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Impact of hypothyroidism on the biomechanics and tomography of the cornea in keratoconus

Dissertation to achieve the degree of a Doctor of Medicine

Faculty of Medicine

UNIVERSITY OF SAARLAND

2021

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Einfluss der Hypothyreose auf die Biomechanik und Tomographie der Hornhaut beim Keratokonus

Dissertation zur Erlangung des Grades eines Doktors der Medizin **der Medizinischen Fakultät**

UNIVERSITÄT DES SAARLANDES 2021

vorgelegt von Sonia Razafimino

geb. am 13. Dezember 1989 in Strasbourg, Frankreich

Tag der Promotion: 17.05.2022

Dekan: Univ.-Prof. Dr. med. Michael D. Menger

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LIST OF ABBREVIATIONS

Anti-TPO	Anti-thyroid peroxidase
ARC	Anterior Radius of Curvature
CT	Corneal Thickness in µm
CH	Corneal Hysteresis
CKI	Central Keratoconus Index
CRF	Corneal Resistance Factor
CXL	Crosslinking
D	Diopters
DALK	Deep Anterior Lamellar Keratoplasty
fT3	Free triiodothyronine
fT4	Free thyroxine
HKC	Homburg Keratoconus Center
HT	Keratoconus patients with hypothyroidism
IHD	Index of Height Decentration
ICRS	Intracorneal Ring Segment
IVA	Index of Vertical Asymmetry
K	Keratometry
KC	Keratoconus
KCI	Klyce/Maeda keratoconus index
KI	Keratoconus Index
KMI	Keratoconus Match Index
KSI	Klyce/Smolek Keratoconus Index
LASIK	Laser-Assisted In Situ Keratomileusis
pIOL	Phakic intraocular lens implantation
PKP	Penetrating Keratoplasty
PRC	Posterior Radius of Curvature
PRK	Photorefractive Keratectomy
SAI	Surface Asymmetry Index

SD	Standard Deviation
SKC	Severe Keratoconus
SRI	Surface Regularity Index
TGD	Thyroid Gland Dysfunction
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating hormone
T4	Thyroxine
T3	Triiodothyronine
WHT	Keratoconus patients without hypothyroidism

ZUSAMMENFASSUNG

Hintergrund und Zweck: Die Ätiologie des Keratokonus ist multifaktoriell, letztendlich aber bislang unklar. Einige frühere klinische Beobachtungen legen nahe, dass die Hypothyreose eine Rolle bei der Entstehung und Ausprägung des Keratokonus spielt. Das Ziel unserer Studie war es, die tomographischen und biomechanischen Parameter zwischen Keratoconus-Patienten mit und ohne Hypothyreose zu vergleichen, in einer Querschnitts- und Längschnitt-Betrachtung aus dem Homburger Keratokonuszentrum bei Einschluss und nach einem Jahr.

Methoden: 28 Patienten mit Keratokonus und Hypothyreose (Gruppe HT) und 56 Keratokonus-Patienten ohne Schilddrüsenfunktionsstörung (Gruppe WHT), die geschlechts- und altersmäßig übereinstimmten, wurden analysiert. Das Durchschnittsalter betrug 40,3 Jahre (von 14 bis 57) in der Gruppe HT und 40,3 Jahre (von 14 bis 57) in der Gruppe WHT. Die ophthalmologischen Routineuntersuchungen bestanden aus Hornhauttomographie und biomechanischen Parametern. Wir analysierten die folgenden Keratoconus-Parameter aus der Pentacam (Pentacam HR, Oculus, Wetzlar, Deutschland): Astigmatismus, Rmin (minimaler Radius), Kmax (steilster Krümmungsradius), ARC (vordere korneale Krümmungsradien), PRC (hintere korneale Krümmungsradien), TP (minimale Hornhautdicke), IVA (Index of Vertical Asymmetry), IHD (Index of Heigh Decentration), KI (Keratokonus Index), CKI (Central Keratoconus Index) und sowie aus dem Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA) die CH (korneale Hysterese), den CRF (kornealen Resistance-Faktor) sowie den KMI (Keratokonus-Match-Index).

Ergebnisse: Die Ergebnisse der tomographischen und biomechanischen Werte zeigten sowohl in der Querschnitts- als auch in der Längsschnittanalyse keine signifikanten Unterschiede zwischen der HT- und WHT-Gruppe.

Schlussfolgerung: Die Schwere des KK scheint basierend auf biomechanischen und tomographischen Parametern nicht von der Hypothyreose beeinflusst zu sein.

SUMMARY

Background and Purpose: The etiology of Keratoconus is supposedly multifactorial but remains ultimately unknown. Previous scientific observations suggested that hypothyroidism might play a role in the development and progression of Keratoconus. The purpose of this study was to analyze the tomographic and biomechanical parameters in Keratoconus patients with or without hypothyroidism at the time of inclusion (cross-sectional aspect) in the Homburg Keratoconus Center (HKC) and after one-year follow-up (longitudinal aspect).

Methods: 28 patients with Keratoconus and hypothyroidism (group HT) and 56 patients Keratoconus patients without thyroid dysfunction (group WHT) with matching sex and age were analyzed. Mean age was 40.3 years (range 14-57) in the group HT and 40.3 years (range 14-57) in the WHT group. Routine ophthalmic examinations consisted of corneal tomography and biomechanical parameters. We extracted the following Keratoconus parameters from the Pentacam (Pentacam HR, Oculus, Wetzlar, Germany): astigmatism, Rmin (mean of the minimum radius of curvature), Kmax (maximum Keratometry), ARC (Anterior Radius of Curvature), PRC (Posterior Radius of Curvature), TP (Thinnest Pachymetry), IVA (Index of vertical asymmetry), IHD (Index of Height Decentration), KI (Keratoconus Index) and, CKI (Central Keratoconus Index). From the Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA) we extracted the CH (Corneal Hysteresis), CRF (Corneal Resistance factor) and KMI (Keratoconus Match Index).

Results: The comparison of the tomographic and biomechanical values from cross-sectional and longitudinal analyses showed no significant differences between the HT and WHT groups.

Conclusion: The severity of KC based on tomographical and biomechanical parameters does not seem to depend on the presence of hypothyroidism.

1. Introduction

1.1. Corneal anatomy

1.1.1. General aspects

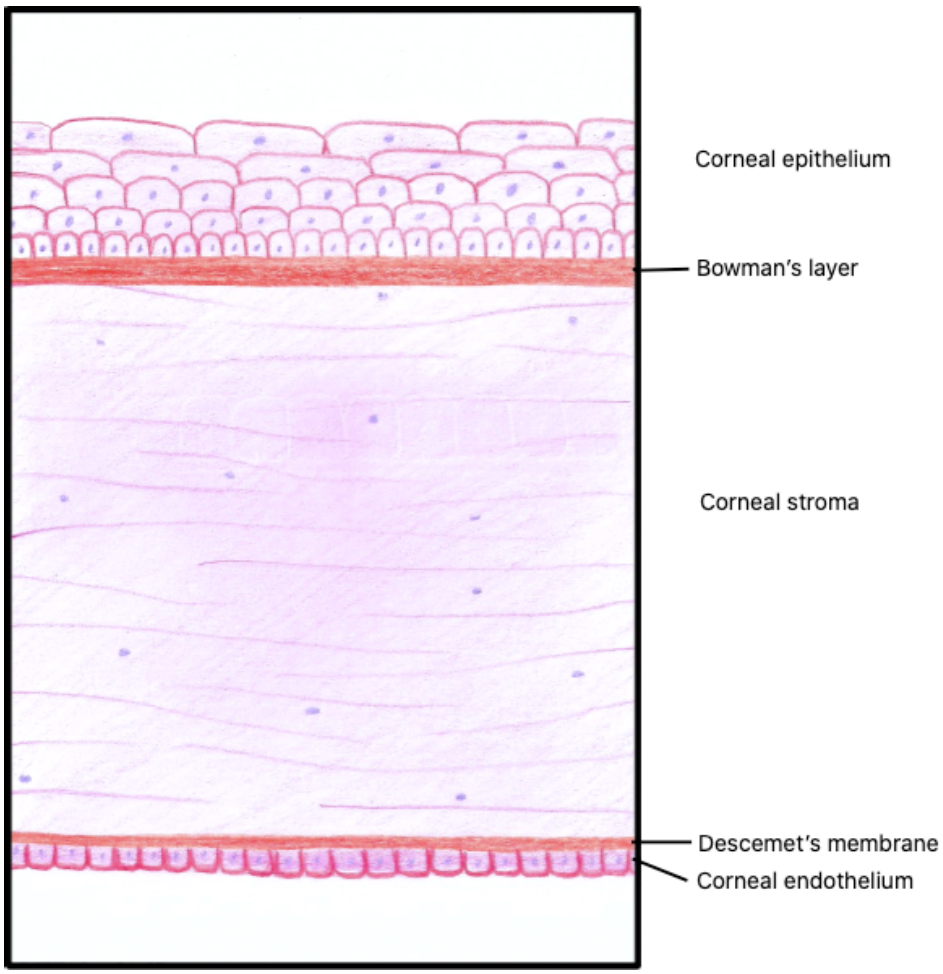


Figure 1 : Schema of the corneal layers in a normal cornea.

The cornea is a transparent avascular connective tissue which acts as the primary infectious and structural barrier for the eye. Together with the overlying tear film, it provides a proper anterior refractive surface for the eye. Its clarity is the result of multiple factors including the structural anatomy and physiology of its cellular components. The maintenance of corneal shape and transparency is critical for optimizing the eye's refractive power [122].

The cornea is the foremost transparent, strongly curved section that occupies the center of the anterior pole of the ocular globe. Transparency of the cornea is essential for the necessary normal functioning

of the eye. The cornea in itself has no blood vessels, it consists of one continuous homogeneous structure. To improve the optical properties of the cornea, it is covered by a tear film consisting of tear fluid. The cornea is approximately 1000 µm thick at its periphery and 500 µm thick centrally [21]. In the average adult, the horizontal diameter of the cornea is 11,5 to 12,0 mm [97]. In humans, the cornea forms a positive lens with the refractive power of about 42 diopters (D) and constitutes the main refractive element of the eye.

The human cornea consists of 5 layers, 3 cellular (the epithelium, stroma and endothelium) and 2 interfaces (Bowman's layer, Descemet's membrane). Figure 1 presents the schema of the corneal layers in a normal functioning cornea.

1.1.2. Corneal Layers

Epithelium

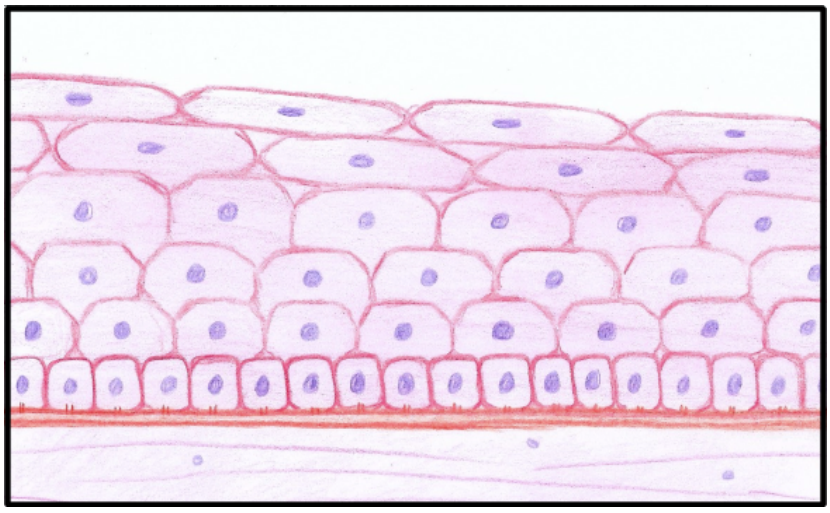


Figure 2: Schema of the corneal epithelium in a normal cornea.

The corneal epithelium is the outermost peripheral layer of the cornea and creates the first barrier to the outside environment. It is a squamous nonkeratinized epithelium of about 40 to 50 µm [21]. Figure 2 shows the schema of the corneal epithelium. The epithelium is composed of three different types of layers: A single layer of basal cells adhere to the underlying basement membrane using hemidesmosomes and are bound together with tight junctions. Located above them are two or three layers of wing cells and the superficial cells, which consist of 2–3 layers made up of flat polygonal cells. The microplicae and microvilli on the surface increase the surface area and they are involved in

the exchanges between the cornea and the precorneal tear film. In addition, highly resistant tight junctions between adjacent epithelial cells provide a protective layer and form an effective barrier against fluid loss and pathogen penetration [21]. The corneal epithelium and overlying tear film maintain a symbiotic relationship both anatomically and physiologically. The epithelial basement membrane, approximately 0.05 µm thick, is composed of type IV collagen and laminin [21].

Bowman's layer

The Bowman's layer or anterior limiting lamina is about 10-15 µm thick and localized at the interface between the corneal epithelium and the stroma. It consists of randomly dispersed collagen fibrils and is strictly acellular. When disrupted, it will not regenerate, and scar tissue can form [21].

Stroma

The stroma is about 500 µm and makes up approximately 80% of the total corneal thickness in the human eye [21]. It is comprised of collagen-producing keratocytes and a precise organization of the collagen fibers and the extracellular matrix [76]. The collagen fibers are arranged in parallel bundles called fibrils, and these fibrils are packed in parallel ranged layers or lamellae [21]. Collagen types are mainly I but also V [30]. Extracellular matrix consists of proteoglycans that run along and between the collagen fibrils. The major stromal proteoglycans are keratan sulfate or chondroitin sulfate/dermatan sulfate side chains, which assist in regulating hydration and structural properties. The keratocytes are located between the corneal lamellae and synthesize both collagen and proteoglycans [21]. Within each lamella, collagen fibrils are parallel, tightly packed, and highly uniform in diameter. This organized architecture is responsible for the transparency of the cornea, and any disruption in this organization leads to a severely opaque cornea [45].

The Dua's layer is an airtight, extremely tear-resistant layer approximately 15 µm thick, which is located between the stroma and the Descemet's membrane. It contains five to eight compact thin lamellae of type I collagen and has an abundance of type VI (long spacing) collagen [23].

Descemet's membrane

Descemet's membrane or lamina limitans posterior is a membrane which separates the endothelial layer from the corneal stroma. Similarly to Bowman's layer, it is an acellular layer and therefore not continuous with the collagen fibrils of the stroma [21].

Endothelium

The corneal endothelium is comprised of a single layer of mostly hexagonal cells and appears as a honeycomb-shaped mosaic when viewed from the posterior side, as shown in Figure 3 [21]. The main function of the endothelium is to regulate the water content of the corneal stroma. The endothelial cell density and tomography continue to change throughout its lifespan. From the second to eighth decade of life, the cell density declines from 3000 to 4000 cells/mm^2 to around 2600 cells/mm^2 [128].

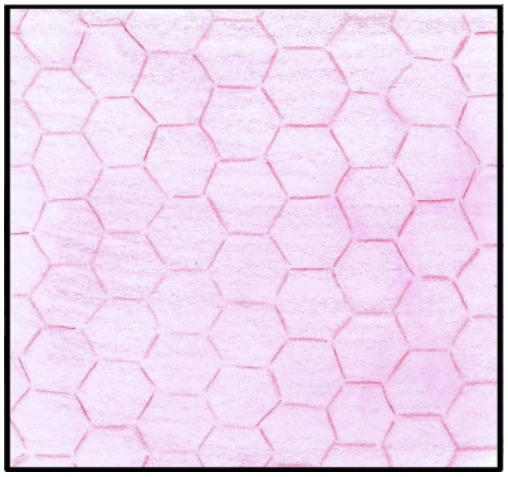


Figure 3: Schema of the corneal endothelium in a normal cornea.

1.2. Keratoconus

1.2.1. Definition and epidemiology

The keratoconus (KC) was first described in the literature more than 150 years ago by Nottingham in 1854 [84] and further detailed in 1998 by Rabinowitz [90]. It derives from the Greek words (cornea) and konos (cone) and is the most common primary corneal ectasia. It was first described as a non-inflammatory corneal thinning disorder characterized by progressive central or paracentral corneal stroma thinning and loss of structural integrity, leading to a protrusion of the cornea (cone shape appearance in KC) and in the most advanced cases, to corneal scarring [65]. More recent reports suggest an additional underlying inflammatory process [39,79]. The corneal protrusion causes high myopia and irregular astigmatism, which result in distorted and therefore decreased vision. The KC is mostly bilateral [61,129] and asymmetric [13,131].

It usually commences over the course of the second decade of life, during puberty [61,90] and progresses until the third or fourth decade. However, its progression differs among patients; it has also been found to develop both earlier but also later in life [3,90,93].

Incidence and prevalence observed in the general population are estimated to be between 50 and 230 per 100 000, and 5.4 per 10 000, respectively [61,65,90,96]. The prevalence of KC varies greatly worldwide; it was reported at 0.0003% in Russia [53], 0.0086% in Denmark [83], 0.249% in Iran [132] and 2.3% in central India [56]. The prevalence of KC changes with geographical variation, perhaps partly due to the way the data is collected but may also be due to accessibility to advanced technology. It will not be surprising to notice an increase in the incidence and prevalence detection rates of KC over the years given the significant improvement in topographic, tomographic and biomechanical detection devices.

KC affects both genders [90,127], but most of the recent publications report a male preponderance [36,130]. KC is known to affect all ethnicities [61,90,96,120,123].

1.2.2. Etiology

Although many different pathways including biochemical, genetic, mechanical and often multifactorial origins have been investigated, the etiology of KC remains unclear [18,90].

The most common presentation of KC is a sporadic disorder [61,90], but it has long been recognized that a significant minority of patients have a family medical history [29,37,95]. A positive family medical history has been reported in 6-8% of the cases and its prevalence in first-degree relatives is 15 to 67 times higher than those in general population [121].

In an - as yet unknown - proportion of patients, one of the etiological factors can certainly be attributed to genetic make-up [24,54,88,90,91]. The genetic is evidenced by the familial inheritance of the genetic condition, its discordance between monozygotic and dizygotic twins and its association with other known genetic disorders such as Down's syndrome and Marfan syndrome. Possible genetic predisposition towards increasing sensitivity to apoptotic mediators by keratocytes has also been hypothesized [24].

KC is most commonly an isolated disorder, although several reports describe an association with Down's syndrome, Leber congenital amaurosis, mitral valve prolapse and connective tissue diseases [8,101,110].

Associations have also been made with atopy and eye rubbing [7,41,77,82,90,92,123], which can be related to a biomechanical effect. It has been established that most KC patients with disease progression rub their eyes excessively [60]. Most authors described eye rubbing as an aggravating factor in predisposed patients [111,124]. Moran et al. even suggest eye-rubbing to be the root cause of developing KC [81]. This association may be due to the activation of wound healing processes and signaling pathways secondary to mechanical stress, in the form of epithelial trauma but, also

mechanical trauma of keratocytes or even increased hydrostatic pressure [78]. Contact lens wearing is another form of corneal microtrauma which could eventually induce a KC [2,74].

1.2.3. Histopathology

Maintenance of corneal shape and transparency is critical for optimizing the eye's refractive power. Researchers have used a variety of different advanced techniques to evaluate major morphological corneal changes in KC patients. Morphological changes have been outlined in every layer of the keratoconic cornea. From a histopathological point of view, there are three notable signs which typically characterize KC: stromal corneal thinning, Bowman's layer breaks, and iron deposits within the corneal epithelium's basal layer [65,90].

In vivo confocal microscopy show that superficial epithelial cells located at the apex of the cone are extremely elongated and arranged in a whorl-like fashion [104]. Basal epithelial density is decreased in both moderate and advanced KC in comparison to normal corneas [122]. Teng et al. observed with an electron microscope that the keratoconic basement membrane assumes an irregular appearance and localized breaks [114]. The keratoconic cornea exhibited sharply edged ruptures and fragmentation in the Bowman's layer in comparison to normal corneas, in which the surface of the Bowman's layer remained smooth and collagen fibrils regularly arranged. The coexistence of a proliferative collagenous tissue derived from the anterior stroma just beneath the Bowman's layer was also observed [98,102].

In the stroma, a decrease in the number of lamellae and keratocytes is detected but the thickness of collagen lamellae remains unaltered [113]. Changes in the orientation of collagen fibrils within the lamellae have also been noticed [19,80]. Studies carried out using confocal microscopy have demonstrated a reduction in the number of keratocytes in KC compared to normal subjects. The reduction seems to be more pronounced in more advanced disease [28,67,104].

Ruptures and folds in the Descemet's membrane were described in KC and cause an acute corneal hydrops [126]. The endothelium is generally unaffected by the disease [122] although pleomorphism and endothelial cells pointing towards the cone have been reported [90,102] and eventually a change in endothelial cells density in KC patients in comparison with a normal cornea [49]. It has also been demonstrated that corneal nerves in KC have thicker fiber bundles, reduced density, and subepithelial plexus compared to normal subjects [35,89].

1.2.4. Clinical features and diagnosis

Table 1: Characteristic signs of keratoconus

Retinoscopy :
- Myopic astigmatism - Scissors reflex
Slit lamp findings :
- Stromal thinning can be centrally or paracentrally most commonly inferiorly or inferotemporally - Central or eccentric corneal protrusion - Fleischer ring - Vogt's striae - Enlarged corneal nerves - Anterior stromal scars
External signs :
- Munson's sign - Rizzuti's sign

The ocular symptoms and signs of KC are variable and depend on the stage of the progression of the disorder. At incipient stages, also referred as subclinical, the KC rarely produce symptoms and thus can go unnoticed unless specific tests (corneal tomography and/or biomechanical assessments) are undertaken for diagnosis. In advanced cases it leads to a significant distortion in vision accompanied by profound visual loss, which consequently can no longer be compensated for by wearing of spectacles.

In KC the stromal thinning can be centrally or paracentrally, most commonly inferiorly or inferotemporally and which leads to a conical protrusion. In moderate and advanced cases, an iron line partially or completely surrounding the cone can appear (Fleischer ring [34,90]) as well as fine vertical lines in the deep stroma and Descemet's membrane, which are parallel to the axis of the cone and disappear transiently on gentle digital pressure (Vogt's striae [72]). Other accompanying signs may include anterior stromal scars, enlarged corneal nerves, increased intensity of the corneal endothelial reflex and subepithelial fibrillary lines [90].

Munson's sign (V-shaped conformation of the lower lid in downgaze) and Rizzuti's sign (sharply focused beam of light near the nasal limbus, produced by lateral illumination of the cornea) are useful external signs but only seen in advanced cases [72].

Breaks in the Descemet's membrane have been described in severe KC, causing acute stromal edema, known as corneal hydrops, sudden vision loss and significant pain [116]. The Table 1 summarizes the clinical characteristic signs that can be encountered in KC.

1.2.5. Classification

Table 2: Staging of keratoconus according to the standard Amsler-Krumeich keratoconus classification based on the spectacle refraction, central keratometry, presence of scarring and central corneal thickness.

Stage I	Eccentric steepening Myopia/astigmatism <5.00 D Mean K <48.0 D
Stage II	Myopia/astigmatism >5.00 D but < 8.00D Mean K <53.0 D Absence of scarring Minimal apical corneal thickness <400µm
Stage III	Myopia/astigmatism >8.00 D but < 10.00D Mean K >53.0 D Absence of scarring Minimal apical corneal thickness <400µm but > 300µm
Stage IV	Refraction not possible Mean K >55.0 D Central corneal scarring Minimal apical corneal thickness <300µm
Stage is determined if one of the characteristics applies, stage I being the least severe and IV the more severe. D: Diopters, K: Keratometry	

For the therapeutic decision and for studies, the classification of KC into stages is essential. Unfortunately, the oldest and most widely classification for the staging of KC has not been thus fas sufficient. Both the Amsler-Krumeich classification [3,66] and, the classification for the Collaborative Longitudinal Evaluation of Keratoconus Study (CLEK) [120] do not include topographic or tomographic criteria.

KC can be classified in 4 stages using the Amsler-Krumeich classification (Table 2). The 4 stages are based on spectacle refraction, central keratometry, presence or absence of scarring and central corneal thickness. The Table 3 shows the CLEK classification depending on the Kmax value.

Table 3: Staging of keratoconus according to the Collaborative Longitudinal Evaluation of Keratoconus Study Classification based on the Kmax (maximum Keratometry).

Stage	Kmax
Mild	≤ 45.00 D
Moderate	45.00 – 52.00 D
Advanced	52.00D
D: Diopters	

There are a variety of KC indices available today - especially for early detection and classification. Experienced clinicians base their (early) diagnosis mainly on topographic and tomographic indices from the Pentacam (OCULUS Optikgeräte GmbH, Wetzlar, Germany), the TMS-5 (Tomey GmbH, Nagoya, Japan) and the anterior segment OCT CASIA 2 (Tomey GmbH, Nagoya, Japan). Biomechanical parameters have also been found to be useful for the detection of KC, using the Ocular Response Analyzer (ORA) (Reichert Inc., Depew, USA) and more recently the Corneal Visualization Scheimpflug Technology (CORVIS ST, OCULUS Optikgeräte GmbH, Wetzlar, Germany) [68].

The ABCD classification recently published by Belin and Duncan represents a useful and clear method of documenting the stages and progression of KC [9,36]. It includes a graduation of the “Anterior Radius of Curvature (ARC)” (A), the “Posterior Radius of Curvature (PRC)” (B), the “Corneal pachymetry at thinnest point” (C) and the “Distance best-corrected vision with spectacles” (D). In contrast to the classical Amsler-Krumeich classification, the ABCD classification has a different severity of 0-4 for all four parameters. A “-“ is added if no scars are visible, “+” for scars that leave iris details visible and “++” for scars that hide iris details (Table 4).

Table 4: ABCD Keratoconus grading system by Belin and Duncan, based on the ARC (Anterior radius of curvature), PCR (Posterior radius of curvature), thinnest pachymetry using Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) as well as BCVA (best corrected visual acuity) and cornea's scarring [9].

ABCD Criteria	A ARC (3mm zone)	B PRC (3mm zone)	C Thinnest pachymetry (µm)	D BCVA	Scarring
Stage 0	> 7.25 mm (<46.5 D)	> 5.90mm	>490µm	≥20/20 (≥1.0)	-
Stage I	> 7.05 mm (<48.0 D)	>5.70mm	>450µm	<20/20 (<1.0)	-,+,++
Stage II	> 6.35 mm (<53.0 D)	>5.15mm	>400µm	<20/40 (<0.5)	-,+,++
Stage III	> 6.15 mm (<55.0 D)	≥4.95mm	>300µm	<20/100 (<0.2)	-,+,++
Stage IV	≤6.15 mm (≥55.0 D)	<4.95mm	≤300µm	<20/400 (<0.05)	-,+,++

Scarring - clear, no scarring (-), scarring, iris details visible (+), scarring, iris obscured (++);

D: Diopters, also shown for anterior radius of curvature

ARC: Anterior Radius of Curvature

PRC: Posterior Radius of Curvature

BCVA: Best Corrected Visual Acuity

1.2.6. Treatment

KC management varies depending on the severity of the disorder. In the less severe cases, the initial option is to wear spectacles to improve vision. In mild to advanced cases, spectacles alone do not provide sufficient vision, due to irregular astigmatism and the increase in myopia. The wearing of gas permeable and hybrid or rigid oxygen permeable contact lenses becomes necessary in these cases [99].

When it is no longer possible to correct the vision with contact lenses, it is possible to perform a keratoplasty (penetrating keratoplasty (PKP) or deep anterior lamellar keratoplasty (DALK)) [51,99,109].

In the case of contact lens intolerance, intracorneal ring segments (ICRS) can be implanted to flatten and regularize the cornea. This not only improves vision, but also improves contact lens tolerance [99].

Other surgical procedures such as corneal crosslinking are used to stabilize the deformation of the cornea [99]. Phakic intraocular lens implantation (pIOL) is also described to improve the vision [11,52,96]. Photorefractive keratectomy (PRK) before/during/after CXL has been proposed as a refractive surgical laser ablative procedure [59]. However, due to its "unpredictability", this method has not been found suitable into the stepwise therapy schema of KC in Germany. The same is true for the so-called "Bowman's layer Transplantation" [117,119]. All other refractive surgery procedures are contraindicated in KC.

1.3. Hypothyroidism

1.3.1. Definition

Hypothyroidism is defined as a thyroid hormone deficiency. The definition of hypothyroidism is based on statistical reference ranges in relevant biochemical parameters due to the large variation in clinical presentation and general absence of symptom specificity. It is increasingly a matter of debate. Overt or clinical primary hypothyroidism is defined on an increase in TSH (by lifting the negative feedback of thyroid hormones on the pituitary thyroid cells). If the TSH is above the reference range, the free thyroxine (fT4) will then be determined in a second step to help refine the diagnosis. If the concentration in fT4 is "inappropriately" normal, it is described as frustrated (or subclinical) hypothyroidism and in this case, the TSH is moderately increased, most often between 4 and 10 mUI/L. If the fT4 is below the range reference, it is an overt (or clinical) hypothyroidism, in which case the TSH is usually higher, above 10 mUI/L. Primary hypothyroidism is the most common etiology of hypothyroidism. The prevalence of overt hypothyroidism in the general population varies between 0.2% and 5.3% in Europe [5,40]. Moreover, hypothyroidism is more common in patients with autoimmune diseases. The existing reference ranges of TSH and fT4 used to define thyroid dysfunction is a matter for debate. For this study we apply the reference ranges as predefined by the Central University Laboratory of the Saarland University [133]. The reference ranges can also differ with age, sex, and ethnic origin [12,20].

1.3.2. Hormones

Triiodothyronine (T3) and thyroxine (T4), are tyrosine-based hormones produced by the thyroid gland and play a crucial role in the control of energy metabolism, and, influencing cardiovascular function, growth and bone metabolism. They are important for the normal development of gonadal functions and the nervous system. The major form of thyroid hormone in the blood is T4, it is essential for the proper development and differentiation of all cells in the human body, regulating protein, fat, and carbohydrate metabolism, but also affecting how human cells use energetic compounds.

Thyroid-stimulating hormone (TSH)

Definition:

TSH is produced in the pituitary gland and stimulates the thyroid gland to secrete thyroid hormones. TSH secretion is stimulated by a thyrotropin-releasing hormone (TRH) from the hypothalamus and inhibited by thyroid hormones via a negative feedback loop. For an orientation examination of thyroid function, TSH determination alone, without fT3 and fT4, is most often sufficient. The TSH stimulation test (TRH test) is only necessary in doubtful cases of borderline basal values.

Reference values:

The reference values for TSH from the Central University Laboratory of the Saarland University Medical Center are [133]:

- 11 to 18 years old: 0.51 – 4.30 mU/L
- 18 years old and more: 0.27 – 4.20 mU/L
-

Test:

For the quantitative determination of TSH in human serum, 4.7 mL of blood is collected in a tube S-Monovette Serum-Gel. Our laboratory uses an immunological in vitro test: The Electrochemiluminescence Immunoassay “ECLIA” (Figure 4).

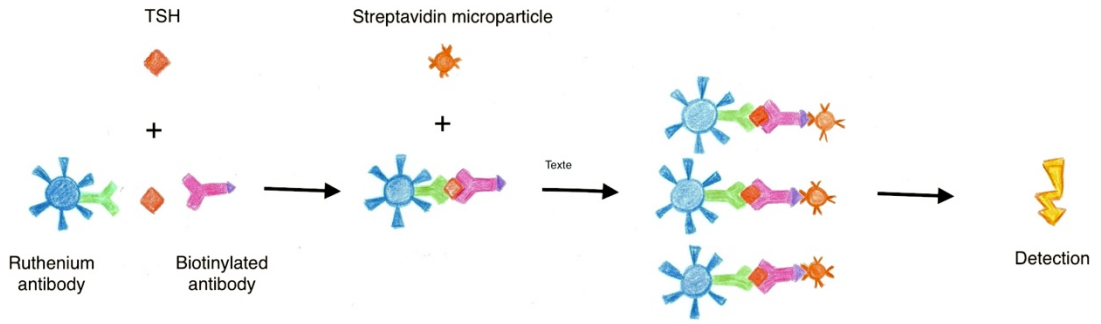


Figure 4: Schematic representation of the ECLIA measurement: Electrochemiluminescence immunoassay for the determination of TSH.

Principe of electrochemiluminescence immunoassay for the quantitative determination of TSH in human serum [134]:

- First step: Incubation of the blood sample with a biotinylated monoclonal TSH-specific antibody and a ruthenium complex-labeled monoclonal TSH-specific antibody. A sandwich complex will be formed between: Biotinylated antibody - TSH – Ruthenium antibody.

- Second step: Incubation and addition of streptavidin-coated paramagnetic microparticles, the immune complex is bound to the solid phase via biotin-streptavidin.

- Third step: The reaction mixture is transferred into the measuring cell, where the microparticles are magnetically fixed on the electrode surface. Afterwards, the unbound substances are washed off. The application of an electrical voltage induces a chemiluminescence emission which is measured by a photomultiplier. The power of the signal detected is directly proportional to the concentration of the TSH contained in the sample.

Disruptive factors of TSH measurement:

- Increased values: Medication with Amiodarone, Benserazide, Clomiphene, Iodide, Lithium, Metoclopramide, Morphine, Neuroleptic, Antithyroid agent.

- Decreased values: Medication with Bromocriptine, Carbamazepine, Corticosteroid, Dopamine, Heparine, L-Dopa.

Free Thyroxine (ft4)

Definition:

T4 is the main thyroid hormone secreted into the blood by the thyroid gland. Together with T3, it plays a crucial role in controlling energy metabolism, influences cardiovascular function, growth, and bone metabolism, and it is important for normal development of gonadal function and the nervous system. T4 circulates in the blood equally as both free and serum-bound hormone. FT4 is the unbound and biologically active form, accounting for only about 0.03% of total T4. The remaining T4 is inactive and bound to serum proteins such as thyroxine-binding globulin (75%), prealbumin (15%), and albumin (10%). The determination of fT4 has the advantage of being independent of variations in the concentration of the binding properties of these proteins, thus eliminating the need for additional determination of a binding parameter. Therefore, fT4 is a useful tool in routine clinical diagnostics for the assessment of thyroid status.

Reference values:

The reference values for fT4 from the Central University Laboratory of the Saarland University Medical Center are [133]:

- 11 to 18 years old: 0.98 – 1.63 ng/dL (12.5-21pmol/L)
- 18 years old and more: 0.93 – 1.70 ng/dL (12-22pmol/L)

Test:

For the quantitative determination of ft4 in human serum, our laboratory also uses an immunological in vitro test: The Electrochemiluminescence Immunoassay “ECLIA”.

Disruptive factors of ft4 measurement:

- Increased values: Medication with Amiodarone, Acetylsalicylic acid, Propanolol.
- Decreased values: Medication with Rifampicin, Anticonvulsants.

Free Triiodothyronine (fT3)

Definition

FT3 is biologically about 5 times more potent than fT4 and 80% derives from conversion of T4 to T3 (25 µg/d). Only about 20% of circulating total T3 comes directly from the thyroid gland. T3 circulates in the blood equally as free and serum-bound hormone. It is the unbound and biologically active form, accounting for only about 0.2-0.4% of total T3. The remaining T3 is inactive and bound to serum proteins. In plasma, T3 is primarily bound to thyroxine-binding globulin. The determination of fT3 has the advantage of being independent of variations in the concentration of binding protein and binding properties, thus eliminating the need for additional determination of a binding parameter.

Reference values:

The reference values for fT3 from our Central University Laboratory of the Saarland University Medical Center are [133]:

- 11 to 18 years old: 2.6-5 pg/mL
- 18 years old and more: 2-4.4 pg/mL

Test:

For the quantitative determination of fT3 in human serum, our laboratory also uses an immunological in vitro test: The Electrochemiluminescence Immunoassay “ECLIA”.

Disruptive factors for ft3 measurement:

- Decreased values: Medication with Phenytoin, Propanolol, Valproate.

1.4. Background and Purpose of our studies

Up until today the exact etiology of KC remains unknown. Numerous therapies have been developed over years of research to manage or slow the progression and deal with complications of KC, but the incomplete understanding of the pathophysiological mechanisms and etiology of KC limits the development of curative therapy. Several clinical observations suggest that hormonal changes, and in particular thyroid disorders, may indeed contribute to the appearance or aggravation of KC. Already,

as far back as 1936, Appelbaum observed the increased prevalence of hypothyroidism in patients with KC, compared to those in the general population [4]. Moreover, cases of KC development have been diagnosed in patients with hypothyroidism secondary to thyroidectomy [62] and more recently in a patient treated with radioactive iodine treatment [71].

KC is known to be mostly progressive during puberty and progresses until the third or fourth decade of life, which may coincide with the hormonal changes described during puberty, when the level of T4-binding globulin is lowest [32]. Several studies reported the progression of the KC during pregnancy which is also associated with major changes of thyroid hormones [10,42,44,55,87,87,103,107]. It must also be noted, that certain disorders frequently associated with hypothyroid, such as Down's syndrome and Alagille syndrome, are more frequently associated with KC [70,90,108].

Biochemical research also suggests that tT4 has an influence on the cornea. The presence of T4 receptors (T4Rs) has been proven in chicken's cornea [14]. Coulombre et al. injected T4 into chicken embryo eggs and demonstrated that thyroid hormones induce a corneal dehydration and transparency during embryonic development [16,17]. Masterson et al. demonstrated that T4 treatment accelerated the development of endothelial cells and had a role in the development of the chicken's cornea [75]. In humans, T4Rs have been found in the lacrimal gland by Thanos et al., confirming that the tear producing gland is a target organ of T4 [22]. Furthermore, T4 levels were found to be higher in the tears of KC patients [115] in particular during the progression of KC [57] but also in the aqueous humor [106].

The purpose of this study was to analyze the tomographic and biomechanical corneal parameters of KC patients with or without hypothyroidism at the time of inclusion (cross-sectional aspect) in the Homburg Keratoconus Center (HKC) and after one-year follow-up (longitudinal aspect).

2. Patients and Methods

2.1. Study design

Composed of a retrospective single-center comparative study conducted at the Department of Ophthalmology, Saarland University Medical Center in Homburg/Saar, Germany. The study was approved by the local ethics committee (Number 157/10) and followed the tenets of the Declaration of Helsinki. Informed written consent for the collection and analysis of data and blood samples was obtained from all subjects [94].

2.2. Patient selection

All patients were recruited from the "Homburger Keratoconus Center" (HKC), which was established in the Department of Ophthalmology at the Saarland University Medical Center in Homburg/Saar, Germany in 2010. More than 2200 patients with KC have been registered between the months of October 2010 and August 2021. For our study, we recruited and sorted 84 patients into two different groups. We included in the first group (HT group) 28 patients with KC and documented hypothyroidism at the time of inclusion in the HKC and according to the criteria described below. Additionally, we included a second group which was sex- and age-matched and consisted of 56 patients with KC but free from thyroid disease (WHT group) [94].

Inclusion criterion for all the patients was KC, which was diagnosed based on clinical and tomographic evaluations [47]. Exclusion criteria included previous history of trauma or ocular surgery, uveitis or other intraocular inflammatory eye diseases, and any other associated corneal diseases. In addition, to eliminate known risk factors for developing KC, we excluded participants with a history of eye rubbing, atopy, vernal keratoconjunctivitis, atopic dermatitis, Down's syndrome, Leber congenital amaurosis, Turner syndrome or congenital rubella. Patients with diabetes mellitus were also excluded because of its known influence on biomechanical corneal parameters [50,64].

In the HT group, we included patients with hypothyroidism based on their medical history. Between October 2010 and January 2020, we detected 180 patients with thyroid dysfunction from the HKC by method of a questionnaire. Twenty-eight of them were included in the study with previously diagnosed hypothyroidism and who are currently under treatment. We excluded 152 patients from the study due to the following exclusion criteria; (8) Graves' disease, (20) Hashimoto's thyroiditis, (6) Hyperthyroidism, (4) Cold nodule, (1) Thyroid carcinoma, (12) Thyroid Operation, (77) unclear or incomplete data about thyroid dysfunction and/or the patients who could not be reached and (24) other exclusion criteria (Figure 5).

In the WHT group, we excluded all patients with a previous thyroid disease or thyroid surgery, and abnormal thyroid parameters according to the reference values of our laboratories (Central University Laboratory of the Saarland University Medical Center): TSH (thyroid-stimulating hormone) 0.27-4.20 mU/L, fT4 (free thyroxine) 0.93-1.70ng/dL, fT3 (free triiodothyronine) 2-4.4pg/mL and antibodies against TPO (Anti-TPO) <34IU/mL [133]. Patients with hormone levels within these ranges were classified as euthyroid. Patients were also excluded if they had incomplete medical records.

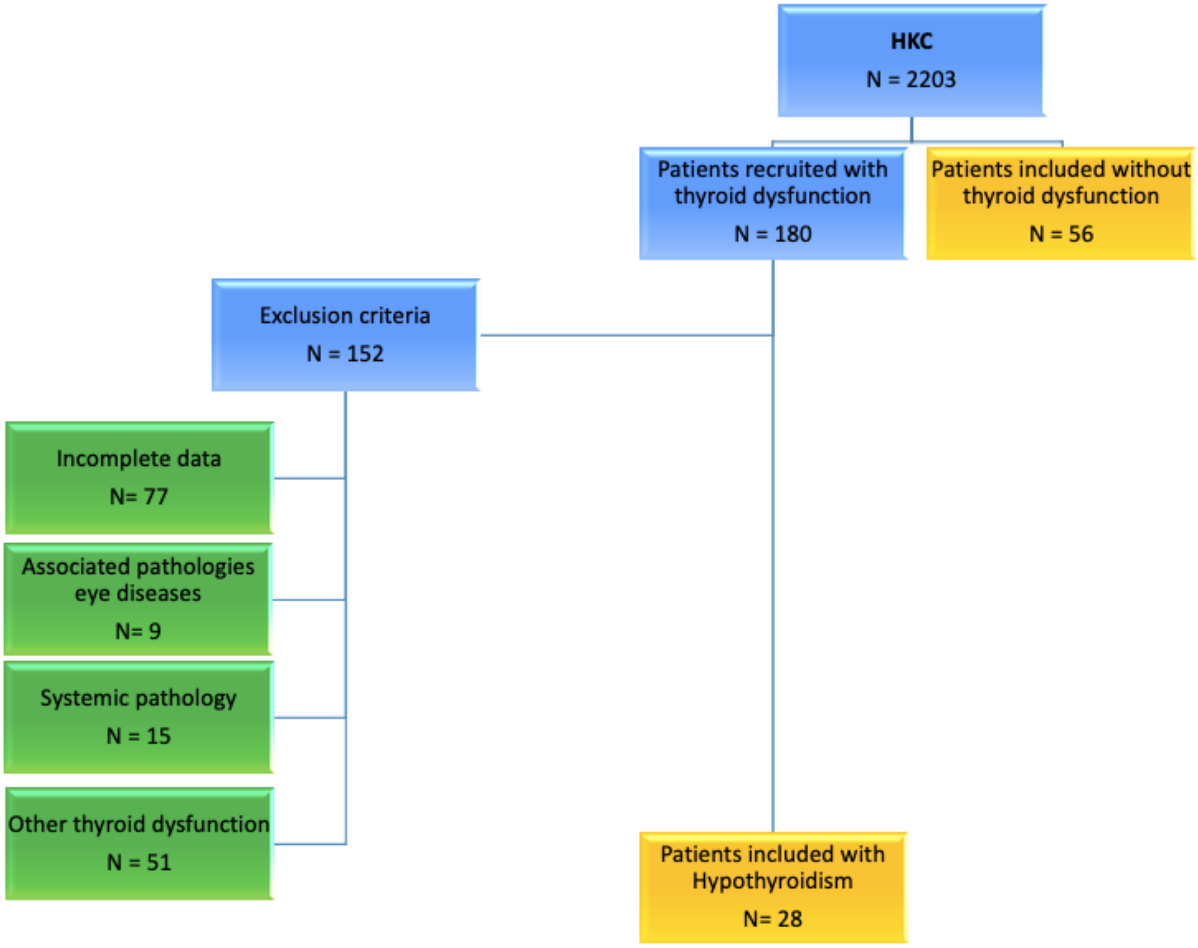
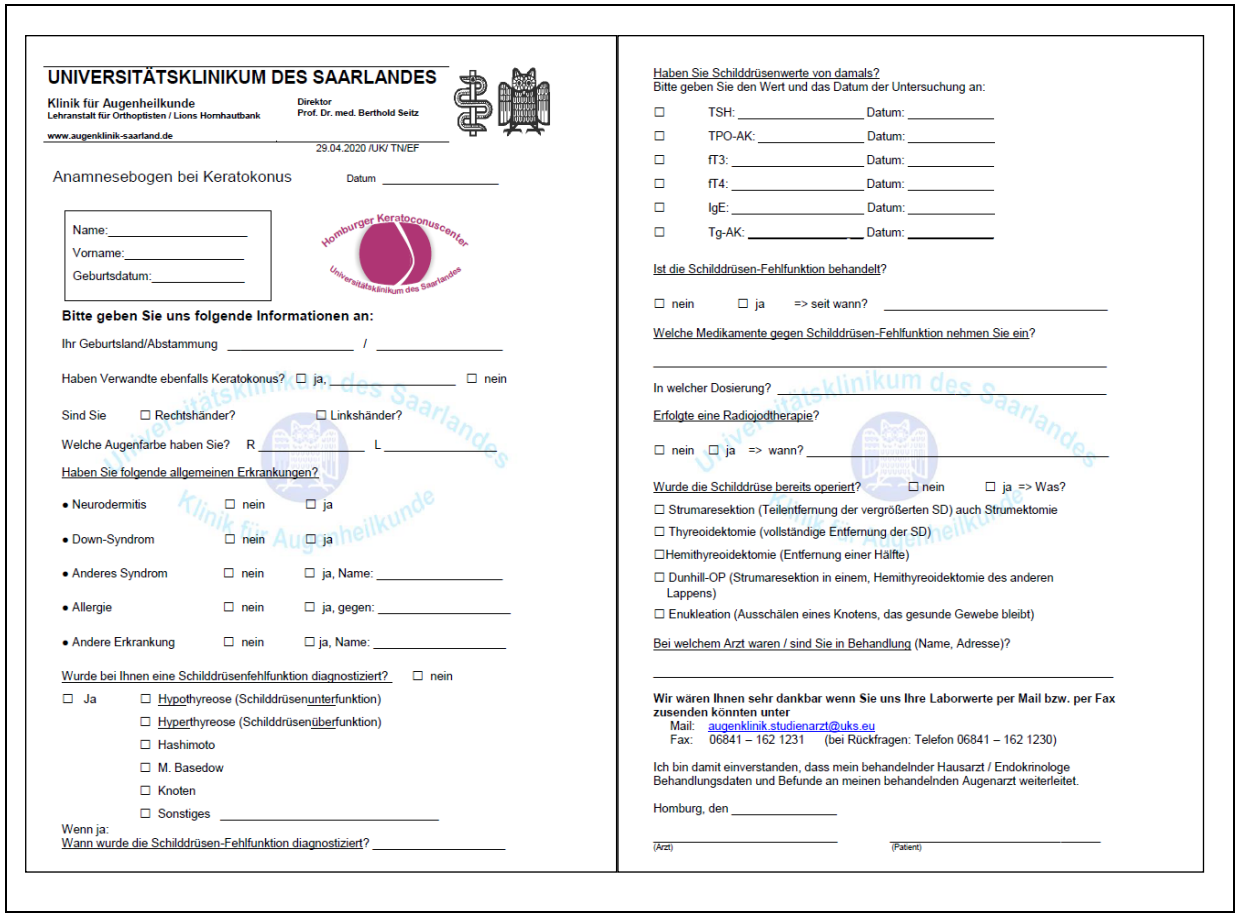


Figure 5: Flowchart of patient inclusion in this study

At the first examination, patients were required to complete a questionnaire regarding thyroid disease in their medical history (Figure 6). Information was requested on the type of thyroid dysfunction, since when the diagnosis was, how and when they had been treated. If cases were unclear, patients also received a further telephone call to clarify details about their thyroid dysfunction. Additionally, all patients were required to have a blood thyroid hormone status at the time of inclusion to the HKC including TSH, fT3, fT4, Anti-TPO to exclude undiagnosed pathologies [94].



UNIVERSITÄTSKLINIKUM DES SAARLANDES

Klinik für Augenheilkunde
Lehranstalt für Orthoptisten / Lions Hornhautbank

Direktor
Prof. Dr. med. Berthold Seitz

www.augenklinik-saarland.de

29.04.2020 /UK/ TN/EF

Anamnesebogen bei Keratokonus Datum _____

Name:_____
Vorname:_____
Geburtsdatum:_____

Homburger Keratoconuscenter
Universitätsklinikum des Saarlandes

Bitte geben Sie uns folgende Informationen an:

Ihr Geburtsland/Abstammung _____ / _____

Haben Verwandte ebenfalls Keratokonus? ☐ ja, _____ ☐ nein

Sind Sie ☐ Rechtshänder? ☐ Linkshänder?

Welche Augenfarbe haben Sie? R _____ L _____

Haben Sie folgende allgemeinen Erkrankungen?

- Neurodermitis ☐ nein ☐ ja
- Down-Syndrom ☐ nein ☐ ja
- Anderes Syndrom ☐ nein ☐ ja, Name: _____
- Allergie ☐ nein ☐ ja, gegen: _____
- Andere Erkrankung ☐ nein ☐ ja, Name: _____

Wurde bei Ihnen eine Schilddrüsenfehlfunktion diagnostiziert? ☐ nein

☐ Ja
- ☐ Hypothyreose (Schilddrüsenunterfunktion)
- ☐ Hyperthyreose (Schilddrüsenüberfunktion)
- ☐ Hashimoto
- ☐ M. Basedow
- ☐ Knoten
- ☐ Sonstiges _____

Wenn ja:
Wann wurde die Schilddrüsen-Fehlfunktion diagnostiziert? _____

Haben Sie Schilddrüsenwerte von damals?
Bitte geben Sie den Wert und das Datum der Untersuchung an:

☐ TSH: _____ Datum: _____
☐ TPO-AK: _____ Datum: _____
☐ fT3: _____ Datum: _____
☐ fT4: _____ Datum: _____
☐ IgE: _____ Datum: _____
☐ Tg-AK: _____ Datum: _____

Ist die Schilddrüsen-Fehlfunktion behandelt?

☐ nein ☐ ja => seit wann? _____

Welche Medikamente gegen Schilddrüsen-Fehlfunktion nehmen Sie ein?

_____

In welcher Dosierung? _____

Erfolgte eine Radiojodtherapie?

☐ nein ☐ ja => wann? _____

Wurde die Schilddrüse bereits operiert? ☐ nein ☐ ja => Was?

☐ Strumaresektion (Teilentfernung der vergrößerten SD) auch Strumektomie
☐ Thyreoidektomie (vollständige Entfernung der SD)
☐ Hemithyreoidektomie (Entfernung einer Hälfte)
☐ Dunhill-OP (Strumaresektion in einem, Hemithyreoidektomie des anderen Lappens)
☐ Enukleation (Ausschälen eines Knotens, das gesunde Gewebe bleibt)

Bei welchem Arzt waren / sind Sie in Behandlung (Name, Adresse)?

_____

Wir wären Ihnen sehr dankbar wenn Sie uns Ihre Laborwerte per Mail bzw. per Fax zusenden könnten unter
Mail: augenklinik.studienarzt@uks.eu
Fax: 06841 – 162 1231 (bei Rückfragen: Telefon 06841 – 162 1230)

Ich bin damit einverstanden, dass mein behandelnder Hausarzt / Endokrinologe Behandlungsdaten und Befunde an meinen behandelnden Augenarzt weiterleitet.

Homburg, den _____

(Arzt) (Patient)

Figure 6: Questionnaire regarding thyroid dysfunction within the HKC.

2.3. Methods of investigation

Every patient underwent a routine ophthalmic examination (contact lens wearing was stopped at least three days before the examination) consisting of corneal tomography, pachymetry and biomechanical parameters. We extracted the following KC parameters from the Pentacam (Pentacam HR, Oculus, Wetzlar, Germany) [46]: astigmatism, Rmin (mean of the minimum radius of curvature), Kmax (maximum Keratometry), ARC (Anterior Radius of Curvature), PRC (Posterior Radius of Curvature), TP (Thinnest Pachymetry), IVA (Index of vertical asymmetry), IHD (Index of Height Decentration), KI (Keratokonus Index) and CKI (Central Keratoconus Index) [15]. From the Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Deprew, NY, USA) [26], we extracted the CH (Corneal Hysteresis), CRF (Corneal Resistance factor) and KMI (Keratoconus Match Index) [94].

The study was divided into two parts. We first carried out a cross-sectional study to highlight a difference in the tomographic and the biomechanical parameters with respect to KC severity between the patients in the HT group compared to the patients in the WHT group. In addition, we assessed in a

longitudinal analysis, whether corneas in the HT group displayed a more severe progression than corneas in the WHT group after one-year of follow-up.

The cross-sectional and longitudinal analysis were first performed on the eye with the more severe KC stage in each patient between the HT group and the WHT group [27]. The eye selection in each patient was made according to the KC severity, based on the TKC (Tomographical Keratoconus Classification) and on the Kmax (maximal keratometry) if the TKC stage was the same in both eyes. Then, we compared the eye with the less severe KC stage in each patient. In cases where only one eye of a patient was included in the study, we used it for both analyses [94].

2.3.1 Pentacam

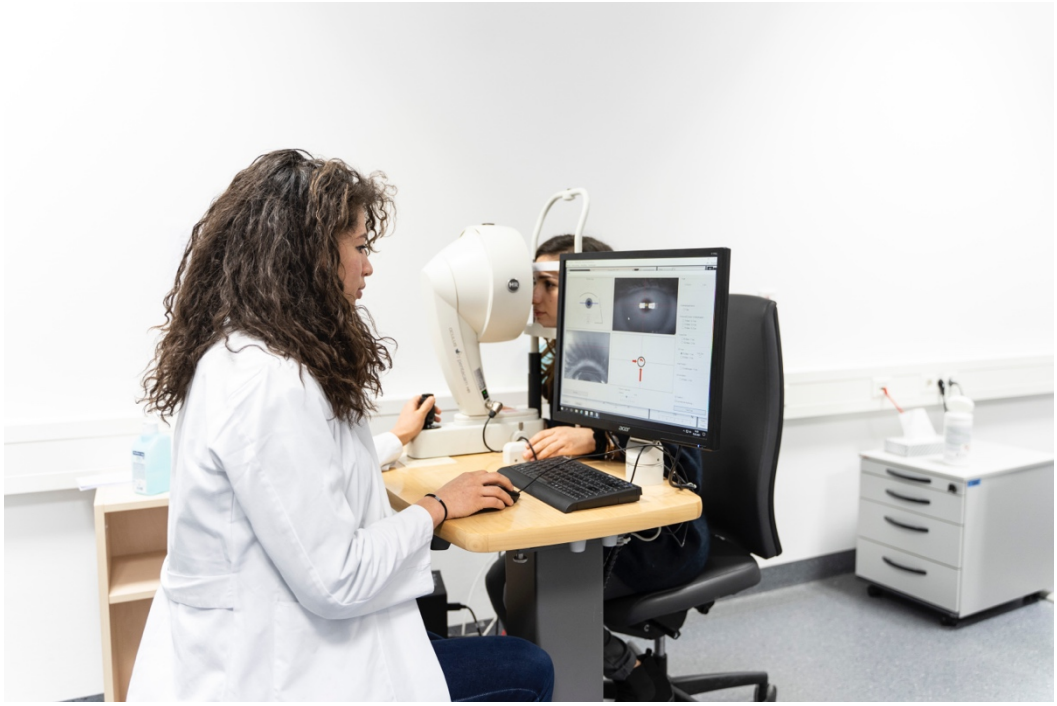


Figure 7: Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany

The Pentacam (Pentacam HR, Oculus Optikgeräte GmbH, Wetzlar, Germany) is based on a rotating Scheimpflug camera, giving three-dimensional images (25,000 distinct elevation points allowing 3D visualisation) to measure anterior and posterior corneal surfaces and other anterior segment structures (Figure 7). It is currently used as a routine tool to detect corneal ectasia such as keratoconus [85].

For the study we analyzed the following parameters:

- **Corneal Astigmatism** (expressed in diopter) is an imperfection in the curvature of the cornea. In KC the astigmatism is increased and irregular.
- **Rmin** (mean of the minimum radius of curvature, expressed in mm) is the smallest radius of sagittal/axial corneal curvature. It denotes the maximum steepness of the cone [58].
- **Kmax** (maximum Keratometry) indicates the degree of central steepening of the central cornea.
- **ARC** (Anterior Radius of Curvature, expressed in mm) is the average anterior radius of curvature in the 3.0mm zone centered on the thinnest location of the cornea [9].
- **PRC** (Posterior Radius of Curvature, expressed in mm) is the average posterior radius of curvature in the 3.0mm zone centered on the thinnest location of the cornea [9].
- **IVA** (Index of vertical asymmetry) is the mean difference between superior and inferior corneal curvature [58].

  IHD (Index of Height Decentration, expressed in µm) reflects the degree of vertical decentration of corneal elevation data [118].
- **KI** (Keratoconus Index) is the ratio between mean radius values in the upper half and lower half of the cornea [58].
- **CKI** (Central Keratoconus Index) is the ratio between mean radius of curvature in a peripheral placido ring and mean radius of curvature of central ring. It increases with severity for central keratoconus [58].

In Table 5, we have gathered the abnormal values of the above-mentioned indices. IVA, ISV, IHD, IHA, KI, CKI are increased in KC, whereas Rmin is decreased.

Table 5: Abnormal and/or tomographic values of the following index [58,118]

Index	Abnormal	Pathologic
Rmin [mm]	<6.71	<6.71
IVA	>0.28	<0.32
IHD [µm]	>0.014	>0.016
KI	>1.07	>1.07
CKI	>1.03	>1.03

2.3.2. Ocular Response Analyzer (ORA)

Figure 8: Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA)

To measure the biomechanical properties of the cornea, the patients were examined with the Ocular Response Analyzer (ORA, Reichert Ophthalmic Instruments, Depew, NY, USA) (Figure 8).

It provides data allowing KC diagnosis and classification by assessing corneal hysteresis and resistance [48,68]. The following parameters were analyzed: Keratoconus Match Index (KMI), Corneal Hysteresis (CH), and Corneal Resistance Factor (CRF). A previous study found the two latter two values to be significantly lower in KC compared to normal and post-LASIK subjects [26].

The ORA is based on a dynamic bidirectional applanation process. A controlled amount of air pulse such as the traditional air-puff tonometer deforms the cornea (Figure 9), and the kinetics of the cornea during inward and outward movement are analyzed [73].

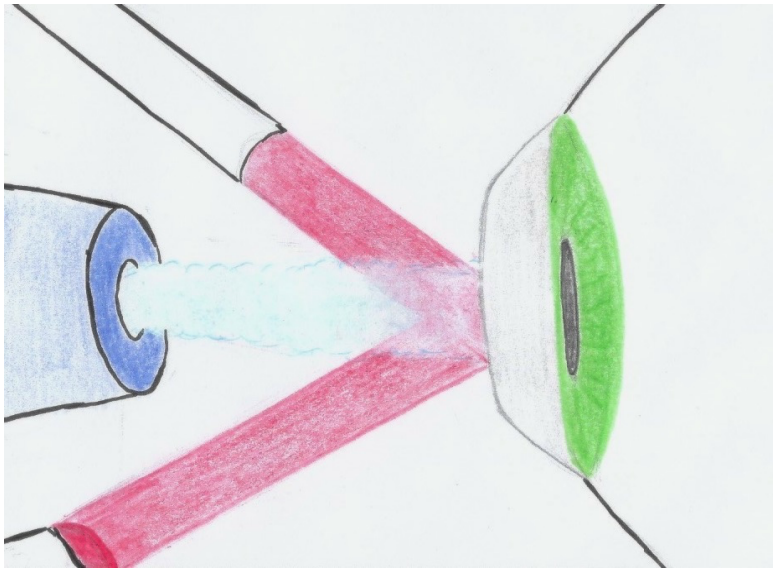


Figure 9: Schema of air pulse causing the corneal applanation.

The air-puff causes the cornea to deform inwards, passing a flattening point or applanation point (peak 1) and taking on a concave shape. The cornea then deforms outwards, passes the applanation point (peak 2) again and returns to its original shape. Due to the viscoelastic properties of the cornea, the return to applanation (peak 2) is slower and the pressures P1 and P2 are therefore different from each other; the pressure during outwards flattening (peak 2) is lower than the pressure during inwards flattening. The greater the difference is between the two measures, the more mechanical energy was absorbed by the cornea [69]. An electrooptical collimator detects light reflecting from the central 3

mm of the cornea throughout the 20-millisecond measurement, with the resulting voltage change being recorded to create the pressure-corneal deformation profile [73] (Figure 10).

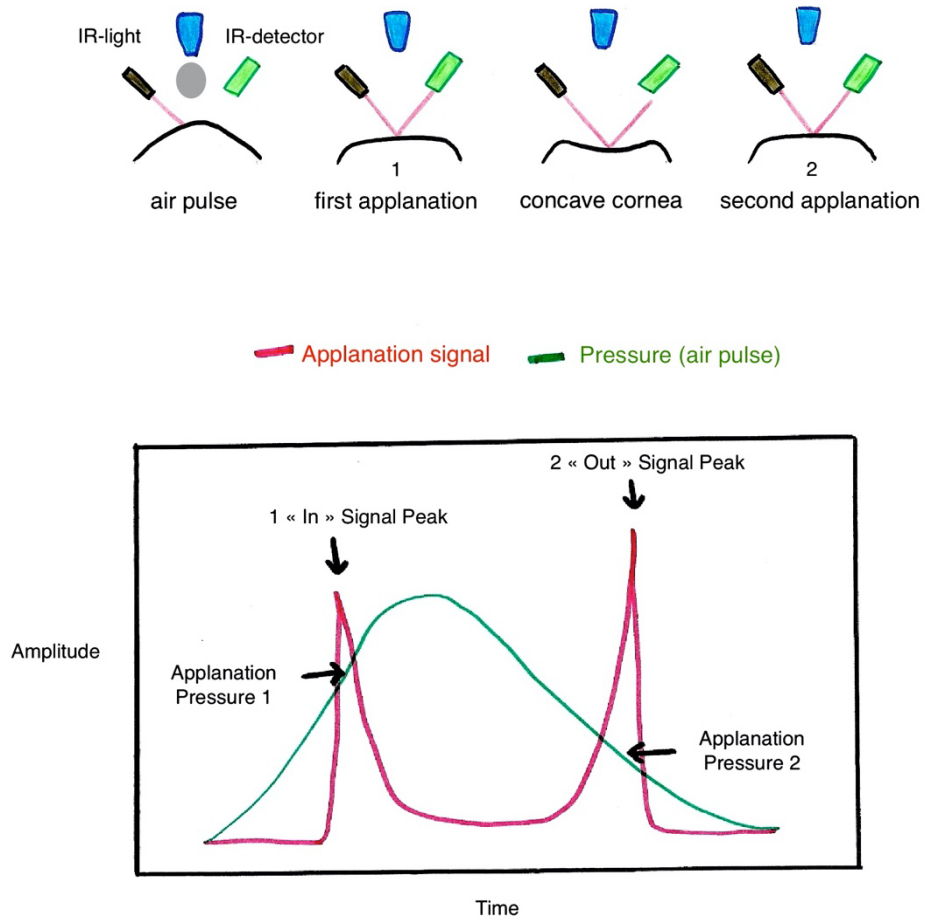


Figure 10: The different steps during a measurement by the ORA (Reichert Ophthalmic Instruments, Depew, NY, USA)

Corneal Hysteresis (CH), (expressed in mmHg) corresponds to the difference between the pressures at the 2 applanation times [125]. It reflects the capability of the corneal tissue to absorb and dissipate energy (damping) due to its viscoelastic properties [73].

Corneal Resistance Factor (CRF), (expressed in mmHg) is based on CH, calculated as a linear function of the pressures at the 2 applanation times using a proprietary algorithm, and is thought to indicate the total resistance of the cornea [86].

Several studies, reported that keratoconic corneas have a lower CH and CRF than normal corneas [63,73,86,100] and appears to be related to the severity of the condition [100,125]. The mean CH and CRF values in normal corneas are around 11mmHg and respectively around 7.5 and 6.5mmHg in a keratoconic cornea [86]. However, in subclinical KC, there is no significant difference in comparison to normal eyes [63,86,100].

The Keratoconus Match Index (KMI) is the outcome of a neural network calculation of seven waveform scores and represents the similarity of the waveform of the examined eye when compared against the same average waveform scores of the KC eyes in the machine's database. The value in a normal cornea is around 1 and <0.7 in the keratoconic cornea [38,68].

2.4 Statistical analysis

All of the data weas collected into a Microsoft excel sheet (Microsoft Corporation, Redmond, Washington, USA) designed for specific data collection and evaluation. Statistical processing using IBM SPSS for windows, version 20 (SPSS, Inc., Chicago, IL, USA) was carried out on the basis of the database created with Microsoft excel sheets.

For the cross-sectional analysis, we analyzed the Pentacam- and ORA-parameters descriptively and the results were expressed as mean ± standard deviation (SD). The corresponding p-values were calculated with t-test for normally distributed parameters, otherwise a non-parametric Mann-Whitney U-test was used. For the longitudinal analysis, we analyzed the Pentacam parameters descriptively and the results were expressed as mean difference (Δ) ± standard deviation (SD) between the first examination and the examination after a one-year of follow-up. The corresponding p-value was also calculated with a t-test when normally distributed or Mann-Whitney U-test otherwise. P-values below 0.05 were considered statistically significant.

3. Results

3.1. Demographic data

For our study, we recruited 84 patients into two groups. In the HT group (with KC and Hypothyroidism), 28 patients were included, of which 19 male and 9 were female (67% male and 33% female), with a mean age of 40.3±10.6 years (range 14-57). The WHT group was selected from the HKC database to match gender and age of the HT group. There were 56 patients, of which 38 male and 18 female (67% male and 33% female), with a mean age of 40.3±10.9 years (range 14-57). In addition, 53% and 55% of patients wore contact lenses in the HT and WHT groups respectively. Table 6 shows the distribution of KC severity according to the TKC. Table 7 displays the values for serum thyroid hormones: TSH, fT3, fT4, anti-TPO between HT and WHT group [94].

Table 6: Keratoconus severity according to the Topographic Keratoconus Classification (TKC) measured with Pentacam (Oculus Optikgeräte GmbH, Wetzlar Germany) comparing the HT (with hypothyroidism) and the WHT group (without thyroid dysfunction) (N=84).

Grade	Group NH (N = 28 eyes)	Group WHT (N = 56 eyes)
1	7 (25%)	15 (27.5%)
2	13 (46.5%)	21 (37.5%)
3	6 (21.5%)	18 (32%)
4	2 (7%)	2 (3.5%)
Classification into grade 1 (suspect), 2 (mild), 3 (moderate), or 4 (severe)		

Table 7: Mean ± standard deviation (SD) values and corresponding p-values for serum thyroid hormones: TSH (thyroid-stimulating hormone), fT3 (free triiodthyronin), fT4 (free thyroxine), anti-TPO (thyroid peroxidase antibodies) comparing keratoconus (KC) patients with hypothyroidism (HT) and without thyroid dysfunction (WHT).

Disease group	TSH	fT3	fT4
Group HT	1.80±1.37	3.36±0.53	1.38±0.21
Group WHT	1.44±0.63	3.38±0.56	1.24±0.14
P Value	0.39*	0.22*	0.01*
P* values compare HT and WHT groups with Independent-samples-Mann-Whitney U test			

3.2. Comparison between the HT and WHT groups concerning the eye with the more severe KC stage in each patient

We compared the corneal tomographic and biomechanical parameters in the eye with the more severe KC stage in each patient between the KC patients in the HT group and WHT group at the time of inclusion in the HKC (Table 8 and 9). We also compared the change of the tomographic parameters in the eye with the more severe KC stage in each patient between the HT and the WHT group from the first preliminary examination to a one-year follow-up (Table 8). We did not detect any significant differences between the HT and WHT group neither at baseline nor after a one-year of follow-up [94].

Table 8 : Mean ± standard deviation values and corresponding p-values for astigmatism, Rmin (mean of the minimum radius of curvature), Kmax (maximum Keratometry), ARC (Anterior Radius of Curvature), PRC (Posterior Radius of Curvature), TP (Thinnest Pachymetry), IVA (Index of vertical asymmetry), IHD (Index of Height Decentration), KI (Keratokonus Index) and CKI (Central Keratoconus Index) using Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) in the more severe eye between keratoconus (KC) patients with hypothyroidism (HT) and without thyroid dysfunction (WHT) at the first examination baseline. Mean difference (Δ) ± standard deviation of the same tomographic values between the first examination and the one-year follow-up.

	Cross-sectional study at the time of first examination			Longitudinal study (mean difference between first examination and at one-year follow-up)		
	With Hypothyroidism Group HT (N= 28 eyes)	Without Hypothyroidism Group WHT (N=56 eyes)	P-Value	With Hypothyroidism Group HT (N= 28 eyes)	Without Hypothyroidism Group WHT (N=56 eyes)	P-Value
Astigmatism [D]	2.84±1.64	2.88±1.83	0.37[2]	-0.08±1.10	-0-05±0.64	0.60[2]
Rmin [mm]	7.32±0.51	7.33±0.59	0.77[2]	-0.03±0.20	0.04±0.25	0.12[2]
Kmax [D]	52.73±6.06	53.09±6.35	0.63[2]	-0.35±1.79	-0.21±1.92	0.46[2]
ARC [mm]	6.95±0.58	6.95±062	0.30[2]	0.06±0.15	0.01±0.22	0.35[2]
PRC [mm]	5.31±0.65	5.18±0.71	0.61[2]	0.04±0.21	-0.01±0.33	0.90[2]
TP [µm]	479.7±53.82	472.1±46.20	0.19[2]	4.96±15.02	2.67±13.35	0.43[2]
IVA	0.80±045	0.83±0.45	0.48[2]	-0.08±0.14	-0.01±0.15	0.75[2]
IHD	0.10±0.07	0.21±0.89	0.95[1]	-0.01±0.025	0.11±0.89	0.76[1]
KI	1.17±0.15	1.19±0.14	0.41[1]	-0.02±0.04	-0.01±0.05	0.70[1]
CKI	1.03±0.05	1.03±0.04	0.44[1]	0.01±0.02	-0.01±0.01	0.07[1]

[1] P-values compare the HT and WHT group with non-parametric test (Independent-samples-Mann-Whitney-U-test)

[2] P-values compare the HT and WHT group with parametric test (t-test)

Table 9 : Mean ± standard deviation values and corresponding p-values for CH (Corneal Hysteresis), CRF (Corneal Resistance Factor) and KMI (Keratoconus Match Index) using the Ocular Response Analyzer (ORA, Reichert Ophthalmic instruments, Depew, NY, USA) in the more severe eyes comparing keratoconus (KC) patients with hypothyroidism (HT) and without thyroid dysfunction (WHT) at the time of first examination.

	With Hypothyroidism Group HT (N = 28 eyes)	Without Hypothyroidism Group WHT (N=56 eyes)	P Value
CH	8.36±1.84	8.37±2.00	0.98[2]
CRF	7.61±1.94	7.42±2.40	0.65[2]
KMI	0.16±0.42	0.09±0.36	0.47[2]

[1] P-Values compare the HT and WHT group with non-parametric test (Independent-samples-Mann-Whitney-U-test)

[2] P-Values compare the HT and WHT group with parametric test (t-test)

3.3. Comparison between HT and WHT groups concerning the eye with the less severe KC stage in each patient

In Table 10 and 11, we also compared the mean ± SD of tomographic and biomechanical values in the less severe KC eyes between the HT and the WHT group at the first examination. Then, we compared the mean difference (Δ) ± SD of tomographic values in the less severe eyes from each patient between the HT and the WHT group between the first examination and after the one-year follow-up. There were no significant differences between the HT and WHT group [94].

Table 10: Mean ± standard deviation values and corresponding p-values for astigmatism, Rmin (mean of the minimum radius of curvature), Kmax (maximum Keratometry), ARC (Anterior Radius of Curvature), PRC (Posterior Radius of Curvature), TP (Thinnest Pachymetry), IVA (Index of vertical asymmetry), IHD (Index of Height Decentration), KI (Keratokonus Index) and CKI (Central Keratoconus Index) using Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) in the less severe eyes between keratoconus (KC) patients with hypothyroidism (HT) and without thyroid dysfunction (WHT) at the first examination. Mean difference (Δ) ± standard deviation of the same tomographical values between the first examination and after one-year of follow-up.

	Cross-sectional study at the time of first examination			Longitudinal study (mean difference between first examination and at one-year follow-up)		
	With Hypothyroidism Group HT (N= 28 eyes)	Without Hypothyroidism Group WHT (N=56)	P-Value	With Hypothyroidism Group HT (N= 28 eyes)	Without Hypothyroidism Group WHT (N=56)	P-Value
Astigmatism [D]	2,84±1.61	2.59±1.88	0.54[1]	-0.21±1.02	0.01±0.77	0.99[1]
Rmin [mm]	6.52±0.69	6.58±0.73	0.06[2]	0.06±0.20	0.12±0.58	0.25[2]
Kmax [D]	51.8±5.14	51.9±6.23	0.10[1]	0.45±1.82	-0.39±1.87	0.42[1]
ARC [mm]	6.97±0.57	7.01±0.62	0.76[1]	-0.06±0.16	0.08±0.47	0.24[1]
PRC [mm]	5.32±0.64	5.31±0.73	0.76[1]	0.03±0.21	-0.02±0.39	0.95[1]
TP [µm]	480.5±53.1	477.38±45.1	0.79[2]	3.78±15.83	2.19±12.23	0.40[2]
IVA	0.78±0.44	0.75±0.43	0.76[1]	-0.09±0.15	-0.20±0.15	0.73[1]
IHD	0.08±0.05	0.20±0.89	0.58[1]	-0.13±0.02	0.11±0.89	0.73[1]
KI	1.14±0.11	1.17±0.12	0.65[1]	-0.27±0.50	-0.01±0.05	0.77[1]
CKI	1.02±0.04	1.03±0.04	0.66[1]	0.01±0.02	-0.01±0.01	0.06[1]

[1] P-values compare the HT and WHT group with non-parametric test (Independent-samples-Mann-Whitney-U-test)

[2] P-values compare the HT and WHT group with parametric test (T-test)

Table 11: Mean ± standard deviation values and corresponding p-values for CH (Corneal Hysteresis), CRF (Corneal Resistance Factor) and KMI (Keratoconus Match Index) using the Ocular Response Analyzer (ORA, Reichert Ophthalmic instruments, Depew, NY, USA) in the less severe eyes comparing keratoconus (KC) patients with hypothyroidism (HT) and without thyroid dysfunction (WHT) at the time of first examination.

	With Hypothyroidism Group HT (N = 28 eyes)	Without Hypothyroidism Group WHT (N=56 eyes)	P Value
CH	8.36±1.84	8.26±1.69	0.81[2]
CRF	7.60±1.95	7.34±2.27	0.60[2]
KMI	0.16±0.42	0.17±0.37	0.90[2]

[1] P-values compare the HT and WHT group with non-parametric test (Independent-samples-Mann-Whitney-U-test)

[2] P-values compare the HT and WHT group with parametric test (t-test)

4. Discussion

Keratoconus (KC) was described in detail in 1998 by Rabinowitz [90]. Despite extensive research over the last few decades, the etiology and pathogenesis for development of KC remain insufficiently understood. Several hypotheses propose genetic, environmental, immunological, and biochemical causes and mechanisms.

Over the last decades, there has been increasing evidence that hormonal influences may play a role in the pathogenesis of corneal ectasia. The importance of thyroid hormones in corneal dehydration and transparency during embryonic development was previously described in 1964 [17,18]. Masterson et al. showed that the thyroxine (T4) treatment accelerated the development of endothelial cells and had a role in the development in chicken's cornea [75]. Furthermore, the presence of T4 receptors (T4Rs) has been proven in chicken's cornea [14]. In humans, T4Rs have been found in the lacrimal gland, confirming that the tear producing gland is indeed a target organ of T4 [22]. Some studies have demonstrated that T4 is increased in the tears of KC patients [115], in particular during the progression of KC [57] but also in the aqueous humor [106].

Based on these previous findings, we analyzed the relationship between KC and hypothyroidism in our cross-sectional study and more specifically, the impact of hypothyroidism on corneal tomography and biomechanics. It showed no statistically significant difference neither for tomographic (Astigmatism, Rmin, Kmax, ARC, PRC, TP, IVA, IHD, KI, CKI) nor for biomechanical (KMI, CH, CRF) parameters between the HT group with hypothyroidism and the WHT group without thyroid dysfunction. To our knowledge, this is the first study of its kind introducing this unique design comparing the tomographic and biomechanical parameters in KC patients related to hypothyroidism [94].

Flaskó et al. compared 626 subjects, divided in 4 groups with and without KC, with and without hypothyroidism. They found that approximately 80% of patients diagnosed with KC were classified as euthyroid, whereas only 20% with KC and hypothyroidism and concluded that there was no association between KC and hypothyroidism and even that KC occurs more frequently in euthyroid patients [33]. Alhawari et al. investigated whether KC was associated with autoimmune thyroid disease. They found no significant difference in the prevalence of KC and/or suspected KC between patients with (2.9%) or without autoimmune thyroid disease (3.2%) [1].

On the contrary, previous studies about the prevalence of hypothyroidism in KC patients appear to indicate that the prevalence of KC is higher in patients with hypothyroidism than in a healthy population. Indeed, Appelbaum reported in 1936 that KC patients often show unusual signs of hypothyroidism [4]. El-Massary et al. suggested a higher prevalence of thyroid gland dysfunction among KC patients compared to healthy controls (5.35% and 1.1% respectively). However, the higher prevalence was regardless of the type of thyroid dysfunction. Moreover, the prevalence in their control group was lower than the previously reported prevalence of thyroid dysfunction in adults in the general population (3.82%) in Europe [25,40]. This can possibly be explained by environmental factors when comparing Arab/Middle Eastern countries and Europe.

The results of our study were limited by the small sample size, which may ultimately affect the power and, therefore, the impact of the study. In the HT group of KC patients with hypothyroidism, we solely included 28 patients out of over 180 patients with thyroid dysfunction. It was essential to make up the most homogeneous group possible in order to eliminate possible biases induced by other pathological mechanisms such as pathologies known or suspected to cause a progression of KC, which explains the amount of exclusion criteria. We also excluded Hashimoto's thyroiditis to eliminate the potential influence of antibodies in the development of KC, independently of thyroid values. In addition, 77 KC

patients with thyroid dysfunction were excluded due to a lack of data (patients lost to follow-up and unreachable).

Selection bias related to the Department of Ophthalmology of our hospital which is specialized in corneal pathologies has to be taken into account. A certain number of patients are regularly followed by their “treating” or “primary care” ophthalmologist in the context of their KC and are referred to us only if there is a suspicion of KC progression and/or with indication for sugery. Patients who underwent surgery between the first appointment and the follow-up at one-year had to be excluded from the longitudinal analysis, because the tomographic and biomechanical values could not be compared on an already operated eye. Beyond this, other patients are referred to us for a second medical opinion after surgery. Patients who have already undergone surgery once were excluded from our study because a comparison of the corneal parameters is not reliable on a previously operated eye and, therefore, the worse advanced or progressive cases were excluded.

In the HT group, the patients with hypothyroidism were selected by means of the medical questionnaire and were already being medically treated, which may lead to a bias due to normalization of thyroid values. Ethically, it was not possible to leave them untreated in order to accurately analyze the effect of hypothyroidism on the cornea during a one-year follow-up period. However, we surmised that in case hypothyroidism led to an irreversible worsening of KC, there would be a significant difference in KC severity at the first baseline examination. Secondly, the possible fluctuation of thyroid values despite treatment could have led to a worsening of the KC in the longitudinal analysis, which was not the case. The hypothesis that hypothyroidism does reversibly induce corneal changes in the acute phase, has already been highlighted [42,43]. Bahçeci et al. described an increase in corneal thickness during the acute phase of hypothyroidism in normal cornea patients, which decreased with hormonal treatment. After stabilization of the thyroid parameters (after 9 months), the cornea returned to its original thickness [6]. Other authors also proved transient and reversible corneal changes in KC, associated with hormonal changes during gestation [42,55].

The lack of a difference between our two groups with or without hypothyroidism could also be explained by the hypothesis of a single local T4 effect on the cornea, independent of the thyroid dysfunction (serum’s level). This may be consistent with the finding of Kahàn et al. who demonstrated that tear T4 levels were higher during the progression of KC and declined when corneal curvature reached a steady value [57], and the finding of Stachon et al. which highlighted an increase of the T4

level in KC patients in the aqueous humor in comparison to patients with healthy corneas [106]. In our study neither tear nor aqueous humor thyroxin was measured.

The higher prevalence of KC patients in some disorders such as Down's syndrome could also be explained by the fact that they tend to often rub their eyes.

Regarding the reported progression tendency of KC during pregnancy, several hypotheses have been put forward. In our study we concluded that hypothyroidism does not, in fact lead to irreversible changes in the cornea. This does not exclude the possibility of KC aggravation in an acute phase of thyroid disease, which would be consistent with the studies cited above [42,43,55]. A second hypothesis could well be that the corneal changes during gestation are not due to thyroid changes but instead to hormonal changes such as estrogen or progesterone change. Based on their report that women have a higher tendency to develop keratectasia after laser-assisted in situ keratomileusis (LASIK) than men, Spoerl et al. evaluated the influence of estrogen receptors in porcine corneas and showed the influence of estrogen in the biomechanical stability of the cornea [105]. The expression of sex steroid hormone receptors in the human cornea were also reported by Suzuki et al. [112]. However, the study of Fink et al. opposes this last hypothesis. They investigated the influence of gender and hormone status on the severity and progression of KC and could not verify any significant differences between midlife males, "hormone-active" females, and "hormone-inactive" females [31].

To conclude, the severity of keratoconus based on biomechanical and tomographic values does not seem to depend on thyroid dysfunction.

5. Bibliography

1. Alhawari HH, Khader YS, Alhawari HH, Alomari AF, Abbasi HN, El-Faouri MS, Al Bdour MD (2018) Autoimmune Thyroid Disease and Keratoconus: Is There an Association? Int J Endocrinol 2018:7907512

2. Alipour F, Letafatnejad M, Beheshtnejad AH, Mohammadi S-F, Ghaffary SR, Hassanpoor N, Yaseri M (2016) Corneal biomechanical findings in contact lens induced corneal warpage. J Ophthalmol 2016:5603763

3. Amsler M (1946) Kératocône classique et kératocône fruste; arguments unitaires. Ophthalmologica 111:96–101

4. Appelbaum A (1936) Keratoconus. Arch Ophthalmol 15:900–921

5. Asvold BO, Vatten LJ, Bjøro T (2013) Changes in the prevalence of hypothyroidism: the HUNT Study in Norway. Eur J Endocrinol 169:613–620

6. Bahçeci UA, Ozdek S, Pehlivanli Z, Yetkin I, Onol M (2005) Changes in intraocular pressure and corneal and retinal nerve fiber layer thicknesses in hypothyroidism. Eur J Ophthalmol 15:556–561

7. Bawazeer AM, Hodge WG, Lorimer B (2000) Atopy and keratoconus: a multivariate analysis. Br J Ophthalmol 84:834–836

8. Beardsley TL, Foulks GN (1982) An association of keratoconus and mitral valve prolapse. Ophthalmology 89:35–37

9. Belin MW, Duncan JK (2016) Keratoconus: The ABCD Grading System. Klin Monbl Augenheilkd 233:701–707

10. Bilgihan K, Hondur A, Sul S, Ozturk S (2011) Pregnancy-induced progression of keratoconus. Cornea 30:991–994

11. Budo C, Bartels MC, van Rij G (2005) Implantation of Artisan toric phakic intraocular lenses for the correction of astigmatism and spherical errors in patients with keratoconus. J Refract Surg 21:218–222

12. Chaker L, Bianco AC, Jonklaas J, Peeters RP (2017) Hypothyroidism. Lancet 390:1550–1562

13. Chopra I, Jain AK (2005) Between eye asymmetry in keratoconus in an Indian population. Clin Exp Optom 88:146–152

14. Conrad AH, Zhang Y, Walker AR, Olberding LA, Hanzlick A, Zimmer AJ, Morffi R, Conrad GW (2006) Thyroxine affects expression of KSPG-related genes, the carbonic anhydrase II gene, and KS sulfation in the embryonic chicken cornea. Invest Ophthalmol Vis Sci 47:120–132

15. Correia FF, Ramos I, Lopes B, Salomão MQ, Luz A, Correa RO, Belin MW, Ambrósio RJ (2012) Topometric and tomographic indices for the diagnosis of keratoconus. Int J Kerat Ect Cor Dis 1:92–99

16. Coulombre AJ, Coulombre JL (1958) Corneal development. I. Corneal transparency. J Cell Comp Physiol 51:1–11

17. Coulombre AJ, Coulombre JL (1964) Corneal development. III The role of thyroid in dehydratation and the development of transparency. Exp Eye Res 3:105–114

18. Davidson AE, Hayes S, Hardcastle AJ, Tuft SJ (2014) The pathogenesis of keratoconus. Eye (Lond) 28:189–195

19. Daxer A, Fratzl P (1997) Collagen fibril orientation in the human corneal stroma and its implication in keratoconus. Invest Ophthalmol Vis Sci 38:121–129

20. Dayan CM (2001) Interpretation of thyroid function tests. Lancet 357:619–624

21. DelMonte DW, Kim T (2011) Anatomy and physiology of the cornea. J Cataract Refract Surg 37:588–598

22. Dias AC, Módulo CM, Jorge AG, Braz AM, Jordão AA, Filho RB, de Paula JS, Rocha EM (2007) Influence of thyroid hormone on thyroid hormone receptor beta-1 expression and lacrimal gland and ocular surface morphology. Invest Ophthalmol Vis Sci 48:3038–3042

23. Dua HS, Faraj LA, Said DG, Gray T, Lowe J (2013) Human corneal anatomy redefined: a novel pre-Descemet's layer (Dua's layer). Ophthalmology 120:1778–1785

24. Edwards M, McGhee CN, Dean S (2001) The genetics of keratoconus. Clin Exp Ophthalmol 29:345–351

25. El-Massry A, Doheim MF, Iqbal M, Fawzy O, Said OM, Yousif MO, Badawi AE, Tawfik A, Abousamra A (2020) Association between keratoconus and thyroid gland dysfunction: A cross-sectional case-control study. J Refract Surg 36:253–257

26. Emre S, Doganay S, Yologlu S (2007) Evaluation of anterior segment parameters in keratoconic eyes measured with the Pentacam system. J Cataract Refract Surg 33:1708–1712

27. Eppig T, Spira-Eppig C, Goebels S, Seitz B, El-Husseiny M, Lenhart M, Papavasileiou K, Szentmáry N, Langenbucher A (2018) Asymmetry between left and right eyes in keratoconus patients increases with the severity of the worse eye. Curr Eye Res 43:848–855

28. Erie JC, Patel SV, McLaren JW, Nau CB, Hodge DO, Bourne WM (2002) Keratocyte density in keratoconus. A confocal microscopy study. Am J Ophthalmol 134:689–695

29. Etzine S (1954) Conical cornea in identical twins. S Afr Med J 28:154–155

30. Fini ME, Stramer BM (2005) How the cornea heals: cornea-specific repair mechanisms affecting surgical outcomes. Cornea 24:S2–S11

31. Fink BA, Sinnott LT, Wagner H, Friedman C, Zadnik K, Group CS (2010) The influence of gender and hormone status on the severity and progression of keratoconus. Cornea 29:65–72

32. Fisher DA, Sack J, Oddie TH, Pekary AE, Hershman JM, Lam RW, Parslow ME (1977) Serum T4, TBG, T3 uptake, T3, reverse T3, and TSH concentrations in children 1 to 15 years of age. J Clin Endocrinol Metab 45:191–198

33. Flaskó Z, Zemova E, Eppig T, Módis L, Langenbucher A, Wagenpfeil S, Seitz B, Szentmáry N (2019) Hypothyroidism is not associated with keratoconus disease: analysis of 626 subjects. J Ophthalmol 2019:3268595

34. Fleischer B (1906) Über Keratokonus und eigenartige Pigmentbildung in der Kornea. München Med Wschr 53:625–6

35. Flockerzi E, Daas L, Seitz B (2020) Structural changes in the corneal subbasal nerve plexus in keratoconus. Acta Ophthalmol 98:e928–e932

36. Flockerzi E, Xanthopoulou K, Goebels SC, Zemova E, Razafimino S, Hamon L, Jullien T, Klühspies U, Eppig T, Langenbucher A, Seitz B (2021) Keratoconus staging by decades: a baseline ABCD classification of 1000 patients in the Homburg Keratoconus Center. Br J Ophthalmol 105:1069–1075

37. Forstot SL, Goldstein JH, Damiano RE, Dukes DK (1988) Familial keratoconus. Am J Ophthalmol 105:92–93

38. Fraenkel D, Hamon L, Daas L, Flockerzi E, Suffo S, Eppig T, Seitz B (2021) Tomographically normal partner eye in very asymmetrical corneal ectasia: biomechanical analysis. J Cataract Refract Surg 47:366–372

39. Galvis V, Sherwin T, Tello A, Merayo J, Barrera R, Acera A (2015) Keratoconus: an inflammatory disorder? Eye 29:843–859

40. Garmendia Madariaga A, Santos Palacios S, Guillén-Grima F, Galofré JC (2014) The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. J Clin Endocrinol Metab 99:923–931

41. Gasset AR, Hinson WA, Frias JL (1978) Keratoconus and atopic diseases. Ann Ophthalmol 10:991–994

42. Gatzioufas Z, Thanos S (2008) Acute keratoconus induced by hypothyroxinemia during pregnancy. J Endocrinol Invest 31:262–266

43. Gatzioufas Z, Panos GD, Brugnolli E, Hafezi F (2014) Corneal topographical and biomechanical variations associated with hypothyroidism. J Refract Surg 30:78–79

44. Gatzioufas Z, Panos GD, Gkaragkani E, Georgoulas S, Angunawela R (2017) Recurrence of keratoconus after deep anterior lamellar keratoplasty following pregnancy. Int J Ophthalmol 10:1011–1013

45. Giugliani R, Federhen A, Rojas MVM, Vieira T, Artigalás O, Pinto LL, Azevedo AC, Acosta A, Bonfim C, Lourenço CM, Kim CA, Horovitz D, Bonfim D, Norato D, Marinho D, Palhares D, Santos ES, Ribeiro E, Valadares E, Guarany F, de Lucca GR, Pimentel H, de Souza IN, Correa J, Fraga JC, Goes JE, Cabral JM, Simionato J, Llerena J, Jardim L, Giuliani L, da Silva LCS, Santos ML, Moreira MA, Kerstenetzky M, Ribeiro M, Ruas N, Barrios P, Aranda P, Honjo R, Boy R, Costa R, Souza C, Alcantara FF, Avilla SGA, Fagondes S, Martins AM (2010) Mucopolysaccharidosis I, II, and VI: Brief review and guidelines for treatment. Genet Mol Biol 33:589–604

46. Gobbe M, Guillon M (2005) Corneal wavefront aberration measurements to detect keratoconus patients. Cont Lens Anterior Eye 28:57–66

47. Goebels S, Seitz B, Langenbucher A (2013) Diagnostik und stadiengerechte Therapie des Keratokonus: Eine Einführung in das Homburger Keratokonuscenter (HKC). Ophthalmologe 110:808–809

48. Goebels S, Eppig T, Wagenpfeil S, Cayless A, Seitz B, Langenbucher A (2017) Complementary keratoconus indices based on topographical interpretation of biomechanical waveform parameters: A supplement to established keratoconus indices. Comput Math Method M 2017:1–7

49. Goebels S, Eppig T, Seitz B, Szentmàry N, Cayless A, Langenbucher A (2018) Endothelial alterations in 712 keratoconus patients. Acta Ophthalmol 96:e134–e139

50. Goldich Y, Barkana Y, Gerber Y, Rasko A, Morad Y, Harstein M, Avni I, Zadok D (2009) Effect of diabetes mellitus on biomechanical parameters of the cornea. J Cataract Refract Surg 35:715–719

51. Gomes JA, Tan D, Rapuano CJ, Belin MW, Ambrósio Jr R, Guell JL, Malecaze F, Nishida K, Sangwan VS (2015) Global consensus on keratoconus and ectatic diseases. Cornea 34:359–369

52. Gore DM, Shortt AJ, Allan BD (2013) New clinical pathways for keratoconus. Eye 27:329–339

53. Gorskova EN, Sevost'ianov EN (1998) Epidemiology of keratoconus in the Urals. Vestnik Oftalmologii 114:38–40

54. Heon E, Greenberg A, Kopp KK, Rootman D, Vincent AL, Billingsley G, Priston M, Dorval KM, Chow RL, McInnes RR (2002) VSX1: a gene for posterior polymorphous dystrophy and keratoconus. Human molecular genetics 11:1029–1036

55. Hoogewoud F, Gatzioufas Z, Hafezi F (2013) Transitory topographical variations in keratoconus during pregnancy. J Refract Surg 29:144–146

56. Jonas JB, Nangia V, Matin A, Kulkarni M, Bhojwani K (2009) Prevalence and associations of keratoconus in rural maharashtra in central India: the central India eye and medical study. Am J Ophthalmol 148:760–765

57. Kahán IL, Varsányi-Nagy M, Tóth M, Nádrai A (1990) The possible role of tear fluid thyroxine in keratoconus development. Exp Eye Res 50:339–343

58. Kanellopoulos AJ, Asimellis G (2013) Revisiting keratoconus diagnosis and progression classification based on evaluation of corneal asymmetry indices, derived from Scheimpflug imaging in keratoconic and suspect cases. Clin Ophthalmol 7:1539–1548

59. Kanellopoulos AJ, Asimellis G (2014) Corneal refractive power and symmetry changes following normalization of ectasias treated with partial topography-guided PTK combined with higher-fluence CXL (the Athens Protocol). J Refract Surg 30:342–346

60. Karseras AG, Ruben M (1976) Aetiology of keratoconus. Br J Ophthalmol 60:522–525

61. Kennedy RH, Bourne WM, Dyer JA (1986) A 48-year clinical and epidemiologic study of keratoconus. Am J Ophthalmol 101:267–273

62. King EF (1953) Keratoconus following thyroidectomy. Trans Ophthalmol Soc UK 73:31–39

63. Kirwan C, O'Malley D, O'Keefe M (2008) Corneal hysteresis and corneal resistance factor in keratoectasia: findings using the Reichert ocular response analyzer. Ophthalmologica 222:334–337

64. Kotecha A, Oddone F, Sinapis C, Elsheikh A, Sinapis D, Sinapis A, Garway-Heath DF (2010) Corneal biomechanical characteristics in patients with diabetes mellitus. J Cataract Refract Surg 36:1822–1828

65. Krachmer JH, Feder RS, Belin MW (1984) Keratoconus and related noninflammatory corneal thinning disorders. Surv Ophthalmol 28:293–322

66. Krumeich JH, Daniel J (1997) Lebend-Epikeratophakie und tiefe lamelläre Keratoplastik zur stadiengerechten chirurgischen Behandlung des Keratokonus (KK) I-III. Klin Monbl Augenheilkd 211:94–100

67. Ku JYF, Niederer RL, Patel DV, Sherwin T, McGhee CNJ (2008) Laser scanning in vivo confocal analysis of keratocyte density in keratoconus. Ophthalmology 115:845–850

68. Labiris G, Gatzioufas Z, Sideroudi H, Giarmoukakis A, Kozobolis V, Seitz B (2013) Biomechanical diagnosis of keratoconus: evaluation of the keratoconus match index and the keratoconus match probability. Acta Ophthalmol 91:e258-262

69. Landoulsi H, Saad A, Haddad NN, Guilbert E, Gatinel D (2013) Repeatability of Ocular Response Analyzer waveform parameters in normal eyes and eyes after refractive surgery. J Refract Surg 29:709–714

70. Lang GE, Naumann GO (1991) Keratoconus in Alagille syndrome. Klin Monbl Augenheilkd 198:555–557

71. Lee R, Hafezi F, Randleman JB (2018) Bilateral keratoconus induced by secondary hypothyroidism after radioactive iodine therapy. J Refract Surg 34:351–353

72. Li X, Rabinowitz YS, Rasheed K, Yang H (2004) Longitudinal study of the normal eyes in unilateral keratoconus patients. Ophthalmology 111:440–446

73. Luce DA (2005) Determining in vivo biomechanical properties of the cornea with an ocular response analyzer. J Cataract Refract Surg 31:156–162

74. Macsai MS, Varley GA, Krachmer JH (1990) Development of keratoconus after contact Lens wear: patient characteristics. Arch Ophthalmol 108:534–538

75. Masterson E, Edelhauser HF, Van Horn DL (1977) The role of thyroid hormone in the development of the chick corneal endothelium and epithelium. Invest Ophthalmol Vis Sci 16:105–115

76. Maurice DM (1957) The structure and transparency of the cornea. J Physiol 136:263–286

77. McMonnies CW (2007) Abnormal rubbing and keratectasia. Eye Contact Lens 33:265–271

78. McMonnies CW (2009) Mechanisms of rubbing-related corneal trauma in keratoconus. Cornea 28:607–615

79. McMonnies CW (2015) Inflammation and keratoconus. Optom Vis Sci 92:e35-41

80. Meek KM, Tuft SJ, Huang Y, Gill PS, Hayes S, Newton RH, Bron AJ (2005) Changes in collagen orientation and distribution in keratoconus corneas. Invest Ophthalmol Vis Sci 46:1948–1956

81. Moran S, Gomez L, Zuber K, Gatinel D (2020) A case-control study of keratoconus risk factors. Cornea 39:697–701

82. Naderan M, Rajabi MT, Zarrinbakhsh P, Bakhshi A (2017) Effect of allergic diseases on keratoconus severity. Ocul Immunol Inflamm 25:418–423

83. Nielsen K, Hjortdal J, Aagaard Nohr E, Ehlers N (2007) Incidence and prevalence of keratoconus in Denmark. Acta Ophthalmol Scand 85:890–892

84. Nottingham J (1854) Practical observations on conical cornea: and on the short sight, and other defects of vision connected with it. John Churchill, London,UK

85. Oculus Optikgeräte GmbH (Hrsg.) Oculus Pentacam Interpretation Manual Pentacam/Pentacam HR (3rd edition).

86. Ortiz D, Piñero D, Shabayek MH, Arnalich-Montiel F, Alió JL (2007) Corneal biomechanical properties in normal, post-laser in situ keratomileusis, and keratoconic eyes. J Cataract Refract Surg 33:1371–1375

87. Padmanabhan P, Radhakrishnan A, Natarajan R (2010) Pregnancy-triggered iatrogenic (post-laser in situ keratomileusis) corneal ectasia: a case report. Cornea 29:569–572

88. Parker J, Ko WW, Pavlopoulos G, Wolfe PJ, Rabinowitz YS, Feldman ST (1996) Videokeratography of keratoconus in monozygotic twins. J Refract Surg 12:180–183

89. Patel DV, Ku JYF, Johnson R, McGhee CNJ (2009) Laser scanning in vivo confocal microscopy and quantitative aesthesiometry reveal decreased corneal innervation and sensation in keratoconus. Eye (Lond) 23:586–592

90. Rabinowitz YS (1998) Keratoconus. Surv Ophthalmol 42:297–319

91. Rabinowitz YS (2003) The genetics of keratoconus. Ophthalmol Clin North Am 16:607–620, vii

92. Rahi A, Davies P, Ruben M, Lobascher D, Menon J (1977) Keratoconus and coexisting atopic disease. Br J Ophthalmol 61:761–764

93. Rahman W, Anwar S (2006) An unusual case of keratoconus. J Pediatr Ophthalmol Strabismus 43:373–375

94. Razafimino S, Flockerzi E, Zemova E, Munteanu C, Seit B (in print) Impact of hypothyroidism on tomography and biomechanics in keratoconus - cross-sectional and longitudinal assessment within the HKC at the time of inclusion and after one year. Klin Monatsbl Augenheilkd

95. Redmond KB (1968) The role of heredity in keratoconus. Trans Ophthalmol Soc Aust 27:52–54

96. Romero-Jiménez M, Santodomingo-Rubido J, Wolffsohn JS (2010) Keratoconus: a review. Cont Lens Anterior Eye 33:157–166; quiz 205

97. Rüfer F, Schröder A, Erb C (2005) White-to-white corneal diameter: normal values in healthy humans obtained with the Orbscan II topography system. Cornea 24:259–261

98. Sawaguchi S, Fukuchi T, Abe H, Kaiya T, Sugar J, Yue BY (1998) Three-dimensional scanning electron microscopic study of keratoconus corneas. Arch Ophthalmol 116:62–68

99. Seitz B, Daas L, Hamon L, Xanthopoulou K, Goebels S, Spira-Eppig C, Razafimino S, Szentmáry N, Langenbucher A, Flockerzi E (2021) [Erratum to: Stage-appropriate treatment of keratoconus]. Ophthalmologe doi 10.1007/s00347-021-01472-8

100. Shah S, Laiquzzaman M, Cunliffe I, Mantry S (2006) The use of the Reichert ocular response analyser to establish the relationship between ocular hysteresis, corneal resistance factor and central corneal thickness in normal eyes. Cont Lens Anterior Eye 29:257–262

101. Sharif KW, Casey TA, Coltart J (1992) Prevalence of mitral valve prolapse in keratoconus patients. J R Soc Med 85:446–448

102. Sherwin T, Brookes NH (2004) Morphological changes in keratoconus: pathology or pathogenesis. Clin Exp Ophthalmol 32:211–217

103. Soeters N, Tahzib NG, Bakker L, Van der Lelij A (2012) Two cases of keratoconus diagnosed after pregnancy. Optom Vis Sci 89:112–116

104. Somodi S, Hahnel C, Slowik C, Richter A, Weiss DG, Guthoff R (1996) Confocal in vivo microscopy and confocal laser-scanning fluorescence microscopy in keratoconus. Ger J Ophthalmol 5:518–525

105. Spoerl E, Zubaty V, Raiskup-Wolf F, Pillunat LE (2007) Oestrogen-induced changes in biomechanics in the cornea as a possible reason for keratectasia. Br J Ophthalmol 91:1547–1550

106. Stachon T, Stachon A, Hartmann U, Seitz B, Langenbucher A, Szentmáry N (2017) Urea, Uric Acid, Prolactin and fT4 Concentrations in Aqueous Humor of Keratoconus Patients. Curr Eye Res 42:842–846

107. Stock RA, Thumé T, Bonamigo EL (2017) Acute corneal hydrops during pregnancy with spontaneous resolution after corneal cross-linking for keratoconus: a case report. J Med Case Rep 11:53

108. Stoiber J, Muss W, Ruckhofer J, Grabner G (2003) Acute keratoconus with perforation in a patient with Down's syndrome. Br J Ophthalmol 87:120

109. Stoiber J (2008) Die stadiengerechte Therapie des Keratokonus. Klin Monbl Augenheilkd 225:R121-136; quiz R137-138

110. Street DA, Vinokur ET, Waring GO, Pollak SJ, Clements SD, Perkins JV (1991) Lack of association between keratoconus, mitral valve prolapse, and joint hypermobility. Ophthalmology 98:170–176

111. Sugar J, Macsai MS (2012) What causes keratoconus? Cornea 31:716–719

112. Suzuki T, Kinoshita Y, Tachibana M, Matsushima Y, Kobayashi Y, Adachi W, Sotozono C, Kinoshita S (2001) Expression of sex steroid hormone receptors in human cornea. Curr Eye Res 22:28–33

113. Takahashi A, Nakayasu K, Okisaka S, Kanai A (1990) [Quantitative analysis of collagen fiber in keratoconus]. Nippon Ganka Gakkai Zasshi 94:1068–1073

114. Teng CC (1963) Electron microscope study of the pathology of keratoconus: I. Am J Ophthalmol 55:18–47

115. Thanos S, Oellers P, Meyer Zu Hörste M, Prokosch V, Schlatt S, Seitz B, Gatzioufas Z (2016) Role of thyroxine in the development of keratoconus. Cornea 35:1338–1346

116. Thota S, Miller WL, Bergmanson JPG (2006) Acute corneal hydrops: a case report including confocal and histopathological considerations. Cont Lens Anterior Eye 29:69–73

117. Tong CM, van Dijk K, Melles GRJ (2019) Update on Bowman layer transplantation. Curr Opin Ophthalmol 30:249–255

118. Twa M (2009) Irregular Astigmatism: Diagnosis and Treatment. Optom Vis Sci 86:1209

119. van Dijk K, Liarakos VS, Parker J, Ham L, Lie JT, Groeneveld-van Beek EA, Melles GRJ (2015) Bowman layer transplantation to reduce and stabilize progressive, advanced keratoconus. Ophthalmology 122:909–917

120. Wagner H, Barr JT, Zadnik K (2007) Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study: methods and findings to date. Cont Lens Anterior Eye 30:223–232

121. Wang Y, Rabinowitz YS, Rotter JI, Yang H (2000) Genetic epidemiological study of keratoconus: evidence for major gene determination. Am J Med Genet 93:403–409

122. Weed KH, MacEwen CJ, Cox A, McGhee CNJ (2007) Quantitative analysis of corneal microstructure in keratoconus utilising in vivo confocal microscopy. Eye (Lond) 21:614–623

123. Weed KH, Macewen CJ, McGhee CNJ (2007) The Dundee University Scottish Keratoconus Study II: a prospective study of optical and surgical correction. Ophthalmic Physiol Opt 27:561–567

124. Wilson SE, He YG, Weng J, Li Q, McDowall AW, Vital M, Chwang EL (1996) Epithelial injury induces keratocyte apoptosis: hypothesized role for the interleukin-1 system in the modulation of corneal tissue organization and wound healing. Exp Eye Res 62:325–327

125. Wolffsohn JS, Safeen S, Shah S, Laiquzzaman M (2012) Changes of corneal biomechanics with keratoconus. Cornea 31:849–854

126. Wolter JR, Henderson JW, Clahassey EG (1967) Ruptures of Descemet's membrane in keratoconus causing acute hydrops and posterior keratoconus. Am J Ophthalmol 63:1689–1692

127. Woodward MA, Blachley TS, Stein JD (2016) The association between sociodemographic factors, common systemic diseases, and keratoconus: an analysis of a nationwide heath care claims database. Ophthalmology 123:457-465.e2

128. Yee RW, Matsuda M, Schultz RO, Edelhauser HF (1985) Changes in the normal corneal endothelial cellular pattern as a function of age. Curr Eye Res 4:671–678

129. Zadnik K, Barr JT, Gordon MO, Edrington TB (1996) Biomicroscopic signs and disease severity in keratoconus. Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study Group. Cornea 15:139–146

130. Zadnik K, Barr JT, Edrington TB, Everett DF, Jameson M, McMahon TT, Shin JA, Sterling JL, Wagner H, Gordon MO (1998) Baseline findings in the Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study. Invest Ophthalmol Vis Sci 39:2537–2546

131. Zadnik K, Steger-May K, Fink BA, Joslin CE, Nichols JJ, Rosenstiel CE, Tyler JA, Yu JA, Raasch TW, Schechtman KB, CLEK Study Group. Collaborative Longitudinal Evaluation of Keratoconus (2002) Between-eye asymmetry in keratoconus. Cornea 21:671–679

132. Ziaei H, Jafarinasab MR, Javadi MA, Karimian F, Poorsalman H, Mahdavi M, Shoja MR, Katibeh M (2012) Epidemiology of keratoconus in an Iranian population. Cornea 31:1044–1047

133. (2016) Universitätsklinikum des Saarlandes UKS in Homburg/Saar. Zentrallabor, Referenzwerte. (2016).

134. (2016) Universitätsklinikum des Saarlandes UKS in Homburg/Saar. Zentrallabor, Referenzwerte. ElecsysThyrotropin (TSH) Assay Cobas Roche Diagnostics: instructions.

6. Publications and conference participations

Scientific publications:

- Impact of hypothyroidism on tomography and biomechanics in keratoconus - cross-sectional and longitudinal assessment within the HKC at the time of inclusion and after one year.

Razafimino S, Flockerzi E, Zemova E, Munteanu C, Seitz B. Klin Monatsbl Augenheilkd 2021 August (accepted, published ahead of print)

- Stage-appropriate treatment of keratoconus

Seitz B, Daas L, Hamon L, Xanthopoulou K, Goebels S, Spira-Eppig C, Razafimino S, Szentmáry N, Langenbucher A, Flockerzi E. Ophthalmologe. 2021 Aug 2. doi: 10.1007/s00347-021-01472-8.

- Keratoconus stating by decades: a baseline ABCD classification of 1000 patients in the Homburg Keratoconus Center.

Flockerzi E., Xanthopoulou K., Goebels S., Zemova E., Razafimino S., Hamon L. Julien T., Klüspies U, Eppig T. Langenbuchr A, Seitz B. Br J Ophthalmol. 2020 Aug; 105(8): 1069-1075. Doi:10.1136/bjophthalmol-2020-316789

Conference presentations:

- **Endokrinologische Untersuchungen am Beispiel der Hypothyreose.** Homburger Keratoconus Symposium HKCS, Germany, 04.09.2021.

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7. Acknowledgements

Foremost, I wish to express my deepest gratitude to the **Prof. Dr. med. B. Seitz ML, FEBO** for giving me the opportunity to work in the excellent and stimulating academic environment of the Saarland University, for his patience, for sharing his incomparably knowledge with me, and for always challenging me.

I am very grateful to **Cristian Muteanu** for his valuable guidance through the course of this work, his helpful advice concerning methods and statistical analysis. I also thank **Prof. Dr. S. Wagenpfeil** for his additional advice concerning methods and statistical analysis.

I especially want to thank my colleague and friend at the Department of Ophthalmology **Elias Flockerzi** for his constructive suggestions, advice and help during all phases of my work. I am very grateful for all the efforts you have made to help me through this.

I want to thank my dear friend **Marie Hartmann**, who went through the ups and downs with me. For supporting me days and nights, for giving me courage, and for reminding me of my goal, I sincerely thank you. Cette année est une grâce de plus, même si ce n'est pas facile, profitons de cette grâce et tournons ensemble le gros chapitre de nos années d'internat avec tout ce que ça implique (et de préférence avant 2022 :p)

My thanks also go to my work colleagues and friends, **Elena Zemova**, **Frau Dr. U. Löw**, **Tim Berger**, **Loic Hamon**, **Kassandra Xanthopoulou** and **Ivana Marjanovic**. Thank you for being there. Each in his own way, contributed to improve the quality of my work and to give me strength. I also thank **Lourd McCabe-Brockmann** for her precious help.

I dedicate my last word to **my family**, who have always supported me in all important and less important moments of my life, and to my friend **Emmanuel Feyt,** who I thank for being so patient and self-sacrificing, for all the long discussion even you didn't know what it was all about, for listen to my babbling and for always cheering me up.

This work would not have been possible without the unconditional support of my friends and family.