sci Pollut Res Int* 2021.
17. Dutheil F, Bessonnat B, Pereira B, et al. Napping and cognitive performance during night shifts: a systematic review and meta-analysis. *Sleep* 2020.
18. Dutheil F, Danini B, Bagheri R, et al. Effects of a Short Daytime Nap on the Cognitive Performance: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2021;18:10212.

19. Dutheil F, Esquirol Y, Navel V. Will the COVID-19 pandemic decrease the FatMax? *J Appl Physiol Bethesda Md* 1985 2020;129:1.
20. Dutheil F, Méchin G, Vorilhon P, et al. Breastfeeding after Returning to Work: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2021;18:8631.
21. Dutheil F, Mondillon L, Navel V. PTSD as the second tsunami of the SARS-Cov-2 pandemic. *Psychol Med* 2020:1–2.
22. Dutheil F, Nasir H, Navel V. SARS-CoV-2 Tackles the Tobacco Industry: Comment on “Tobacco Industry Interference Index: Implementation of the World Health Organization’s Framework Convention on Tobacco Control Article 5.3 in India.” *Asia Pac J Public Health* 2020:1010539520944700.
23. Dutheil F, Navel V, Clinchamps M. The Indirect Benefit on Respiratory Health From the World’s Effort to Reduce Transmission of SARS - CoV-2. *Chest* 2020.
24. Dutheil F, Oueslati T, Delamarre L, et al. Myopia and Near Work: A Systematic Review and Meta-Analysis. *Int J Environ Res Public Health* 2023;20:875.
25. Dutheil F, Pélangéon S, Duclos M, et al. Protective Effect on Mortality of Active Commuting to Work: A Systematic Review and Meta-analysis. *Sports Med Auckl NZ* 2020;50:2237–2250.
26. Dutheil F, Trousselard M, Navel V. SARS-CoV-2 as a protective factor for cardiovascular mortality? *Atherosclerosis* 2020.
27. Dutheil F, Zaragoza-Civale L, Pereira B, et al. Prostate Cancer and Asbestos: A Systematic Review and Meta-Analysis. *Perm J* 2020;24.
28. Dutheil F, Zaragoza-Civale L, Pereira B, et al. In Response. *Perm J* 2020;25:1.
29. Franceschino A, Dutheil F, Pereira B, et al. Descemetorhexis Without Endothelial Keratoplasty in Fuchs Endothelial Corneal Dystrophy: A Systematic Review and Meta-Analysis. *Cornea* 2022;41:815–825.
30. Lamat H, Sauvart-Rochat M-P, Tauveron I, et al. Metabolic syndrome and pesticides: A systematic review and meta-analysis. *Environ Pollut Barking Essex* 1987 2022;305:119288.

31. Magnier F, Dutheil F, Pereira B, et al. Preventive treatment of allograft rejection after endothelial keratoplasty: A systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2022;100:e1061–e1073.
32. Metzger M, Navel V, Barrière J-V, et al. Benefits of using corneal topography to choose subjective refraction technique in keratoconus (RE-CON): a prospective comparative crossover clinical study. *Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Klin Exp Ophthalmol* 2021.
33. Metzger M, Navel V, Bouvet M, et al. Complications of Adenoviral Keratoconjunctivitis in Ophthalmologists and Orthoptists: Epidemiology and Risk Factor, A Retrospective Questionnaire Analysis. *Cornea* 2022;41:339–346.
34. Millot C, Pereira B, Miallaret S, et al. Statistical Analysis of Absenteeism in a University Hospital Center between 2007 and 2019. *Int J Environ Res Public Health* 2022;19:12966.
35. Navel V, Baker JS, Dutheil F. COVID-19 and macular edema: a necessarily blindness? *Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Klin Exp Ophthalmol* 2020.
36. Navel V, Barriere J-V, Joubert R, et al. Irregular Astigmatism Management Using SPOT Scleral Lenses in the Treatment of Corneal Ectasia and Penetrating Keratoplasty. *Eye Contact Lens* 2021;47:401–407.
37. Navel V, Beze S, Dutheil F. COVID-19, sweat, tears... and myopia? *Clin Exp Optom* 2020.
38. Navel V, Chiambaretta F, Dutheil F. Response to Re: COVID-19, sweat, tears... and myopia? *Clin Exp Optom* 2020;103:718.
39. Navel V, Chiambaretta F, Dutheil F. Will environmental impacts of social distancing due to the pandemic caused by SARS-CoV-2 decrease allergic disease? *J Allergy Clin Immunol* 2020.
40. Navel V, Chiambaretta F, Dutheil F. Haemorrhagic conjunctivitis with pseudomembranous related to SARS-CoV-2. *Am J Ophthalmol Case Rep* 2020:100735.
41. Navel V, Chiambaretta F, Dutheil F. Reply. *J Allergy Clin Immunol* 2020.
42. Navel V, Chiambaretta F, Dutheil F. Coronavirus: good or bad news for ocular diseases? *BMJ Open Ophthalmol* 2020;5:e000495.
43. Navel V, Chiambaretta F, Dutheil F. Reply. *J Allergy Clin Immunol* 2020.

44. Navel V, Malecaze J, Belville C, et al. Dysregulation of Receptor for Advanced Glycation End Products (RAGE) Expression as a Biomarker of Keratoconus Mitra P, ed. *Dis Markers* 2022;2022:1–11.
45. Navel V, Malecaze J, Pereira B, et al. Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2020.
46. Navel V, Mulliez A, Benoist d’Azy C, et al. Efficacy of treatments for Demodex blepharitis: A systematic review and meta-analysis. *Ocul Surf* 2019;17:655–669.
47. Navel V, Sapin V, Henrioux F, et al. Oxidative and antioxidative stress markers in dry eye disease: A systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2021.
48. Picard M, Tauveron I, Magdasy S, et al. Effect of exercise training on heart rate variability in type 2 diabetes mellitus patients: A systematic review and meta-analysis. *PloS One* 2021;16:e0251863.
49. Roumeau I, Coutu A, Navel V, et al. Efficacy of medical treatments for vernal keratoconjunctivitis: A systematic review and meta-analysis. *J Allergy Clin Immunol* 2021:S0091-6749(21)00548–0.
50. Roumeau S, Dutheil F, Sapin V, et al. Efficacy of treatments for neurotrophic keratopathy: a systematic review and meta-analysis. *Graefes Arch Clin Exp Ophthalmol Albrecht Von Graefes Arch Klin Exp Ophthalmol* 2022;260:2623–2637.





## 7. Bibliographie

1. Bierhaus A, Humpert PM, Morcos M, et al. Understanding RAGE, the receptor for advanced glycation end products. *J Mol Med* 2005;83:876–886.
2. Hangai S, Kimura Y, Taniguchi T, Yanai H. Signal-transducing innate receptors in tumor immunity. *Cancer Sci* 2021;112:2578–2591.
3. Choltus H, Lavergne M, De Sousa Do Outeiro C, et al. Pathophysiological Implication of Pattern Recognition Receptors in Fetal Membranes Rupture: RAGE and NLRP Inflammasome. *Biomedicines* 2021;9:1123.
4. Sugaya K, Fukagawa T, Matsumoto K, et al. Three Genes in the Human MHC Class III Region near the Junction with the Class II: Gene for Receptor of Advanced Glycosylation End Products, PBX2 Homeobox Gene and a Notch Homolog, Human Counterpart of Mouse Mammary Tumor Gene int-3. *Genomics* 1994;23:408–419.
5. Fritz G. RAGE: a single receptor fits multiple ligands. *Trends Biochem Sci* 2011;36:625–632.
6. Manigrasso MB, Pan J, Rai V, et al. Small Molecule Inhibition of Ligand-Stimulated RAGE-DIAPH1 Signal Transduction. *Sci Rep* 2016;6:22450.
7. Hudson BI, Lippman ME. Targeting RAGE Signaling in Inflammatory Disease. *Annu Rev Med* 2018;69:349–364.
8. Navel V, Malecaze J, Belville C, et al. Dysregulation of Receptor for Advanced Glycation End Products (RAGE) Expression as a Biomarker of Keratoconus Mitra P, ed. *Dis Markers* 2022;2022:1–11.
9. Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced Glycation End Products: Sparking the Development of Diabetic Vascular Injury. *Circulation* 2006;114:597–605.
10. Cerami C, Founds H, Nicholl I, et al. Tobacco smoke is a source of toxic reactive glycation products. *Proc Natl Acad Sci* 1997;94:13915–13920.
11. Ashraf JMohd, Ahmad S, Choi I, et al. Recent advances in detection of AGEs: Immunochemical, bioanalytical and biochemical approaches: Technological Progress in Age Detection. *IUBMB Life* 2015;67:897–913.
12. Dariya B, Nagaraju GP. Advanced glycation end products in diabetes, cancer and phytochemical therapy. *Drug Discov Today* 2020;25:1614–1623.
13. Nowotny K, Jung T, Höhn A, et al. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. *Biomolecules* 2015;5:194–222.
14. Takeuchi M, Kikuchi S, Sasaki N, et al. Involvement of Advanced Glycation End-products (AGEs) in Alzheimers Disease. *Curr Alzheimer Res* 2004;1:39–46.
15. Goodwin GH, Sanders C, Johns EW. A New Group of Chromatin-Associated Proteins with a High Content of Acidic and Basic Amino Acids. *Eur J Biochem* 1973;38:14–19.

16. Erlandsson Harris H, Andersson U. Mini-review: The nuclear protein HMGB1 as a proinflammatory mediator. *Eur J Immunol* 2004;34:1503–1512.
17. Scaffidi P, Misteli T, Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191–195.
18. Kwak MS, Kim HS, Lee B, et al. Immunological Significance of HMGB1 Post-Translational Modification and Redox Biology. *Front Immunol* 2020;11:1189.
19. Gonzalez LL, Garrie K, Turner MD. Role of S100 proteins in health and disease. *Biochim Biophys Acta BBA - Mol Cell Res* 2020;1867:118677.
20. Donato R, Cannon B, Sorci G, et al. Functions of S100 Proteins. *Curr Mol Med* 2013;13:24–57.
21. Ostendorp T, Leclerc E, Galichet A, et al. Structural and functional insights into RAGE activation by multimeric S100B. *EMBO J* 2007;26:3868–3878.
22. Aoki J. Two pathways for lysophosphatidic acid production. *Biochim Biophys Acta BBA - Mol Cell Biol Lipids* 2008;1781:513–518.
23. Cummings JL. Alzheimer's Disease Wood AJJ, ed. *N Engl J Med* 2004;351:56–67.
24. Murphy JE, Tacon D, Tedbury PR, et al. LOX-1 scavenger receptor mediates calcium-dependent recognition of phosphatidylserine and apoptotic cells. *Biochem J* 2006;393:107–115.
25. Teh BK, Yeo JG, Chern LM, Lu J. C1q regulation of dendritic cell development from monocytes with distinct cytokine production and T cell stimulation. *Mol Immunol* 2011;48:1128–1138.
26. Dong H, Zhang Y, Huang Y, Deng H. Pathophysiology of RAGE in inflammatory diseases. *Front Immunol* 2022;13:931473.
27. Jangde N, Ray R, Rai V. RAGE and its ligands: from pathogenesis to therapeutics. *Crit Rev Biochem Mol Biol* 2020;55:555–575.
28. Rai V, Maldonado AY, Burz DS, et al. Signal Transduction in Receptor for Advanced Glycation End Products (RAGE). *J Biol Chem* 2012;287:5133–5144.
29. Delrue C, Speeckaert R, Delanghe JR, Speeckaert MM. The Potential Influence of Advanced Glycation End Products and (s)RAGE in Rheumatic Diseases. *Int J Mol Sci* 2023;24:2894.
30. Rao NL, Kotian GB, Shetty JK, et al. Receptor for Advanced Glycation End Product, Organ Crosstalk, and Pathomechanism Targets for Comprehensive Molecular Therapeutics in Diabetic Ischemic Stroke. *Biomolecules* 2022;12:1712.
31. Ma W-Q, Qu Q-R, Zhao Y, Liu N-F. Association of RAGE gene Gly82Ser polymorphism with coronary artery disease and ischemic stroke: A systematic review and meta-analysis. *Medicine (Baltimore)* 2016;95:e5593.
32. Ostrand-Rosenberg S, Huecksteadt T, Sanders K. The Receptor for Advanced Glycation

Endproducts (RAGE) and Its Ligands S100A8/A9 and High Mobility Group Box Protein 1 (HMGB1) Are Key Regulators of Myeloid-Derived Suppressor Cells. *Cancers* 2023;15:1026.

33. Faiz A, van den Berge M, Vermeulen CJ, et al. AGER expression and alternative splicing in bronchial biopsies of smokers and never smokers. *Respir Res* 2019;20:70.

34. Pisella P-J, Baudouin C, Hoang-Xuan T. Surface oculaire: rapport 2015 [présenté à la] Société française d'ophtalmologie. Issy-les-Moulineaux: Elsevier Masson; 2015.

35. Rohen JW, Steuhl P. Specialized cell types and their regional distribution in the conjunctival epithelium of the cynomolgus monkey. *Graefes Arch Clin Exp Ophthalmol* 1982;218:59–63.

36. Tsubota K, Yokoi N, Watanabe H, et al. A New Perspective on Dry Eye Classification: Proposal by the Asia Dry Eye Society. *Eye Contact Lens Sci Clin Pract* 2020;46:S2–S13.

37. Dua HS, Faraj LA, Said DG, et al. Human Corneal Anatomy Redefined. *Ophthalmology* 2013;120:1778–1785.

38. Parker JS, Dockery PW, Melles GRJ. Bowman Layer Transplantation—A Review. *Asia-Pac J Ophthalmol* 2020;9:565–570.

39. Cotsarelis G, Cheng S-Z, Dong G, et al. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: Implications on epithelial stem cells. *Cell* 1989;57:201–209.

40. Majo F, Rochat A, Nicolas M, et al. Oligopotent stem cells are distributed throughout the mammalian ocular surface. *Nature* 2008;456:250–254.

41. Dua HS, Gomes JAP, Jindal VK, et al. Mucosa specific lymphocytes in the human conjunctiva, corneoscleral limbus and lacrimal gland. *Curr Eye Res* 1994;13:87–93.

42. Craig JP, Nichols KK, Akpek EK, et al. TFOS DEWS II Definition and Classification Report. *Ocul Surf* 2017;15:276–283.

43. Bron AJ, de Paiva CS, Chauhan SK, et al. TFOS DEWS II pathophysiology report. *Ocul Surf* 2017;15:438–510.

44. Pflugfelder SC, de Paiva CS. The Pathophysiology of Dry Eye Disease. *Ophthalmology* 2017;124:S4–S13.

45. Argüeso P, Gipson IK. Epithelial Mucins of the Ocular Surface: Structure, Biosynthesis and Function. *Exp Eye Res* 2001;73:281–289.

46. Baudouin C, Rolando M, Benitez Del Castillo JM, et al. Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Prog Retin Eye Res* 2019;71:68–87.

47. Ducasse A, Ruban J-M, Baggio E, Labrousse M. Paupières et sourcils: anatomie chirurgicale. *EMC - Ophtalmol* 2009;6:1–15.

48. Krachmer JH, Feder RS, Belin MW. Keratoconus and related noninflammatory corneal thinning disorders. *Surv Ophthalmol* 1984;28:293–322.

49. Rabinowitz YS. Keratoconus. *Surv Ophthalmol* 1998;42:297–319.
50. Hashemi H, Heydarian S, Hooshmand E, et al. The Prevalence and Risk Factors for Keratoconus: A Systematic Review and Meta-Analysis. *Cornea* 2020;39:263–270.
51. Ferrari G, Rama P. The keratoconus enigma: A review with emphasis on pathogenesis. *Ocul Surf* 2020;18:363–373.
52. Godefrooij DA, de Wit GA, Uiterwaal CS, et al. Age-specific Incidence and Prevalence of Keratoconus: A Nationwide Registration Study. *Am J Ophthalmol* 2017;175:169–172.
53. Ferdi AC, Nguyen V, Gore DM, et al. Keratoconus Natural Progression. *Ophthalmology* 2019;126:935–945.
54. Esporcatte LPG, Salomão MQ, Neto AB da C, et al. Enhanced Diagnostics for Corneal Ectatic Diseases: The Whats, the Whys, and the Hows. *Diagnostics* 2022;12:3027.
55. Weed KH, MacEwen CJ, McGhee CNJ. The variable expression of keratoconus within monozygotic twins: Dundee University Scottish Keratoconus Study (DUSKS). *Contact Lens Anterior Eye* 2006;29:123–126.
56. Crawford AZ, Zhang J, Gokul A, et al. The Enigma of Environmental Factors in Keratoconus. *Asia-Pac J Ophthalmol* 2020;9:549–556.
57. Torres-Netto EA, Abdshahzadeh H, Abrishamchi R, et al. The Impact of Repetitive and Prolonged Eye Rubbing on Corneal Biomechanics. *J Refract Surg* 2022;38:610–616.
58. Sun E, Kraus CL. The Ophthalmic Manifestations of Down Syndrome. *Children* 2023;10:341.
59. Song M, Fang QY, Seth I, et al. Non-genetic risk factors for keratoconus. *Clin Exp Optom* 2022;1–11.
60. Tsang SH, Sharma T. Leber Congenital Amaurosis. *Adv Exp Med Biol* 2018;1085:131–137.
61. Khawaja AP, Rojas Lopez KE, Hardcastle AJ, et al. Genetic Variants Associated With Corneal Biomechanical Properties and Potentially Conferring Susceptibility to Keratoconus in a Genome-Wide Association Study. *JAMA Ophthalmol* 2019;137:1005.
62. Akhtar S, Bron AJ, Salvi SM, et al. Ultrastructural analysis of collagen fibrils and proteoglycans in keratoconus. *Acta Ophthalmol (Copenh)* 2008;86:764–772.
63. Sykakis E, Carley F, Irion L, et al. An in depth analysis of histopathological characteristics found in keratoconus. *Pathology (Phila)* 2012;44:234–239.
64. Lema I, Duran J. Inflammatory Molecules in the Tears of Patients with Keratoconus. *Ophthalmology* 2005;112:654–659.
65. Lema I, Sobrino T, Duran JA, et al. Subclinical keratoconus and inflammatory molecules from tears. *Br J Ophthalmol* 2009;93:820–824.

66. Lemp MA, Blackman HJ. Ocular surface defense mechanisms. *Ann Ophthalmol* 1981;13:61–63.
67. Navel V, Malecaze J, Pereira B, et al. Oxidative and antioxidative stress markers in keratoconus: a systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2020.
68. Shetty R, Sharma A, Pahuja N, et al. Oxidative stress induces dysregulated autophagy in corneal epithelium of keratoconus patients Trajkovic V, ed. *PLOS ONE* 2017;12:e0184628.
69. McAnena L, Doyle F, O’Keefe M. Cross-linking in children with keratoconus: a systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2017;95:229–239.
70. Gordon MO, Steger-May K, Szczotka-Flynn L, et al. Baseline factors predictive of incident penetrating keratoplasty in keratoconus. *Am J Ophthalmol* 2006;142:923–930.
71. Sykakis E, Karim R, Evans JR, et al. Corneal collagen cross-linking for treating keratoconus Cochrane Eyes and Vision Group, ed. *Cochrane Database Syst Rev* 2015. Available at: <https://doi.wiley.com/10.1002/14651858.CD010621.pub2> [Accessed March 9, 2023].
72. Kobashi H, Rong SS. Corneal Collagen Cross-Linking for Keratoconus: Systematic Review. *BioMed Res Int* 2017;2017:8145651.
73. Song M, Chen T, Moktar A, et al. Diagnosis and Management of Keratoconus—A Narrative Review of Clinicians’ Perspectives. *Children* 2022;9:1973.
74. Baudouin C, Aragona P, Messmer EM, et al. Role of Hyperosmolarity in the Pathogenesis and Management of Dry Eye Disease: Proceedings of the OCEAN Group Meeting. *Ocul Surf* 2013;11:246–258.
75. Stapleton F, Alves M, Bunya VY, et al. TFOS DEWS II Epidemiology Report. *Ocul Surf* 2017;15:334–365.
76. Wu D, Lim BXH, Seah I, et al. Impact of Microplastics on the Ocular Surface. *Int J Mol Sci* 2023;24:3928.
77. Schraufnagel DE, Balmes JR, Cowl CT, et al. Air Pollution and Noncommunicable Diseases. *Chest* 2019;155:417–426.
78. Tsubota K, Nakamori K. Dry Eyes and Video Display Terminals. *N Engl J Med* 1993;328:584–584.
79. Baudouin C, Labbé A, Liang H, et al. Preservatives in eyedrops: the good, the bad and the ugly. *Prog Retin Eye Res* 2010;29:312–334.
80. Stern ME, Beuerman RW, Fox RI, et al. The Pathology of Dry Eye: The Interaction Between the Ocular Surface and Lacrimal Glands. *Cornea* 1998;17:584.
81. Starr CE, Gupta PK, Farid M, et al. An algorithm for the preoperative diagnosis and treatment of ocular surface disorders. *J Cataract Refract Surg* 2019;45:669–684.
82. Wu KY, Kulbay M, Tanasescu C, et al. An Overview of the Dry Eye Disease in Sjögren’s Syndrome Using Our Current Molecular Understanding. *Int J Mol Sci* 2023;24:1580.

83. Sheppard J, Shen Lee B, Periman LM. Dry eye disease: identification and therapeutic strategies for primary care clinicians and clinical specialists. *Ann Med* 2023;55:241–252.
84. Stern ME, Gao J, Siemasko KF, et al. The role of the lacrimal functional unit in the pathophysiology of dry eye. *Exp Eye Res* 2004;78:409–416.
85. Baudouin C. Un nouveau schéma pour mieux comprendre les maladies de la surface oculaire. *J Fr Ophtalmol* 2007;30:239–246.
86. Dmitrieva NI, Bulavin DV, Burg MB. High NaCl causes Mre11 to leave the nucleus, disrupting DNA damage signaling and repair. *Am J Physiol-Ren Physiol* 2003;285:F266–F274.
87. Navel V, Sapin V, Henrioux F, et al. Oxidative and antioxidative stress markers in dry eye disease: A systematic review and meta-analysis. *Acta Ophthalmol (Copenh)* 2021.
88. Jones L, Downie LE, Korb D, et al. TFOS DEWS II Management and Therapy Report. *Ocul Surf* 2017;15:575–628.
89. Navel V, Mulliez A, Benoist d’Azy C, et al. Efficacy of treatments for Demodex blepharitis: A systematic review and meta-analysis. *Ocul Surf* 2019;17:655–669.
90. Kamøy B, Magno M, Nøland ST, et al. Video display terminal use and dry eye: preventive measures and future perspectives. *Acta Ophthalmol (Copenh)* 2022;100:723–739.
91. Kallab M, Szegedi S, Hommer N, et al. Topical Low Dose Preservative-Free Hydrocortisone Reduces Signs and Symptoms in Patients with Chronic Dry Eye: A Randomized Clinical Trial. *Adv Ther* 2020;37:329–341.
92. Hessen M, Akpek EK. Ocular graft-versus-host disease. *Curr Opin Allergy Clin Immunol* 2012;12:540–547.
93. Cui D, Li G, Akpek EK. Autologous serum eye drops for ocular surface disorders. *Curr Opin Allergy Clin Immunol* 2021;21:493–499.
94. Vernhardsdottir RR, Magno MS, Hynnekleiv L, et al. Antibiotic treatment for dry eye disease related to meibomian gland dysfunction and blepharitis – A review. *Ocul Surf* 2022;26:211–221.
95. Ervin A-M, Law A, Pucker AD. Punctal occlusion for dry eye syndrome. *Cochrane Database Syst Rev* 2017;6:CD006775.
96. Zhang J, Han X, Chang J, et al. Soluble RAGE attenuates myocardial I/R injuries via FoxO3–Bnip3 pathway. *Cell Mol Life Sci* 2022;79:269.
97. Mo Y, Chen K. Review: The role of HMGB1 in spinal cord injury. *Front Immunol* 2023;13:1094925.
98. Kempen CG, Deragon MA, Hodges AL, et al. Necroptosis of Lung Epithelial Cells Triggered by Ricin Toxin and Bystander Inflammation. *Cell Physiol Biochem Int J Exp Cell Physiol Biochem Pharmacol* 2023;57:1–14.
99. Molinari P, Caldiroli L, Dozio E, et al. Association of Autofluorescent Advanced Glycation

End Products (AGEs) with Frailty Components in Chronic Kidney Disease (CKD): Data from a Single-Center Cohort Study. *Cells* 2023;12:438.

100. Wu X, Shi X, Chen X, Yin Z. Advanced glycation end products regulate the receptor of AGEs epigenetically. *Front Cell Dev Biol* 2023;11:1062229.

101. Oshitari T. The Pathogenesis and Therapeutic Approaches of Diabetic Neuropathy in the Retina. *Int J Mol Sci* 2021;22:9050.

102. Antonetti DA, Klein R, Gardner TW. Diabetic Retinopathy. *N Engl J Med* 2012;366:1227–1239.

103. Oshitari T. Advanced Glycation End-Products and Diabetic Neuropathy of the Retina. *Int J Mol Sci* 2023;24:2927.

104. Liutkeviciene R, Vilkeviciute A, Kriauciuniene L, et al. Association of genetic variants at CETP, AGER, and CYP4F2 locus with the risk of atrophic age-related macular degeneration. *Mol Genet Genomic Med* 2020;8. Available at: <https://onlinelibrary.wiley.com/doi/10.1002/mgg3.1357> [Accessed March 11, 2023].

105. Tian J, Ishibashi K, Ishibashi K, et al. Advanced glycation endproduct-induced aging of the retinal pigment epithelium and choroid: A comprehensive transcriptional response. *Proc Natl Acad Sci* 2005;102:11846–11851.

106. Nagaraj RH, Linetsky M, Stitt AW. The pathogenic role of Maillard reaction in the aging eye. *Amino Acids* 2012;42:1205–1220.

107. Raghavan CT, Nagaraj RH. AGE-RAGE interaction in the TGF $\beta$ 2-mediated epithelial to mesenchymal transition of human lens epithelial cells. *Glycoconj J* 2016;33:631–643.

108. Raghavan CT, Smuda M, Smith AJO, et al. AGEs in human lens capsule promote the TGF $\beta$ 2-mediated EMT of lens epithelial cells: implications for age-associated fibrosis. *Aging Cell* 2016;15:465–476.

109. Kwon YH, Fingert JH, Kuehn MH, Alward WLM. Primary open-angle glaucoma. *N Engl J Med* 2009;360:1113–1124.

110. Moschos MM, Chatziralli I, Sioziou A, Gazouli M. Receptor of Advanced Glycation End Products Gene Polymorphism and Primary Open-Angle Glaucoma. *Ophthalmic Res* 2017;58:81–84.

111. Tezel G, Luo C, Yang X. Accelerated aging in glaucoma: immunohistochemical assessment of advanced glycation end products in the human retina and optic nerve head. *Invest Ophthalmol Vis Sci* 2007;48:1201–1211.

112. Benoist d’Azy C, Pereira B, Chiambaretta F, Dutheil F. Oxidative and Anti-Oxidative Stress Markers in Chronic Glaucoma: A Systematic Review and Meta-Analysis. *PloS One* 2016;11:e0166915.

113. Inman DM, Lambert WS, Calkins DJ, Horner PJ.  $\alpha$ -Lipoic acid antioxidant treatment limits glaucoma-related retinal ganglion cell death and dysfunction. *PloS One* 2013;8:e65389.



114. Willmann G. Ultraviolet Keratitis: From the Pathophysiological Basis to Prevention and Clinical Management. *High Alt Med Biol* 2015;16:277–282.
115. Han SJ, Min HJ, Yoon SC, et al. HMGB1 in the pathogenesis of ultraviolet-induced ocular surface inflammation. *Cell Death Dis* 2015;6:e1863–e1863.
116. Tong L, Lan W, Lim RR, Chaurasia SS. S100A Proteins as Molecular Targets in the Ocular Surface Inflammatory Diseases. *Ocul Surf* 2014;12:23–31.
117. Al-Swailem S, Xu Z, Wu L, et al. Induction of endothelial RAGE expression in pterygium. *Mol Vis* 2014;20:1740–1748.
118. Nass N, Trau S, Paulsen F, et al. The receptor for advanced glycation end products RAGE is involved in corneal healing. *Ann Anat - Anat Anz* 2017;211:13–20.
119. Gross C, Belville C, Lavergne M, et al. Advanced Glycation End Products and Receptor (RAGE) Promote Wound Healing of Human Corneal Epithelial Cells. *Invest Ophthalmol Vis Sci* 2020;61:14.
120. Wang Z, Handa JT, Green WR, et al. Advanced Glycation End Products and Receptors in Fuchs' Dystrophy Corneas Undergoing Descemet's Stripping with Endothelial Keratoplasty. *Ophthalmology* 2007;114:1453–1460.
121. Reiss GR, Werness PG, Zollman PE, Brubaker RF. Ascorbic Acid Levels in the Aqueous Humor of Nocturnal and Diurnal Mammals. *Arch Ophthalmol* 1986;104:753–755.
122. Miyazaki D, Kandori-Inoue M, Shimizu Y, et al. Role Played by Receptors for Advanced Glycosylation End Products in Corneal Endothelial Cells after HSV-1 Infection. *Int J Mol Sci* 2021;22:5833.
123. Wisse RPL, Kuiper JJW, Gans R, et al. Cytokine Expression in Keratoconus and its Corneal Microenvironment: A Systematic Review. *Ocul Surf* 2015;13:272–283.
124. Gomes JAP, Azar DT, Baudouin C, et al. TFOS DEWS II iatrogenic report. *Ocul Surf* 2017;15:511–538.
125. Soleimani M, Mahdavi Sharif P, Cheraqpour K, et al. Ocular graft-versus-host disease (oGVHD): From A to Z. *Surv Ophthalmol* 2023:S0039625723000425.
126. Ahn H, Ji YW, Jun I, et al. Comparison of Treatment Modalities for Dry Eye in Primary Sjögren's Syndrome. *J Clin Med* 2022;11:463.
127. Messmer EM, Ahmad S, Benitez del Castillo JM, et al. Management of inflammation in dry eye disease: Recommendations from a European panel of experts. *Eur J Ophthalmol* 2022;112067212211414.
128. Perez VL, Mah FS, Willcox M, Pflugfelder S. Anti-Inflammatories in the Treatment of Dry Eye Disease: A Review. *J Ocul Pharmacol Ther* 2023;39:89–101.
129. Guo B, Gopinath B, Watson S, et al. Associations between intake of dietary micro- and macro-nutrients with Dry eye syndrome. *Clin Nutr ESPEN* 2023;54:258–263.

130. Molina-Leyva I, Molina-Leyva A, Bueno-Cavanillas A. Efficacy of nutritional supplementation with omega-3 and omega-6 fatty acids in dry eye syndrome: a systematic review of randomized clinical trials. *Acta Ophthalmol (Copenh)* 2017;95:e677–e685.
131. Kaercher T, Messmer EM, Berninger T, et al. Topical Omega-3 Polyunsaturated Fatty Acids for the Treatment of Dry Eye – Results from a Pilot Randomized Controlled Masked-Observer Study. *Clin Ophthalmol* 2022;Volume 16:4021–4031.
132. Kojima T, Dogru M, Ibrahim OMA, et al. Effects of Oxidative Stress on the Conjunctiva in Cu, Zn-Superoxide Dismutase-1 ( *Sod1* )–Knockout Mice. *Investig Ophthalmology Vis Sci* 2015;56:8382.
133. Kojima T, Wakamatsu TH, Dogru M, et al. Age-Related Dysfunction of the Lacrimal Gland and Oxidative Stress. *Am J Pathol* 2012;180:1879–1896.
134. Fariselli C, Giannaccare G, Fresina M, Versura P. Trehalose/hyaluronate eyedrop effects on ocular surface inflammatory markers and mucin expression in dry eye patients. *Clin Ophthalmol* 2018;Volume 12:1293–1300.
135. Stolz J, Luyckx, Baudouin C. Trehalose: an intriguing disaccharide with potential for medical application in ophthalmology. *Clin Ophthalmol* 2011:577.
136. Collier SA, Madigan MC, Penfold PL. Expression of membrane-type 1 matrix metalloproteinase (MT1-MMP) and MMP-2 in normal and keratoconus corneas. *Curr Eye Res* 2000;21:662–668.
137. Li D-Q, Lokeshwar BL, Solomon A, et al. Regulation of MMP-9 Production by Human Corneal Epithelial Cells. *Exp Eye Res* 2001;73:449–459.
138. Elenjord R, Ljones H, Sundkvist E, et al. Dysregulation of Matrix Metalloproteinases and their Tissue Inhibitors by S100A4. *Connect Tissue Res* 2008;49:185–188.
139. Kijlstra A, Jeurissen SH, Koning KM. Lactoferrin levels in normal human tears. *Br J Ophthalmol* 1983;67:199–202.
140. Regueiro U, López-López M, Varela-Fernández R, et al. Immunomodulatory Effect of Human Lactoferrin on Toll-like Receptors 2 Expression as Therapeutic Approach for Keratoconus. *Int J Mol Sci* 2022;23:12350.
141. Dierick M, Vanrompay D, Devriendt B, Cox E. Lactoferrin, a versatile natural antimicrobial glycoprotein that modulates the host's innate immunity. *Biochem Cell Biol* 2021;99:61–65.
142. López-López M, Regueiro U, Bravo SB, et al. Shotgun Proteomics for the Identification and Profiling of the Tear Proteome of Keratoconus Patients. *Investig Ophthalmology Vis Sci* 2022;63:12.
143. Balasubramanian SA, Pye DC, Willcox MDP. Levels of lactoferrin, secretory IgA and serum albumin in the tear film of people with keratoconus. *Exp Eye Res* 2012;96:132–137.
144. Hussain T, Tan B, Yin Y, et al. Oxidative Stress and Inflammation: What Polyphenols Can Do for Us? *Oxid Med Cell Longev* 2016;2016:1–9.

145. Hage A, Knoeri J, Leveziel L, et al. EYERUBBICS: The Eye Rubbing Cycle Study. *J Clin Med* 2023;12:1529.
146. Acar Eser N, Dikmetas O, Kocabeyoglu S, et al. Evaluation of Keratoconus Disease with Tear Cytokine and Chemokine Levels Before and After Corneal Cross-Linking Treatment. *Ocul Immunol Inflamm* 2023:1–7.
147. Kolozsvári BL, Berta A, Petrovski G, et al. Alterations of Tear Mediators in Patients with Keratoconus after Corneal Crosslinking Associate with Corneal Changes Ljubimov AV, ed. *PLoS ONE* 2013;8:e76333.
148. Lasagni Vitar RM, Bonelli F, Rama P, Ferrari G. Nutritional and Metabolic Imbalance in Keratoconus. *Nutrients* 2022;14:913.
149. Bamdad S, Owji N, Bolkheir A. Association Between Advanced Keratoconus and Serum Levels of Zinc, Calcium, Magnesium, Iron, Copper, and Selenium. *Cornea* 2018;37:1306–1310.
150. Lasagni Vitar RM, Fonteyne P, Knutsson KA, et al. Vitamin D Supplementation Impacts Systemic Biomarkers of Collagen Degradation and Copper Metabolism in Patients With Keratoconus. *Transl Vis Sci Technol* 2022;11:16.
151. Csader S, Korhonen S, Kaarniranta K, Schwab U. The Effect of Dietary Supplementations on Delaying the Progression of Age-Related Macular Degeneration: A Systematic Review and Meta-Analysis. *Nutrients* 2022;14:4273.
152. Chen Y, Mehta G, Vasiliou V. Antioxidant Defenses in the ocular surface. *Ocul Surf* 2009;7:176–185.
153. Pastori V, Tavazzi S, Lecchi M. Lactoferrin-Loaded Contact Lenses: Eye Protection Against Oxidative Stress. *Cornea* 2015;34:693–697.
154. Pastori V, Tavazzi S, Lecchi M. Lactoferrin-loaded contact lenses counteract cytotoxicity caused in vitro by keratoconic tears. *Contact Lens Anterior Eye* 2019;42:253–257.
155. Bielory BP, O’Brien TP, Bielory L. Management of seasonal allergic conjunctivitis: guide to therapy. *Acta Ophthalmol (Copenh)* 2012;90:399–407.
156. Benoist d’Azy C, Pereira B, Chiambaretta F, Dutheil F. Efficacy of Different Procedures of Intra-Corneal Ring Segment Implantation in Keratoconus: a Systematic Review and Meta-Analysis. *Transl Vis Sci Technol* 2019;8:38.
157. Charrin E, Faes C, Sotiaux A, et al. Receptor for Advanced Glycation End Products Antagonism Blunts Kidney Damage in Transgenic Townes Sickle Mice. *Front Physiol* 2019;10:880.
158. Yang H, Liu H, Zeng Q, et al. Inhibition of HMGB1/RAGE-mediated endocytosis by HMGB1 antagonist box A, anti-HMGB1 antibodies, and cholinergic agonists suppresses inflammation. *Mol Med Camb Mass* 2019;25:13.
159. Lewis JB, Mejia C, Jordan C, et al. Inhibition of the receptor for advanced glycation end-products (RAGE) protects from secondhand smoke (SHS)-induced intrauterine growth

restriction IUGR in mice. *Cell Tissue Res* 2017;370:513–521.

160. Lee S, Piao C, Kim G, et al. Production and application of HMGB1 derived recombinant RAGE-antagonist peptide for anti-inflammatory therapy in acute lung injury. *Eur J Pharm Sci* 2018;114:275–284.

161. Audard J, Godet T, Blondonnet R, et al. Inhibition of the Receptor for Advanced Glycation End-Products in Acute Respiratory Distress Syndrome: A Randomised Laboratory Trial in Piglets. *Sci Rep* 2019;9:9227.

162. Blondonnet R, Audard J, Belville C, et al. RAGE inhibition reduces acute lung injury in mice. *Sci Rep* 2017;7:7208.

163. Lutterloh EC, Opal SM, Pittman DD, et al. Inhibition of the RAGE products increases survival in experimental models of severe sepsis and systemic infection. *Crit Care* 2007;11:R122.

164. Christaki E, Opal SM, Keith JC, et al. A Monoclonal Antibody Against RAGE Alters Gene Expression and is Protective in Experimental Models of Sepsis and Pneumococcal Pneumonia. *Shock* 2011;35:492–498.



## **Résumé de la thèse en Français**

Le Récepteur aux Produits Avancés de Glycation (Receptor for Advanced Glycation End products, RAGE) est un récepteur transmembranaire, ubiquitaire, impliqué dans de nombreuses voies d'activation pro-inflammatoires. On lui décrit une forme transmembranaire (fl-RAGE) qui véhicule le signal d'activation intracellulaire, et des formes solubles (s-RAGE et es-RAGE) qui ont un rôle de leurres en séquestrant les ligands. Le rôle de RAGE est bien connu dans la physiopathologie de la rétinopathie diabétique, beaucoup moins dans des pathologies de la surface oculaire comme le kératocône (KC) et le syndrome sec oculaire (dry eye disease, DED). Longtemps défini comme une pathologie cornéenne ectasique et non inflammatoire, le KC semble déclenché ou aggravé par des phénomènes inflammatoires au sein de la surface oculaire, comme l'atteste des travaux récents montrant une élévation des biomarqueurs de l'inflammation (IL-1, IL-6, TNF- $\alpha$ , MMP-9). Par ailleurs, dans le DED, un cercle vicieux inflammatoire au sein de la surface oculaire est reconnu comme mécanisme physiopathologique centrale, avec l'instabilité du film lacrymal et son hyperosmolarité. De plus, en qualité de tissus barrières avec notre environnement, la surface oculaire est quotidiennement exposée au stress oxydatif lié à la pollution atmosphérique ou au rayonnement ultra-violet. Il existe un lien d'activation entre les acteurs de la voie RAGE et le déséquilibre de la balance oxydative dans les tissus. Par ce travail, nous avons montré la surexpression des isoformes de RAGE dans les tissus et larmes de sujets atteints de KC par rapport à des sujets sains. Par ailleurs, dans un cadre formel appliqué aux méta-analyses, nous avons synthétisé la littérature existante pour montrer qu'il existait un déséquilibre de la balance oxydative dans les tissus et larmes de la surface oculaire de patients atteints de KC et de DED. Forts de ces résultats, nous avons développé un modèle d'expérimentation animale (souris C57 Black 6) en parallèle d'une étude menée sur modèle cellulaire (cellules primaires et lignées cellulaires cornéennes et conjonctivales) afin de comprendre l'implication de RAGE dans la physiopathologie du syndrome sec oculaire, et notamment les mécanismes inflammatoires impliqués dans sa survenue et chronicisation.

## **Résumé de la thèse en Anglais**

The Receptor for Advanced Glycation End products (RAGE) is a ubiquitous transmembrane receptor involved in several pro-inflammatory activation pathways. The transmembrane form (fl-RAGE) is described to induce the intracellular activation signal, and soluble forms (s-RAGE and es-RAGE) to act as decoys sequestering the ligands. The role of RAGE is well-known in the pathophysiology of diabetic retinopathy, much less in pathologies of the ocular surface such as keratoconus (KC) and dry eye syndrome (DED). Historically defined as an ectatic and non-inflammatory corneal disease, KC seems to trigger or increase with the inflammatory biomarkers (IL-1, IL-6, TNF- $\alpha$ , MMP-9) in the ocular surface. Moreover, in DED, an inflammatory vicious circle within the ocular surface is recognized as a central pathophysiological mechanism, with the instability of the tear film and its hyperosmolarity. In addition, like others barriers tissues, the ocular surface is daily exposed to oxidative stress (i.e. atmospheric pollution or ultraviolet radiation). It is well-known that the RAGE pathway is linked with the unbalance of the oxidative stress in tissues. Thus, in this work, we showed the overexpression of RAGE isoforms in the tissues and tears of subjects with KC compared to healthy subjects. Moreover, in systematic reviews applied to meta-analyses, we synthesized the existing literature to show that there was an imbalance of the oxidative stress in the tissues and tears of the ocular surface of patients with KC and DED. Finally, we developed an animal experimentation model (C57 Black 6 mice) linked with a study carried out on a cell model (primary cells, corneal and conjunctival cell lines) in order to understand the involvement of RAGE in the physiopathology of dry eye syndrome.