The Analysis of Eye Banking and Corneal Transplantation in New Zealand

Hussain Y Patel

A thesis submitted in partial fulfillment of the requirements
for the degree of Doctor of Medicine, University of Auckland
2007

ABSTRACT

The series of studies comprising this thesis was developed to answer a number of key inter-related questions in regard to eye banking and corneal transplantation in New Zealand.

The source and management of donor tissue procured by the New Zealand National Eye Bank (NZNEB) was analysed. Significant trends were identified with respect to donor demographics, donor procurement source, improved donor tissue processing and storage, decreased biological contamination, and increased utilization of corneal tissue.

Current trends and ethnicity differences in indications for penetrating keratoplasty (PKP) were investigated. Keratoconus was identified as the most common indication for PKP in New Zealand, accounting for a significantly higher proportion of PKPs than other published reports. Keratoconus was the most common indication for PKP throughout all ethnicity groups and was particularly common in the Maori and Polynesian populations. Significant trends were identified including an increase in the number of PKPs for regraft and Fuchs' endothelial dystrophy and a decrease for aphakic or pseudophakic bullous keratopathy and viral keratitis.

Survival and visual outcome following PKP in New Zealand was investigated using univariate and multivariate analysis. Several independent risk factors were identified that influenced outcome of PKP. Active inflammation at PKP, pre-existing vascularisation, pre-operative glaucoma, small or large graft size, intra-operative complications, episodes of reversible rejection and a pre-operative

diagnosis of regraft, trauma or infection resulted in a significantly decreased survival rate. Advancing recipient age, active inflammation at the time of PKP, pre-existing vascularisation, pre-operative glaucoma, episodes of reversible rejection, bullous keratopathy, trauma and non-infective keratitis were associated with poor visual outcome.

Patient characteristics, indications, surgical details, and outcome of paediatric keratoplasty were analysed. Acquired non-traumatic indications accounted for the majority of paediatric keratoplasties in New Zealand. This study highlighted keratoconus as a particularly common indication for paediatric keratoplasty when compared to other countries. Survival and visual outcome was better for acquired compared to congenital indications.

The effects of corneal parameters on the measurement of endothelial cell density (ECD) in the normal eye were analysed. Corneal thickness appears to be negatively correlated to ECD in the normal cornea for all age groups. Corneal diameter is correlated to ECD measurement in children but not in adults. Corneal curvature was not significantly correlated to ECD measurement, but this needs further investigation.

Confocal microscopy and slit scanning topography were used to analyze endothelial morphology and function in the short and long term following PKP. The results of this study are in concordance with other published reports that have identified an accelerated loss of endothelial cells and more rapid development of abnormal endothelial cells in transplanted corneas compared to normal corneas.

ACKNOWLEDGEMENTS

The completion of this thesis would not have been achieved without the help and support of several individuals. Unfortunately it is not possible to mention each and every person who helped along the way, but I would like to thank the following individuals for their significant contribution to this thesis.

First and foremost I would like to thank Professor Charles McGhee for the special opportunity he gave me to undertake a research degree under his supervision in the Department of Ophthalmology, University of Auckland. Professor McGhee provided tremendous help, support and encouragement throughout the entire process of completing this thesis. He was involved in all of the studies that comprise this thesis and his help and advice with these was invaluable. Without Professor McGhee this thesis would never have been accomplished.

The New Zealand National Eye Bank (NZNEB) studies would not have been possible without the significant help of the current NZNEB manager Louise S Moffatt, and technicians Nigel Brooks and Helen Twohill.

I would like to acknowledge the fundamental contribution to this research by A/Prof Gillian Clover, founder and first Scientific Director of the NZNEB; the key role of Dr David Pendergrast, Clinical Director; the Chairman and Trustees of the NZNEB for their continued support, the past Managers, Transplant Coordinators, Scientific Staff; and Ophthalmologists of New Zealand, who have contributed to clinical and scientific aspects of the NZNEB over the last 13 years.

I am particularly grateful to Dr Trevor Sherwin and Dr Sue Ormonde for their invaluable help and guidance with all of the studies in this thesis. I am also indebted to Dr Dipika Patel and Dr Nisha Sachdev for their assistance in the clinical studies that form part of this thesis.

Finally, I would like to thank my mother and father, Amina and Yusuf Patel, and my partner, Manou Moeung, for their encouragement and continued support of my academic pursuits.

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

Chapter 7

Chapter 8

Chapter 9

Chapter 10

LIST OF ABBREVIATIONS

ACD	Anterior chamber depth
ALK	Automated lamellar keratoplasty
ANOVA	Analysis of variance
BCVA	Best corrected visual acuity
BFS	Best fit sphere
BSCVA	Best spectacle corrected visual acuity
BSS	Balanced salt solution
CCT	Central corneal thickness
CI	Confidence interval
CI	Correction Index
COVA	Coefficient of variation for cell area
COVL	Coefficient of variation for cell length
CLs	Contact lens
cm	Centimeter
CSR	Corneoscleral rim
Cyl	Cylinder
D	Dioptre
DPI	Death to preservation interval
ECCE	Extracapsular cataract extraction
ECD	Endothelial cell density
FU	Follow-up
Hz	Hertz
IN	Inferonasal
IOL	Intraocular lens
IT	Inferotemporal
L	Left
LE	Left eye

LK	Lamellar keratoplasty
LogMAR	Base ten logarithm of the minimum angle of resolution
MCA	Mean cell area
MHz	Megahertz
MK	McCarey and Kaufman
m	Month
mJ	Millijoule
mm	Millimetre
µm	Micrometre
nm	Nanometre
NZNEB	New Zealand National Eye Bank
OCM	Organ culture medium
PAR CTS	PAR Corneal Topography System
PC IOL	Posterior chamber intraocular lens
PKP	Penetrating keratoplasty
R	Right
RE	Right eye
RK	Radial keratotomy
RMS	Root mean square
SD	Standard deviation
SIA	Surgically induced astigmatism
SEM	Standard error of the mean
SEQ	Spherical equivalent
SimK	Simulated keratometry
SN	Superonasal
SPSS	Statistical Program for Social Scientists
ST	Superotemporal
TMS	Topographic Modeling System

UBM	Ultrasound biomicroscopy
UCVA	Uncorrected visual acuity
US	Ultrasound
VA	Visual acuity

Section 1

Introduction

Chapter 1

Structure and Function of the Cornea

1.1 Introduction

The cornea is a transparent, avascular tissue that forms the anterior one-sixth of the outer fibrous coat of the eyeball. The cornea has essential optical and protective functions. The cornea acts like a window allowing the transmission of light to the retina, and it forms the principal refractive surface of the eye. The cornea also provides a resistant, mechanically tough, and chemically resistant barrier between the eye and the environment. The cornea has become highly specialised to meet these diverse functions, and this chapter provides a brief outline of its unique structural and functional features.

1.2 Gross corneal anatomy

The cornea is elliptical in shape when viewed anteriorly, with the average horizontal diameter 11.7 mm and vertical diameter 10.6 mm.[1] When viewed posteriorly, the cornea is circular with an average diameter of 11.7 mm.[1] The central third of the anterior surface (the optical zone, 4 mm diameter) is approximately spherical (radius of curvature 7.8 mm) and provides the majority of optical function.[2] The periphery of the anterior surface flattens asymmetrically (greater nasally than temporally).[3] This asymmetry is an important consideration when performing surgical procedures and with contact lens fitting.[4] The posterior surface is almost spherical with a radius of curvature of 6.8 mm.[3] The mean corneal thickness is greater peripherally (0.67 mm) than centrally (0.52 mm).[5]

The periphery of the cornea is continuous with the sclera (remaining five-sixth of outer fibrous coat of the eyeball), episcleral tissue and conjunctiva, and they merge in a 1-2 mm wide transitional zone termed the limbus. There is an extensive vascular supply to the limbus,[6] which has an important role in supplying the peripheral cornea. The tear film covers the anterior surface of the cornea and the posterior surface is directly bathed by aqueous humor.

1.3 Microscopic corneal anatomy

The cornea is composed of five distinct tissue layers, which are (from anterior to posterior): 1) corneal epithelium, 2) Bowman's layer, 3) corneal stroma, 4) Descemet's membrane, 5) and corneal endothelium (figure 1:1).

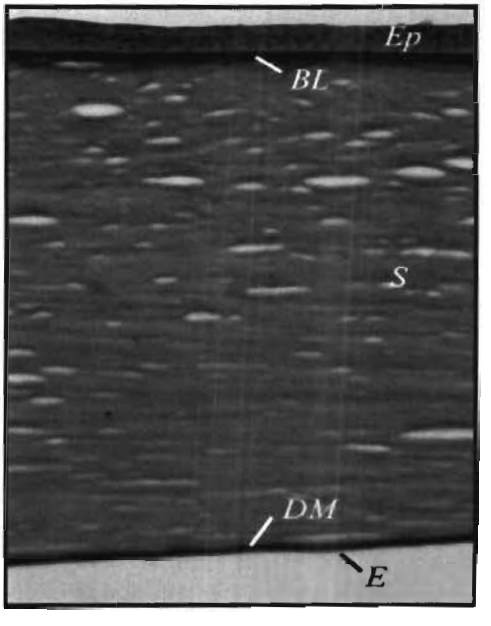


Figure 1:1 ***Microscopic appearance of the cornea. (Ep = Epithelium, BL = Bowman's layer, S = Stroma, DM = Descemet's membrane, E = Endothelium)***

1.3.1 Corneal epithelium

The corneal epithelium is 50-90 µm thick, consists of 5-6 layers of cells, and represents approximately 10% of the total corneal thickness.[7] The epithelium is stratified, squamous and non-keratinized. The epithelium consists of 3-4 superficial flattened squamous cell layers, 1-3 mid-epithelial 'wing' cell layers, and 1 basal cell layer.[8, 9] The basal layer consists of columnar cells, arranged in a palisade-like manner, strongly attached to the basal lamina of the corneal epithelium via hemidesmosomal-like structures.[9] Peripherally, the corneal epithelium is continuous with that of the conjunctiva.[10]

The corneal epithelium is self-renewing with complete turnover occurring in 5-7 days.[11] Mitosis is confined to the basal cell layer. The daughter cells migrate anteriorly, differentiating into wing and then squamous cells before desquamation from the apical surface. Basal cells are renewed by the centripetal migration of new basal cells originating from the limbal stem cells.[11]

1.3.2 Bowman's layer

Bowman's layer is an acellular homogenous zone, 8-14 µm thick, lying immediately subjacent to the basal lamina of the corneal epithelium.[12] It is composed of collagen fibrils (types I, III, V, and VI) and associated proteoglycans densely woven in a random manner into a felt-like matrix. In the posterior region of this layer the collagen fibrils become progressively more orderly, blending with

those of the anterior stroma.[12-15] Bowman's layer is perforated in many places by unmyelinated nerves in transit to the corneal epithelium.[13]

Bowman's layer has several functions: it provides a strong barrier against infective agents and surface tumours; it provides resistance to mechanical trauma; and plays a role in maintaining the smooth anterior surface of the cornea.[16]

1.3.3 Stroma

The stroma is a dense connective tissue layer that constitutes approximately 90% of the corneal thickness. The major components of the stroma are collagen lamellae (approximately 200-300), ground substance and collagen-producing fibroblasts (keratocytes).[17, 18]

The collagen lamellae are arranged in layers parallel with each other and with the corneal surfaces.[18] The arrangement is more regular in the posterior stroma than in the anterior stroma. In the posterior stroma the lamellae form strap-like ribbons which run at right angles to those in consecutive layers.[19] In the anterior stroma the lamellae are narrow and interweaved. Each stromal lamella comprises a band of collagen fibrils arranged in parallel.[20] The predominant collagen in the stroma is type I (50-55%) with smaller amounts of type III, V, and VI.[21] The collagen fibrils have a small (30 nm) and consistent diameter, and there is a highly uniform interfibrillar distance. [21] [22] These characteristic features are

essential for the cornea to achieve its dual properties of transparency and high tensile strength.

The collagen fibrils are embedded in a proteoglycan ground substance, which has an important role in maintaining interfibrillar spacing.[21] The primary glycosaminoglycans of the stroma are keratin sulphate and dermatan sulphate.[21] Keratocytes only occupy 2-3% of the stromal volume but are of major importance for the production and turnover of collagen and ground substance.[16] They are located predominantly between collagen lamellae and are long, flattened, stellate cells. The keratocytes are organised as a syncytium, forming a communication network through their branching processes.[23]

1.3.4 Descemet's membrane

Descemet's membrane is the basement membrane of the corneal endothelium. It is secreted by the endothelium and first appears in the second month of gestation.[24] Its thickness increases throughout life from 3-4 μm at birth to 10-12 μm by late adulthood.[12] Two distinct regions can be discerned by electron microscopy; the anterior one-third is produced in fetal life and displays an irregular banded pattern, the posterior two-thirds is formed after birth and has a homogenous granular structure.[25] Descemet's membrane is composed of characteristic basement membrane components: glycoproteins such as laminin and fibronectin, type IV collagen, and lesser amounts of type V, VIII, IX, and XII collagen.[26]

In the ageing cornea there may be focal overproduction of basement membrane material by endothelial cells, resulting in peripheral excrescence's called Hassal-Henle warts.[27] Descemet's membrane may also be involved in pathological states of the endothelium, such as with Fuchs' endothelial dystrophy and posterior polymorphous dystrophy.[27] Descemet's membrane is not strongly attached to the stroma and can be detached in pathological conditions or dissected surgically.[26]

1.3.5 Endothelium

The human corneal endothelium is a single layer of cuboidal, hexagonal cells, which line the posterior corneal surface.[28] The corneal endothelium is not a true endothelium; it is derived from the neural crest and therefore of neuroectodermal rather than vascular origin.[26]

The endothelial cells are approximately 4-6 μm in height and 20 μm in width.[28] Neighbouring endothelial cells integrate into each other by lateral wall folds and finger-like projections. The cells are firmly bound together by occluding junctions and communicate extensively through numerous gap junctions.[29] The basal cell membrane is attached to Descemet's membrane by modified hemidesmosomes. The apical cell membrane is relatively flat and exhibits microvilli. The intracellular space of the cells is occupied by a large nucleus and numerous organelles, including mitochondria, endoplasmic reticulum and Golgi's apparatus.[30] These features reflect the high metabolic activity of endothelial cells.

The cornea is supplied with a relatively fixed population of approximately 500,000 endothelial cells at birth.[31] Although mitosis can occur in young endothelial cells, it is infrequent in the adult, and injured cells are not replaced. A gradual decrease in endothelial cell density and an increase in size variation (polymegathism) and shape variation (pleomorphism) occur with age.[31] At birth, endothelial cell densities range from 3500 to 4000 cells/mm^2, while the adult cornea normally has cell densities of 1400 to 3500 cell/mm^2.[32] After injury, damaged cells are replaced by the spreading of cells from adjacent zones, the cells increasing in area but decreasing in height.[30]

1.4 Corneal innervation

The cornea is one of the most densely innervated tissues in the body - 100 times more sensitive than the conjunctiva and 300 times more sensitive than skin.[33] Sensory nerves are derived from the ophthalmic division of the trigeminal nerve and reach the cornea mainly via the long ciliary nerves.

Approximately 60-80 myelinated branches enter the anterior corneal stroma. After 2-3 mm they lose their myelin sheaths and divide into 2 groups - anterior and posterior. The anterior nerves (40-50) pass through the substance of the stroma and form a dense subepithelial plexus. From this plexus, the nerves penetrate Bowman's layer and supply terminal endings to the corneal epithelium. The posterior group of nerve fibres supplies the posterior stroma.[34, 35] A supply to Descemet's membrane or the endothelium has not been demonstrated. A sparse

sympathetic innervation derived from the superior cervical ganglion is also present in the human cornea, although its role is unknown. Parasympathetic innervation has not been identified in the human cornea.[34, 35]

1.5 Tear film

The preocular tear film covers the anterior surface of the cornea and has several important functions: it creates a smooth optical surface, essential for the refractive properties of the cornea; it bathes and lubricates the ocular surface, preventing corneal dehydration; it is the primary source of oxygen and other nutrients to the cornea; and it provides a protective extracellular environment for the corneal epithelium.[28 32]

The tear film consists of three layers: a superficial lipid layer (0.1 µm), produced by the Meibomian glands and the glands of Moll and Zeis; an aqueous layer (7.0 µm), produced by the main and accessory lacrimal gland; and a mucin layer (0.02-0.05 µm), produced by conjunctival goblet cells.[28 32] The normal tear thickness is about 7-10 µm and the normal tear volume about 6-8 µl.[36] Water comprises approximately 98% of the total tear film volume. Important factors contained within the tear film include: electrolytes, glucose, immunoglobulins, lactoferrin, lysosome, albumin, histamine, growth factors, interleukins, and prostaglandins.[36]

1.6 Basic corneal physiology

1.6.1 Corneal nutrition and metabolism

The cells of the cornea require considerable metabolic activity in order to maintain normal corneal function. Carbohydrate metabolism, with glucose as the main substrate, is the primary source of energy for corneal cells.[37] Most of the glucose is derived from the aqueous humor, with a small amount (10%) from the limbal vasculature and tear film. The corneal epithelial cells are also able to store glycogen, which is used when free glucose is insufficient.[37] The main metabolic pathways for glucose utilization are the anaerobic Embden-Meyerhof pathway (glycolysis) and the aerobic Krebs cycle.[38, 39] A small fraction of glucose is also diverted to the hexose monophosphate shunt.

The oxygen supply to the corneal epithelium is predominantly derived from the atmosphere, via diffusion form the tear film.[40] The endothelium and stroma are supplied with oxygen derived from the aqueous humor.[40] The oxygen content of tears (155 mmHg) is significantly greater than that of the aqueous humor (40 mmHg), corresponding to the epithelium's higher demand for oxygen.[40] During sleep or closed-eye conditions, oxygen is delivered to the cornea via the highly vascularised superior palpebral conjunctivae.

1.6.2 Control of stromal hydration

The control of stromal hydration is essential for corneal transparency. Although the corneal stroma is the most hydrated tissue in the body (78-80% water), it is

normally maintained in a relatively dehydrated state, in comparison to its ability to absorb water.[41] The stroma has an inherent tendency to imbibe water and swell. This is called the imbibition pressure and is due to the water attracting properties of the proteoglycans in the extracellular matrix.[36, 41] The stromal swelling must be counteracted in order to maintain normal corneal hydration.[42, 43] This is achieved through the metabolic pump function of the endothelium, and the barrier function of both the corneal epithelium and endothelium.[44]

The metabolic pump of the corneal endothelium plays the greatest role in active dehydration of the stroma.[44] The corneal stroma has a Na^+ concentration of 179 m/EqL, with a Na^+ activity of 134 m/EqL (45 m/EqL bound).[45] The aqueous humor contains only unbound Na^+ with an activity of 143 m/EqL. Therefore, a Na^+ gradient exists such that water is removed from the stroma by osmosis.[45, 46] This Na^+ gradient is generated by the active metabolic pumping of Na^+ from endothelial cells into the extracellular space by Na^+/K^+ ATPase (located on lateral membranes). [46] Other essential components of endothelial ion transport include a $HCO3^-$ pump and a Na^+/H^+ exchanger. Carbonic anhydrase is also present in endothelial cells and converts metabolic CO2 to $HCO3^-$.[46] Although the complete details have yet to be elucidated, the above components can be assembled into a model that explains ion transport and osmotic water flow across the endothelium (figure 1:2).

Both the corneal epithelium and endothelium act as barriers to the movement of ions and water into the stroma.[47, 48] The greatest resistance to electrolyte diffusion lies in the epithelium, primarily the surface layers.[49] The epithelial cell outer membranes are relatively impermeable to the passage of ions, and the epithelial cells are connected to surrounding cells by tight junctions which also significantly impede ion flow.[49] The barrier function of the endothelium is maintained by the elaborate interdigitations of cell borders and the occlusive tight junctions between adjacent cells.[47-49]

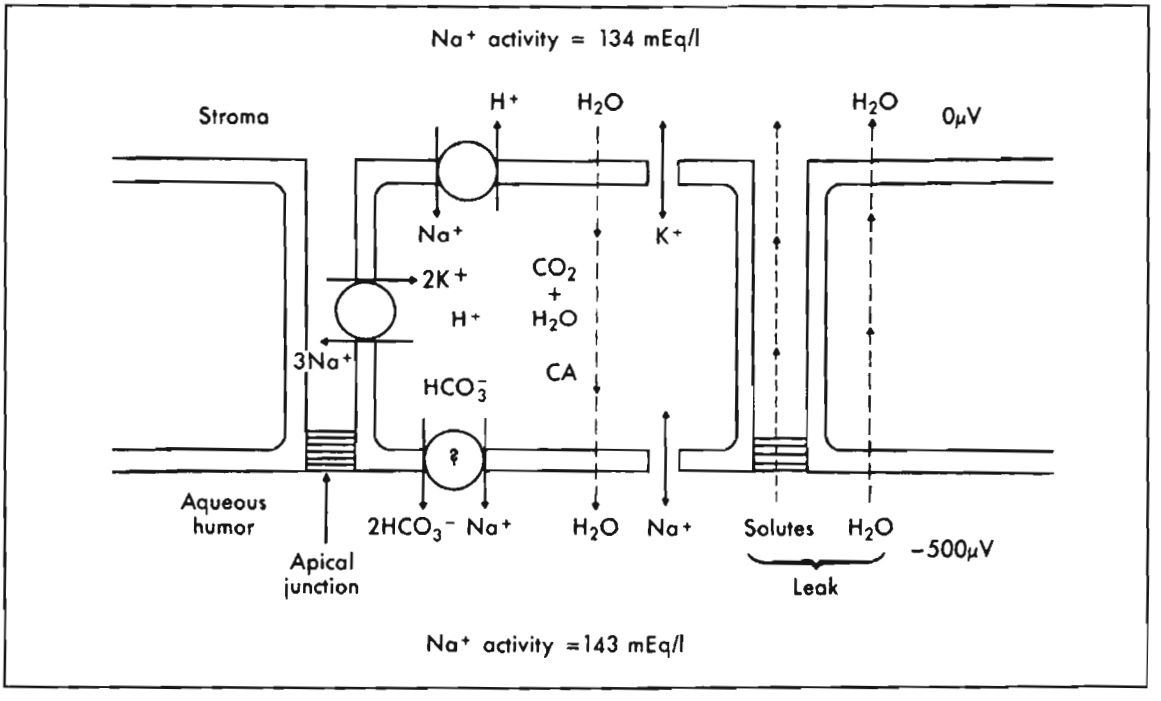


Figure 1:2 ***Model of ion and water transport across the corneal endothelium. Activity of the metabolic pump sets up an osmotic gradient resulting in the movement of water from the stroma to the aqueous humor balancing the leak of fluid from the aqueous humor into the stroma. CA = carbonic anhydrase. (Based on models proposed in Jentsch TJ, et al: Curr Eye Research 4:361, 1985 and Kuang K, et al: Exp Eye Research 50: 487, 1990)***

1.7 Corneal wound healing

The corneal epithelium heals by a combination of three separate processes: basal cell migration, mitosis and differentiation.[50, 51] Following a corneal abrasion, the basal cells at the wounds edge begin preparation for migration. They retract, thicken, and lose their hemidesmosomal attachments to the basal membrane. There is increased synthesis of glycoproteins, glycolipids and various proteins. Cell migration begins with 5-6 hours of injury and progresses at a constant rate of 60-80 μm/hr until the entire wound area is covered. [52, 53] As the plate of basal cells move centripetally, the overlying epithelial layer thins and follows the basal cells. New epithelial cells are produced by mitosis to replace the migrating cells. Mitosis occurs in a 3-5 mm zone behind the leading edge of the wound.[52, 53] Following wound closure, basal cells reform adhesion complexes to the basement membrane and the epithelium thickens.

The stroma heals more slowly than other connective tissues due to its avascular nature. Following injury, keratocytes undergo migration, proliferation, and transformation into myofibroblasts.[54, 55] This process is stimulated by the release of certain cytokines, such as interleukin (IL)-1, Fas ligand and tumour necrosis factor.[54,55] Simultaneously, inflammatory cells invade the injured stroma, attracted by chemotactic factors. Stromal regeneration is dependent on a cytokine-mediated interaction between epithelial cells and keratocytes.[54]

The fibroblasts produce collagen, glycoproteins and proteoglycans which form the new extracellular matrix.[56] Within the first week following injury, single collagen bundles are seen along the surface of fibroblasts.[57] By week three a dense network of fibrils is present, some arranged in tight parallel arrays and others randomly oriented. At 4 months no discernable pattern of collagen deposition is seen, but by 2 years the lamellar collagen pattern is re-approximated.[57] The strength of corneal scars never reaches that of uninjured tissue. Bowman's layer has no regenerative abilities and heals by scarring.

Endothelial cells in humans and other primates have minimal or no capacity to replicate by mitosis and therefore endothelial wound healing is largely dependent on enlargement and movement of surrounding cells to cover a wound site.[58-60] This is in contrast to rabbits wherein endothelial cells are capable of extensive mitosis.[60] Human endothelial wound repair is thus achieved by endothelial cells sliding over the stromal or Descemet's surface, or more usually over a fibronectin sub-matrix.[60] The endothelium is responsible for the deposition of a new Descemet's layer throughout the wound area.

Chapter 2

Contemporary Eye Bank Techniques for Corneal Storage

2.1 Moist Chamber Storage

In 1937, Filatov[61] first described the use of moist chamber storage of enucleated donor eyes. In this method the enucleated eye is placed in a sterile glass chamber and moistened by a saline or antibiotic solution. The corneas are stored at 4°C for up to 48 hours (optimal period 24 hours).[62] This moist chamber storage was used successfully for over forty years (and is still used in some countries). The main advantages of this technique are its simplicity and low cost. The primary disadvantage is the extremely limited storage duration of 24-48 hours.[63] This is because the endothelium of a cornea stored on the globe is subjected to the toxic accumulation of metabolic waste and necrotic tissue in the stagnant aqueous humor.[64]

2.2 Hypothermic Corneal Storage

2.2.1 MK Medium

One of the most significant advancements in eye banking was the introduction of corneal preservation medium for the storage of excised corneal-scleral donor rims.[65] In 1974, McCarey and Kaufman[65] identified that New Zealand rabbit cornea stored in modified tissue culture medium at 4°C appeared to be viable for up to 14 days. The McCarey and Kaufman medium (MK medium) consisted of an existing tissue culture medium, TC199, modified by the addition of dextran as a osmotic agent to compensate for the inactivity of the cornea's normal water removal mechanism at 4°C.[63, 65] Laboratory studies identified that the human corneal endothelium remained viable for up to 4 days when stored in MK

medium,[62] although, several studies reported that there was no significant difference in the success rates of corneal transplants whether the donor corneas were stored using MK medium (up to 72 hours) or the moist chamber technique.[66-69] However, MK medium increased reliable storage duration to 2-3 days and rapidly became the storage method of choice for eye banks.[63]

2.2.2 Storage media containing Chondroitin Sulphate

Chondroitin sulphate was identified as the essential ingredient required to prolong corneal storage duration at 4°C.[62] Chondroitin sulphate is presumed to play an important role in the intracellular redox system as an antioxidant, and as a membrane and growth factor stabilizer.[62] In 1985, Kaufman et al[70] introduced K-Sol (Alcon Laboratories, Inc., Fort Worth, Texas, U.S.A.), a 4-(2-hydroxyethyl)-1-piperazineethanesufonic acid (HEPES)-buffered tissue culture medium containing 2.5% chondroitin sulphate.[71] K-Sol successfully increased corneal storage duration at 4°C to 7-10 days.[70, 72-74]

One of the problems identified with chondroitin sulphate-containing medium was the tendency for corneas to swell,[71] which had not been a significant factor with dextran-containing medium. An improved medium, Dexsol (Chiron Vision Corporation, Irvine, CA, U.S.A.) was introduced that contained both chondroitin sulphate and dextran. In 1991, Optisol (Bausch & Lomb Surgical, Irvine, CA, U.S.A.) was introduced, which was a combination of the best characteristics of K-Sol and Dexsol. Kaufman et al[75] in a comparative study between Optisol and

Dexsol identified improved endothelial cell morphology and thinness of the Optisol corneas after 14 days of storage. Lass et al[76] in a subsequent clinical study comparing paired corneas stored in Optisol and Dexsol identified no significant difference in postoperative clinical or endothelial morphometric parameters. At present the storage medium most commonly used in the United States is Optisol GS (Bausch & Lomb Surgical), which contains both gentamicin and streptomycin for broad spectrum antimicrobial activity.[77] Although original publications suggested Optisol may be suitable for up to 14 days storage, the maximum storage duration accepted by most eye banks is 7-10 days.[63]

2.3 Organ Culture

The organ culture method of corneal storage was first introduced in 1976 by Doughman et al,[78] and has since been further developed by researchers in the United States and Europe.[79-81] The organ culture method uses storage temperatures between 31-37°C and theoretically allows corneal storage for up to 35 days.[63] Several studies have shown that endothelial cell morphology and density are well maintained even up to 35 days of storage with organ culture.[82-88] Bourne et al[89] identified no significant difference in post-surgical endothelial cell survival or eventual corneal thickness between donor corneas stored for a mean of 21 days in organ culture medium and those stored for a mean of 39 hours in MK medium. The major difference was an initial increased thickness of the corneal stroma in the organ-cultured group, but this resolved after 3 weeks.[89]

Various studies have reported primary graft failure rates ranging from 0 to 3% with organ culture storage.[82, 86, 87, 89, 90]

Reported advantages of the longer preservation period with organ culture storage include improved microbiological screening and elimination of infectious agents, time for donor tissue typing for high risk recipients, and improved management of corneal tissue – allowing corneal transplantation to be performed as an elective surgical procedure.[91] These advantages have seen organ culture adopted as the preferred method of corneal storage in the United Kingdom and Europe.[83, 92-94] However, the disadvantages of increased cost and greater technical complexity associated with organ culture storage have limited its use in other parts of the world.[83, 92-94]

Chapter 3

Current Techniques in Corneal Transplantation

3.1 Penetrating keratoplasty

Penetrating keratoplasty (PKP) is the most common corneal transplant procedure performed and occurs when the full-thickness diseased host corneal tissue is excised and replaced with full thickness healthy donor corneal tissue.[95] The first successful PKP was performed in 1905 by Zirm,[96] and through the contributions of many others has evolved significantly over the last century.[97] By the 1970s PKP was a relatively commonplace procedure with increasingly refined techniques.[98] Over the last 30 years there have been further advancements in surgical technique, including improvements in trephination, suturing, combined procedures, adjunctive aids, and treatment of ensuing refractive errors.[98]

The initial step in PKP is preparation of the donor corneal button. Hand-held trephines and corneal endothelial punch systems are the two current techniques most commonly used for trephination of the donor cornea.[99] The hand-held trephine technique is most commonly used in developing countries due to its reduced cost.[95] This technique typically uses a Teflon cutting block with a concave depression into which the donor cornea is placed, with the epithelial surface downwards.[99, 100] Trephination from the endothelial surface has been shown to result in less endothelial damage and cleaner cuts than trephination from the epithelial surface.[100, 101] A variety of different trephines have been developed, but the standard trephine consists of a disposable circular stainless steel blade on a handle. The donor cornea is cut by punching rather than rotating the blade as this results in less endothelial damage.[100, 101]

A variety of corneal endothelial punch systems have been developed including the Barron vacuum donor corneal punch, the IOWA PK Press corneal punch, and the Rothman-Gilbard corneal punch.[95] The basic method of "punching" is the same for all the trephine systems. The corneoscleral button is placed endothelial side up on a cutting block and a barrel mounted circular trephine is brought down through the central part of the system in a single motion cutting the donor cornea.[95] The Barron vacuum donor corneal punch system is slightly different, having a syringe with a spring loaded plunger attached to the system via silicone tubing, which creates suction that firmly holds the donor button in place during trephining.[95]

Non-mechanical trephination of the donor cornea involving excimer laser has also been described, although this is not in widespread use at present.[102, 103]

Prior to trephination of the recipient cornea, accurate marking of the cornea is essential to ensure correct graft centering and donor/recipient alignment. This helps to reduce post-operative astigmatism. A variety of techniques have been developed, including the use of radial keratometry markers and methods for placing circular or central ink marks.[104, 105]

Hand-held trephines, suction trephines and laser trephines are also current techniques for trephination of the recipient cornea. An important difference is that trephination of the recipient cornea occurs from the epithelial surface; whereas

that of the donor cornea is generally performed from the endothelial surface.[98] Several studies have shown that donor buttons trephined from the endothelial surface are smaller by approximately 0.25 mm than those trephined from the epithelial surface.[106-108] Damiano et al[109] also showed that anterior trephination causes inward sloping of the corneal edges, whereas posterior trephination gives the opposite effect, leaving a larger Descemet's membrane diameter from a posterior than an anterior cut. Suction trephines, like the Hessburg-Barron vacuum trephine introduced in 1980, have been shown to improve the control of trephination.[110] The suction trephines are claimed to create a sharper, deeper and more perpendicular incision than hand-held trephines.[110] Studies have shown that with this technique, any posterior beveling may be countered by using a diamond blade or corneoscleral scissors for the posterior stroma.[111] Excimer laser has recently been used for trephination of the recipient cornea.[102, 103, 112] Initial studies show a minor reduction in both post-operative astigmatism and myopia in laser cut than in mechanically cut eyes, however, access to an excimer laser in a full theatre environment in remains limited.[102]

Prior to suturing of the donor cornea, typically viscoelastic is inserted into the anterior chamber. Viscoelastic has been shown to protect the endothelium and other intraocular structures during penetrating keratoplasty.[113, 114] Donor endothelial cell loss after keratoplasty has been shown to be lessened by the use of viscoelastics, especially in the phakic eye.[113-116]

A wide variety of techniques, largely surgeon preference, have developed for suturing the donor corneal button to the recipient corneal opening. These include interrupted, continuous (torque, no-torque, or anti-torque patterns), continuous combined with interrupted, and double continuous suturing techniques.[117-122] The use of interrupted sutures has the advantage of selective suture removal in cases of suture-related post-operative astigmatism.[123] The advantages of a continuous technique include fewer knots to stimulate vascularization, the ability to adjust loop tension under intra-operative keratometric control and post-operatively at the slit-lamp, and adjustment can be completed without suture removal.[124] The advantage of a combined interrupted and continuous technique is that the continuous suture maintains wound apposition whenever the interrupted sutures are removed. The claimed advantage of a double continuous technique is that the remaining continuous suture maintains wound apposition if one of the sutures is removed.[124]

The most common suture material used is Nylon because of its ease of handling and its monofilament character, which permits a smooth passage through corneal tissue therefore causing less tissue reactivity.[125] Nylon maintains its tensile strength for more than a year allowing adequate wound healing. The most common diameter used is 10-0 because of its ease of placement and tensile strength.[125] Some surgeons prefer to use Polyester (Mersilene; Ethicon, Inc., NJ, USA) or Polypropylene (Prolene; Ethicon, Inc.) sutures as an alternative to Nylon, as they do not degrade as quickly allowing greater refractive stability.[126]

3.2 Lamellar keratoplasty

Lamellar keratoplasty (LK) is an alternative technique to PKP and is where only the diseased part of the cornea is replaced, leaving the recipient's normal anatomical layers intact.[127] There are a variety of indications for LK. Optical LK can be used in cases of superficial corneal scars and irregular/ectatic corneas. Tectonic LK can be used in cases of peripheral corneal thinning or ectatic corneal conditions. Therapeutic LK can be performed in cases of recurrent pterygium and conjunctival intraepithelial neoplasia, to remove and replace the affected corneal tissue and to arrest the pathological process.[127, 128] The advantages of LK over PKP include a reduction in the risk of intraocular complications and of irreversible rejection.[127] The Australian Graft Registry reported that LK (4% of transplants) had a significantly lower risk of allograft rejection compared with PKP at 5-years following surgery.[129] Another advantage of LK is the less stringent criteria for the quality of donor corneas.[130]

The different types of LK include anterior LK, deep LK and posterior LK. Anterior LK is the more-established technique and involves removal of the diseased portion of the anterior stromal lamellae and replacement with partial thickness donor cornea consisting of stroma, Bowman's layer, and in some cases epithelium.[127] Anterior LK is therefore indicated for corneal disorders that involve the anterior layers of the cornea only. It may also be used for strengthening the cornea, for example, in corneal thinning disorders.[127]

Deep anterior LK (DALK) is used for patients with corneal stromal disease and normal endothelium.[131] The technique involves removal of the stromal pathology (leaving Descemet's membrane and endothelium intact) and transplanting a complementary donor stromal button.[132]

Posterior LK involves selective transplantation of only the posterior corneal tissue (endothelium and posterior stroma), with the anterior layers of the recipient cornea preserved.[132] This technique is of value in patients with corneal decompensation caused by diseased endothelium.[133] Both deep and posterior LK are relatively new techniques and further clinical studies are needed to evaluate whether they reduce astigmatism, graft rejection, interface scarring, improve visual outcomes, and are viable alternatives to PKP.[132, 134, 135]

Chapter 4

The Indications and Outcome of Penetrating Keratoplasty

4.1 Indications for penetrating keratoplasty

The indications for PKP can be divided into four general categories: optical, tectonic, therapeutic and cosmetic.[136, 137]

4.1.1 Optical keratoplasty

Optical keratoplasty is performed with the primary purpose of improving visual acuity. There is significant worldwide variation in indications for optical keratoplasty. In the majority of North American studies, aphakic or pseudophakic bullous keratopathy is the most common indication reported (accounting for between 20-39% of transplants), followed by keratoconus and regraft procedures.[138-144] In contrast, keratoconus is the most common indication reported in the majority of European and Australian studies (accounting for 21-37%), followed by aphakic or pseudophakic bullous keratopathy and regraft procedures.[145-149] Other indications less commonly reported were Fuchs' endothelial dystrophy, viral keratitis, trauma, stromal dystrophies and microbial keratitis.[138-149] In developing countries, corneal scarring secondary to infection, trauma and malnutrition is the most common indication for optical keratoplasty.[150, 151]

Significant trends in relation to the indications for PKP have been identified over the last decade. Several authors have reported a decreasing trend in the number of PKPs for bullous keratopathy, with reasons cited including improvements in cataract surgery technique, better intraocular lens technology, use of

viscoelastics, and a reduction in anterior chamber intraocular lens use.[138, 139, 145, 152-154] An increasing trend in the number of PKPs for regraft procedures has also been reported, with an expanding recipient pool cited as the likely reason.[139, 141, 142, 146, 154-156] A decrease in PKPs for viral keratitis has also been identified, with advancement in the medical management of herpetic eye disease believed to be responsible for this.[140-142, 154]

4.1.2 Tectonic keratoplasty

Tectonic keratoplasty is performed to restore altered corneal structure. Although improvement in visual acuity remains a relevant consideration, restoration of ocular anatomy and physiology are the principal reasons for tectonic keratoplasty.[136, 137] The reported indications for tectonic keratoplasty include corneal perforation, corneal melting associated with autoimmune disorders, post-traumatic loss of corneal tissue, corneal thinning/ectasia, corneal fistula and pellucid marginal degeneration.[157, 158]

4.1.3 Therapeutic keratoplasty

Therapeutic keratoplasty involves tissue substitution for refractory corneal disease. A common indication for therapeutic keratoplasty is infective keratitis, where keratoplasty is performed to eliminate the infectious load in cases with keratitis unresponsive to antimicrobial therapy.[159] Another common indication of therapeutic keratoplasty is to relieve pain associated with bullous keratopathy epithelial changes.[159] Therapeutic keratoplasties often have the added

advantages of improved clarity, and subsequently, improved visual acuity.[136, 137] Therapeutic keratoplasty is always considered the last option when all other treatment modalities have failed.

4.1.4 Cosmetic keratoplasty

Cosmetic keratoplasty is performed to restore the normal appearance of the eye, without hope of visual improvement.[160] Indications for cosmetic keratoplasty include unsightly corneal scars or deposits. As there are highly successful alternatives, such as painted soft contact lenses, corneal tattooing, and enucleation or evisceration with a prosthesis or cosmetic shield, cosmetic keratoplasty has become a relatively rare procedure.[136, 137]

4.2 Outcome of Penetrating keratoplasty

4.2.1 Survival outcome

Corneal transplant survival is defined as the maintenance of a clear corneal graft. Overall one-year survival rates reported in the literature range from 80-91%.[149, 161-171] Reported 5-year survival rates range from 65-90% and 10-survival rates from 59-82%.[149, 163, 165, 170-173] The most common reasons reported for transplant failure are irreversible allograft rejection, primary endothelial failure, post-operative glaucoma, vascularization, and infection.[149, 165, 168, 172, 174]

The prognosis of penetrating keratoplasty depends on multiple factors, with the indication for keratoplasty being the most important one.[136, 137] Buxton et al[137] have devised a classification system to predict the expected survival outcome based on pre-operative diagnosis. Group 1 (excellent prognosis, > 90% survival) consists of corneas with central corneal disease and normal peripheral architecture. This group includes the following diagnoses: keratoconus, central or paracentral inactive scars, granular or macular dystrophies, and central Fuchs' dystrophy. Group 2 (very good prognosis, 80-90% survival) consists of relatively avascular corneal disorders extending partially or totally to the periphery with a relatively stable ocular surface. Diagnoses in this group include advanced Fuchs' dystrophy, pseudophakic and aphakic bullous keratopathy, inactive herpetic keratitis, iridocorneal endothelial syndromes, inactive interstitial keratitis, and lattice dystrophy. Group 3 (fair prognosis, 50-80% survival) consists of corneal disorders characterized by extremes of corneal thickness and involving a large part of the peripheral cornea. Diagnoses in this group include keratoglobus, pellucid marginal degeneration, active herpes simplex, active fungal keratitis, mild chemical injury, and congenital hereditary endothelial dystrophy. Group 4 (poor prognosis, 0-50% survival) corneal disorders are characterized by one or more of the following: severe fibrovascular replacement of the cornea, conjunctival ischaemia, anterior chamber obliteration, loss of corneal sensation, and advanced dry eye. Diagnoses in this group include ocular pemphigoid, Stevens Johnson syndrome, congenital glaucoma, anterior chamber cleavage

syndromes, neuroparalytic or neurotrophic disease, epithelial downgrowth, and multiple graft failures.

Several other risk factors for decreased penetrating keratoplasty survival have been reported in the literature. The most commonly recognized risk factors are pre-existing vascularisation, anterior segment inflammation at the time of keratoplasty, pre-operative glaucoma, previous ipsilateral grafts, previous intraocular surgery, small or large graft size, and episodes of reversible rejection.[149, 161-163, 174-178] The Australian Graft Registry[163] and the Corneal Transplant Follow-up Study[161] also reported improved survival rates with more experienced surgeons. The majority of published reports identified no association between donor-related factors (age, source, death to preservation interval, endothelial cell density, and storage duration) and survival outcome.[149, 161-163, 174-182] Recipient gender and age has also been extensively investigated, with conflicting reports in the literature. Some studies identified male gender and advanced recipient age as risk factors, however, other studies identified no such association.[149, 174, 175, 183]

4.2.2 Visual outcome

Visual outcome following penetrating keratoplasty is usually measured in terms of improvement in visual acuity and by the amount of astigmatism. The Australian Corneal Graft Registry[149] reported that overall 47% of the 7335 corneal transplants included in their study achieved a post-operative visual acuity of 6/18

(20/60) or better. The large UK Corneal Transplant Follow-up Study[161] and a recent report from the Swedish Graft Registry[184] published comparable results. Several factors have been identified that have an influence on post-operative visual acuity. Pre-operative diagnosis has been recognized as the most important factor.[149, 161-163, 172, 174, 184-186] Most published reports identified keratoconus as the pre-operative diagnosis with the best post-operative visual acuity outcome. This was followed by Fuchs' endothelial dystrophy and viral keratitis. Bullous keratopathy, trauma and regraft were commonly reported to be associated with poor post-operative visual acuity.[149, 161-163, 172, 174, 184-186] Other factors identified to be associated with poor post-operative visual acuity included the presence of associated ocular pathology, poor pre-operative visual acuity, pre-operative glaucoma, pre-existing vascularisation, and advanced recipient age.[149, 161-163, 172, 174, 184-186]

Post-operative astigmatism can also limit post-operative visual outcome following keratoplasty. The Australian Graft Registry[149] reported a median degree of post-operative astigmatism of 5 dioptres, with approximately one-third of patients having 3 dioptres or less of astigmatism. Other studies report that between 27-57% of patients achieved 3 dioptres or less of astigmatism at one-year post-operatively.[161, 184] Several factors are associated with post-keratoplasty astigmatism.[187] Pre-operative factors include donor button astigmatism, recipient corneal condition (thickness, oedema, and vascularization), previous keratoplasty and recipient astigmatism. Surgical factors include trephination error, suture

technique, eccentric graft, and donor/recipient disparity. Post-operative factors include focal wound vascularisation, donor/recipient melting, suture-related problems (suture erosion, compression, and torque), timing of suture removal and technique, wound dehiscence or override, and wound healing.

4.3 Paediatric keratoplasty

Paediatric keratoplasty is a difficult undertaking which presents a wide range of challenges pre-operatively, intra-operatively and post-operatively.[188-193] The presence of amblyopia, associated ocular pathology, and greater severity of disease may significantly limit visual outcome.[188-193] The surgical procedure is technically more complex due to the decreased rigidity and increased elasticity of the infant cornea and sclera, the smaller size of the infant eye, the increased intra-operative fibrin formation and the positive vitreous pressure.[188-193] Post-operative follow-up and management may be more complicated, and graft rejection is often difficult to detect and treat.[188-193]

The indications for paediatric keratoplasty vary significantly in the literature. The proportion of paediatric keratoplasties performed for congenital indications ranged from 14-64%, for acquired non-traumatic 19-80%, and for acquired traumatic 6-29%.[188-191, 194] The most common congenital indication reported was Peters' anomaly followed by congenital hereditary endothelial dystrophy. The most common acquired non-traumatic indication was post-infectious corneal

scarring. The most common acquired traumatic indication was penetrating trauma.[188-191, 194]

The reported survival rates for paediatric keratoplasty vary significantly in the literature, with overall one-year survival rates ranging from 46 to 80%.[188-191, 195, 196] Unsurprisingly, the indication for keratoplasty had a significant impact on survival rate. Reported one-year survival rates for congenital indications (29-80%) was significantly lower than that for acquired non-traumatic (40-85%) and acquired traumatic indications (56-84%).[188-191, 195, 196] Several factors have been identified which increase the risk of corneal transplant failure in paediatric keratoplasty. Performance of an additional surgical procedure at the time of keratoplasty is most significantly associated with increased failure rate, with other factors including pre-operative glaucoma, associated ocular conditions, and corneal vascularisation.[188-191, 196-202] Poor visual outcome in a surviving transplant (clear graft) is a well recognized occurrence in paediatric keratoplasty and is most commonly a result of amblyopia, non-corneal ocular abnormalities, and post-operative astigmatism.[188-191, 197, 198] A higher prevalence of amblyopia and associated ocular abnormalities within the congenital indication group is thought to account for the significantly poorer visual outcome reported in these patients.[188-191, 200-202]

Section 1 References

1. Alkemade PP. The deep structures of the limbus and cornea. Ophthalmalogica 1970;160:85-95.

2. Hemenger RP, Tomlinson A, Oliver K. Optical consequences of asymmetries in normal corneas. Ophthalm Physiol Optics 1996;16:124-9.

3. Masters BR. Shape visualization of the anterior and posterior human cornea in vivo. Ger J Ophthalmol 1996;5:504-7.

4. Schwiegerling J, Greivenkamp JE. Using corneal height maps and polynomial decomposition to determine corneal abberations. Optom Vis Sci 1997;74:906-16.

5. Alsbirk PH. Corneal thickness: Age variation, sex differences, and oculometric correlations. Acta Ophthalmol 1978;56:95-104.

6. Goldberg MF, Bron AJ. Limbal palisades of Vogt. Trans Am Ophthalol Soc 1982;48.

7. Ehlers N. Some comparitive studies on the mammalian corneal epithelium. Acta Ophthalmol 1970;48:821.

8. Tanaka M, Ohnishi Y, Kuwabara T. Membrane structure of corneal epithelium. Jap J Ophthalmol 1983;27:434-43.

9. Pfister RR, Burstein NL. The normal and abnormal human corneal epithelial surface: a scanning electron microscope study. Invest Ophthalmol Vis Sci 1977;16:614-22.

10. Teng CC. The fine structure of the corneal epithelium and basement membrane of the rabbit. Am J Ophthalmol 1961;51:278.

11. Hanna C, Bicknell DS, O'Brien J. Cell turnover in the adult human eye. Arch Ophthalmol 1961;65:695.

12. Rodrigues MM. Cornea. In: Jakobiec FA, ed. Ocular anatomy, embryology, and teratology. Philidelphia: Harper and Row, 1982.

13. Wilson SE, Hang SW. Bowman's layer structure and function: critical or dispensable to corneal function? A hypothesis. Cornea 2000;19:417-20.

14. Patel S, Reinstein DZ, Silverman RH, Coleman DJ. The shape of Bowman's layer in the human cornea. J Refract Surg 1998;14:636-40.

15. Bettman JW. Nature of Bowman's layer. New Eng J of Med 1970;282:344.

16. Seiler T, Matallana M, Sendler S, et al. Does Bowman's layer determine the biomechanical properties of the cornea? Refract Corneal Surg 1992;8:139-42.

17. Hamada R, Pouliquen Y. Microstructure of the dark cell in human corneal epithelium. Nippon Ganka Gakkai Zasshi 1974;78(2):61-78.

18. Hogan MJ, Alvarada JA, Weddell JE. The cornea. In: Histology of the human eye. Philidelphia: WB Saunders, 1971.

19. Kokott W. Uber mechanisch-funktionaelle strukturen des Auges. A von Grafes Arch Ophthalmol 1938;138:424.

20. Bron AJ, Tripathi RC. Anterior corneal mosaic. Further observations. Br J Ophthalmol 1969;53:760.

21. Klintworth GK. The cornea - structure and macromolecules in health and disease. A review. Am J Pathol 1977;89:718-808.

22. Olsen T. Light scattering of the human cornea. Invest Ophthalmol Vis Sci 1982;23:81-6.

23. Moller-Pedersen T, Ehlers N. A three-dimensional study of the human corneal keratocyte density. Curr Eye Res 1995;14:459-64.

24. Preziosi VA. The periphery of Descemet's membrane: a staining technique. Arch Ophthalmol 1969;82:144.

25. Goldman JN, Kuwabara T. Histopathology of corneal oedema. Intern Ophthalmol Clinics 1968;8:561-79.

26. Jue B, Maurice DM. The mechanical properties of the rabbit and human cornea. J Biomechanics 1986;19:847-53.

27. Leslie WJ. Senescent changes of the cornea. J Am Optom Assoc 1978;49:774-9.

28. Kuwabara T. Current concepts in anatomy and histology of the cornea. Contact lens Intraoc Lens Med J 1978;4:101.

29. Kreutziger GO. Lateral membrane morphology and gap junction structure in rabbit corneal endothelium. Exp Eye Res 1976;23:285.

30. Aboalchamat B, Engelmann K, Bohnk M, et al. Morphological and functional analysis of immortalized human corneal endothelial cells after transplantation. Exp Eye Res 1999;69:547-53.

31. Shaw EL, Rao GN, Arthur E, et al. The functional reserve of corneal endothelium. Trans Am Acad Ophthalmol Otolaryngol 1978;85:640.

32. Klyce SD, Beverman RW. Structure and function of the cornea. In: Kaufman HE, Barron BA, McDonald MB, eds. The Cornea, 2nd Ed. Boston: Butterworth-Heinemann, 1998.

33. Belmonte C, Acosta MC, Schmelz M, et al. Measurement of corneal sensitivity to mechanical and chemical stimulation with a carbon dioxide esthesiometer. Invest Ophthalmol Vis Sci 1999;40:513-19.

34. Muller LJ, Pels L, Vrensen GF. Ultrastructural organisation of human corneal nerves. Invest Ophthalmol Vis Sci 1996;37:476-88.

35. Muller LJ, Vrensen GF, Pels L, et al. Architecture of human corneal nerves. Invest Ophthalmol Vis Sci 1997;38:985-94.

36. Tomlinson A, Craig JP, Lowther G. The biophysical role in tear regulation. Adv Exp Med Biol 1998;438:371-80.

37. Thoft RA, Friend J, Dohlman CH. Corneal glucose concentration. Flux in the presence and absence of epithelium. Arch Ophthalmol 1971;85(4):467-72.

38. Kinoshita JH. Some aspects of carbohydrate metabolism of the cornea. Invest Ophthalmol 1962;1:178.

39. Thoft RA, Friend J. Corneal epithelial glucose metabolism. Arch Ophthalmol 1971;88:55.

40. Benjamin WJ, Ruben CM. Human corneal oxygen demands at superior, central, and inferior sites. J Am Optom Assoc 1995;66(7):423-8.

41. Quantock AJ, Meek KM, Brittain P, et al. Alteration of the stromal architecture and depletion of keratan sulphate proteoglycans in oedematous human corneas: histological, immunochemical and X-ray diffraction evidence. Tissue Cell 1991;23(5):593-606.

42. Dohlman CH, Hedbys BO, Mishima S. The swelling pressure of the corneal stroma. Invest Ophthalmol Vis Sci 1962;1:158.

43. Hedbys BO, Dohlman CH. A new method for the determination of the swelling pressure of the corneal stroma in vitro. Exp Eye Res 1963;1:122.

44. Maurice DM. The location of the fluid pump in the cornea. J Physiol (Lond) 1972;221:43.

45. Barfort P, Maurice D. Electrical potential and fluid transport across the corneal endothelium. Exp Eye Res 1974;19(1):11-9.

46. Fischborg J. The mechanism of fluid and electrolyte transport across the corneal endothelium: critical revision and update of a model. Curr Eye Res 1985;4:351.

47. Maurice DM. The permeability to sodium ions of the living rabbits cornea. J Physiol (Lond) 1981;112:367.

48. Mishima S, Hedbys BO. The permeability of the corneal epithelium and endothelium. Exp Eye Res 1967;6:10.

49. Kikkawa Y. Corneal potential studied on excised cornea. Exp Eye Res 1966;5(1):31-6.

50. Terry JE, Dewart MR. Epithelial wound healing. J Am Optom Assoc 1980;51(5):439-44.

51. Geggel HS, Friend J, Thoft RA. Conjunctival epithelial wound healing. Invest Ophthalmol Vis Sci 1984;25(7):860-3.

52. Collin HB, Anderson JA, Richard NR, Binder PS. In vitro model for corneal wound healing; organ-cultured human corneas. Curr Eye Res 1995;14(5):331-9.

53. Chung EH, DeGregorio PG, Wasson M, Zieske JD. Epithelial regeneration after limbus-to-limbus debridement. Expression of alpha-enolase in stem and transient amplifying cells. Invest Ophthalmol Vis Sci 1995;36(7):1336-43.

54. Nagy ZZ, Nemeth J, Suveges I, Csakany B. [Examination of subepithelial scar formation after photorefractive keratectomy with the ultrasound biomicroscope]. Klin Monatsbl Augenheilkd 1996;209(5):283-5.

55. Wilson SE, Mohan RR, Hong JW, et al. The wound healing response after laser in situ keratomileusis and photorefractive keratectomy: elusive control of biological variability and effect on custom laser vision correction. Arch Ophthalmol 2001;119(6):889-96.

56. Frueh BE, Cadez R, Bohnke M. In vivo confocal microscopy after photorefractive keratectomy in humans. A prospective, long-term study. Arch Ophthalmol 1998;116(11):1425-31.

57. Raj CV, Freeman IL, Church RL, Brown SI. Biochemical characterization of procollagen-collagen synthesized by rabbit corneal endothelial cells in culture. Invest Ophthalmol Vis Sci 1979;18(1):75-84.

58. Moller-Pedersen T. A comparative study of human corneal keratocyte and endothelial cell density during aging. Cornea 1997;16(3):333-8.

59. Ratkay-Traub I, Hopp B, Bor Z, et al. Regeneration of rabbit cornea following excimer laser photorefractive keratectomy: a study on gap junctions, epithelial junctions and epidermal growth factor receptor expression in correlation with cell proliferation. Exp Eye Res 2001;73(3):291-302.

60. Olsen EG, Davanger M. The healing of human corneal endothelium. An in vitro study. Acta Ophthalmol (Copenh) 1984;62(6):885-92.

61. Filatov VP. Transplantation of the cornea. Arch Ophthalmol 1935;13:321-47.

62. Szaflik J, Liberek I, Brix M. Corneal storage methods. Transplantation proceedings 2000;32:1424-5.

63. Pollock G. Eye banking - a practical guide. In: Vajpayee RB, ed. Corneal transplantation. New Delhi: Jaypee Brothers Ltd, 2002.

64. Bito LZ, Salvador EV. Intraocular fluid dynamics. Post-mortem changes in solute concentration. Exp Eye Res 1970;10:273-87.

65. McCarey BE, Kaufman HE. Improved corneal storage. Invest Ophthalmol 1974;13:165-73.

66. McCarey BE, Meyer RF, Kaufman HE. Improved corneal storage for penetrating keratoplasties in humans. Ann Ophthalmol 1976;8:1488-92.

67. Stainer GA, Brightbill FS, Calkins B. A comparison of corneal storage in moist chamber and McCarey-Kaufman medium in human keratoplasty. Ophthalmology 1981;88:46-9.

68. Bigar F, Kaufman HE, McCarey BE, et al. Improved corneal storage for penetrating keratoplasties in man. Am J Ophthalmol 1975;79:115-20.

69. Aquavella JV, Horm DLV, Haggerty CJ. Corneal preservation using MK medium. Am J Ophthalmol 1975;80:791-99.

70. Kaufman HE, Varnell ED, Kaufman S, et al. K-Sol corneal preservation. Am J Ophthalmol 1985;100:299-300.

71. Chu W. The past twenty-five years in eye banking. Cornea 2000;19:754-65.

72. Keates RH, Rabin B. Extending corneal storage with 2.5% chondroitin sulphate. Ophthalmic surgery 1988;19:817-20.

73. Busin M, Yau C, Avni I, et al. Comparison of K-Sol and M-K medium for corneal storage. Results of penetrating keratoplasty in rabbits. Br J Ophthalmol 1986;70:860.

74. Stein RM, Bourne WM, Campbell RJ. Chondroitin sulphate for corneal preservation at 4°C. Arch Ophthalmol 1986;104:1358.

75. Kaufman HE, Beuerman RW, Steinman TL, et al. Optisol corneal storage medium. Arch Ophthalmol 1991;109:864-8.

76. Lass JH, Bourne WM, Sugar A, et al. A randomized prospective, double-masked clinical trial of Optisol vs Dexsol corneal storage media. Arch Ophthalmol 1992;110:1401.

77. Smith TM, Popplewell J, Nakamura T, et al. Efficacy and safety of gentamicin and streptomycin in Optisol GS, a preservation medium for donor corneas. Cornea 1995;14:49-54.

78. Doughman DJ, Harris JE, Schmitt MK. Penetrating keratoplasty using 37°C organ cultured corneas. Trans Am Acad Ophthalmol Otolaryngol 1976;81:778-93.

79. Sperling S. Human corneal endothelium in organ culture. The influence of temperature and medium of incubation. Acta Ophthalmol 1979;57:269.

80. Easty DL, Carter CA, Lewkowicz-Moss SJ. Corneal cell culture and organ culture. Trans Ophthalmol Soc UK 1986;105:385.

81. Armitage WJ, Easty DL. Factors influencing the suitability of organ-cultured corneas for transplantation. Invest Ophthalmol Vis Sci 1997;38:16-24.

82. Doughman D. Prolonged donor corneal preservation in organ culture: long term clinical evaluation. Trans Am Ophthalmic Soc 1980;78:567.

83. Prost L, Halfaker B, Holland E. Quality of corneal tissue in the greater than 75 year age group. Cornea 1997;16:507-11.

84. Ehlers N. Corneal banking and grafting. The background to the Danish Eye Bank System, where corneas await their patients. Acta Ophthalmica Scand 2002;80:572-8.

85. Doughman D, Lindstrom R, Skelnick D, et al. Long-term organ cultured storage: Minnesota system. In: Brightbill F, ed. In Corneal surgery: theory, technique and tissue, 2nd ed. St Louis: Mosby, 1993.

86. Doughman D, Horn DV, Harris J. The ultrastructure of the human organ cultured cornea. Arch Ophthalmol 1974;92:516.

87. Lindstrom R, Doughman D, Horn DV, et al. A metabolic and electron microscope study of human organ cultured cornea. Am J Ophthalmol 1976;82:72.

88. Doughman D, Horn DV, Harris J. Endothelium of the human organ cultured cornea: an electron microscope study. Trans Am Ophthalmic Soc 1973;71:304.

89. Bourne W, Lindstrom R, Doughman D. Endothelial cell survival on transplanted human corneas preserved by organ culture with 1.35% chondroitin sulphate. Am J Ophthalmol 1985;100:789-83.

90. Keates R. Organ culture corneal preservation. A preliminary report. Dev Ophthalmol;11:44.

91. European Eye Bank Association Directory. 12th Ed. 2004.

92. Tullo A, Dyer P. Corneal transplantation in Britain. Brit Med J 1995;310:1347-8.

93. Szaflik J, Liberik I, Brix M. Corneal storage methods. Trans Proc 2000;32:1424-5.

94. Pel E, Beekhuis W, Volker-Dieben HJ. Long term tissue storage for keratoplasty. In: Brightbill F, ed. Corneal surgery: theory, technique and tissue, 3rd ed. St Louis: Mosby, 1999;106:897-905.

95. Vajpayee R, Melki S, Sharma N. Technique of Penetrating Keratoplasty. In: Vajpayee R, ed. Corneal Transplantation. New Delhi: Jaypee Brothers, 2002

96. Zirm E. Eine erfolgreiche totale Keratoplastik. Arch Ophthalmol 1906;64:580-93.

97. Castroviejo R. Preliminary report of a new method of corneal transplant. Proc Staff Meet Mayo Clin 1931;6:417-18.

98. Sugar A, Sugar J. Techniques in Penetrating Keratoplasty: A quarter century of development. Cornea 2000;19:603-10.

99. Sugar J. Phakic Keratoplasty. In: Brightbill F, ed. Corneal Surgery: Theory, Technique and Tissue, 3rd ed. St Louis: Mosby-Year Book Inc, 1999;36:279-283

100. Brightbill F, Calkins B. New forceps and cutting block for donor corneas. Am J Ophthalmol 1980;89:744-5.

101. Tanne E. A new donor cutting block for penetrating keratoplasty. Ophthalmic Surg 1981;12:371-2.

102. Sietz B, Langenbucher A, Kus M, et al. Non-mechanical corneal trephination with the excimer laser improves outcome after penetrating keratoplasty. Ophthalmol 1999;106:1156-64.

103. Langenbucher A, Sietz B, Kus M, et al. Graft decentration in penetrating keratoplasty; non-mechanical trephination with the excimer laser versus the motor trephine. Ophthalmic Surg Lasers 1998;29:106-13.

104. Dunker G, Nolle B. A new cornea-marking device for penetrating keratoplasty and refractive corneal procedures. Ophthalmologica 1995;209:25-6.

105. Duronio J, O'Brien T, Gottsch J. A combined marker and trephination instrument for penetrating keratoplasty. Arch Ophthalmol 1998;116:958-59.

106. Barron B. Penetrating keratoplasty. In: Kaufman H, Barron B, McDonald M, eds. The Cornea, 2nd Ed. Boston: Butterworth-Heinemann, 1998.

107. Vajpayee R, Ramu M, Panda A, et al. Oversized grafts in children. Ophthalmol 1999;106:829-32.

108. Olsen R. Variation in corneal graft size related to trephine technique. Arch Ophthalmol 1979;97:1323-5.

109. Damiano R, Horn DV, Schultz R. Trephination of donor corneal buttons: a scanning electron microscope study. Ann Ophthalmol 1978;10:479-85.

110. Hessburg P, Barron M. A disposable corneal trephine. Ophthalmic Surg 1980;11:730-3.

111. Duffin R, Olson R, Ohrloff C. Analysis of the Hessburg-Barron vacuum trephine. Ophthalmic Surg 1984;15:51-4.

112. Kuchle M, Nguyen N, Seitz B, et al. Blood-aqueous barrier after mechanical or non-mechanical excimer laser trephination in penetrating keratoplasty. Am J Ophthalmol 1998;125:177-81.

113. Liesegang T. Viscoelastic substances in Ophthalmology. Surv Ophthalmol 1990;34:268-93.

114. Bahn C, Grosserode R, Musch D, et al. Effect of 1% sodium hyaluronate on a non-regenerating corneal endothelium. Invest Ophthalmol Vis Sci 1986;27:1485-94.

115. Musch D, Meyer R, Sugar A, et al. Endothelial rejection following penetrating keratoplasty using Healon or BSS. Cornea 1990;9:305-11.

116. McDermott M, Hazlett L, Barrett R, et al. Viscoelastic adherence to corneal endothelium following phacoemulsification. J Cataract Refract Surg 1988;24:678-83.

117. McNeill J, Kaufman H. A double running suture technique for keratoplasty: earlier visual rehabilitation. Ophthalmic Surg 1977;8:58-61.

118. Davison J, Bourne W. Results of penetrating keratoplasty using a double running continuous suture technique. Arch Ophthalmol 1981;99:1591-5.

119. McNeil J, Wessels I. Adjustment of single continuous suture to control astigmatism after penetrating keratoplasty. Refract Corneal Surg 1989;5:216-23.

120. Musch D, Meyer R, Sugar A, et al. Corneal astigmatism after penetrating keratoplasty. The role of suture technique. Ophthalmol 1989;96:698-703.

121. Meter WV, Gussler J, Soloman K, et al. Post-keratoplasty astigmatism control: single continuous suture adjustment versus selective interrupted suture removal. Ophthalmol 1991;98:177-83.

122. Assil K, Zarnegar S, Schanzlin D. Visual outcome after penetrating keratoplasty with double continuous or combined interrupted and continuous suture wound closure. Am J Ophthalmol 1992;114:63-71.

123. Pradera I, Ibrahim O, Waring G. Refractive results of successful penetrating keratoplasty, intraocular lens implanation with selective suture removal. Refract corneal surg 1989;5:231-9.

124. Haight D. Sutures and suturing technique. In: Brightbill F, ed. Corneal Surgery: Theory, Technique and Tissue, 3rd ed. St Louis: Mosby-Year Book Inc, 1999;44:336-347.

125. Sudan R, Ahuja R. Suture materials and needles. In: Vaypayee R, ed. Corneal Transplantation. New Delhi: Jaypee Brothers, 2002.

126. Ramselaar J, Beekhuis W, Rijnefeld W. Mersilene, a new suture for penetrating keratoplasty. Doc Ophthalmo;82:89-101.

127. Sharma N, Vajpayee R, Ahuja R. Lamellar keratoplasty. In: Vajpayee R, ed. Corneal Transplantation. New Delhi: Jaypee Brothers, 2002.

128. Sturbaum C, Eve R, Goosey J. Lamellar Grafting. In: Brightbill F, ed. Corneal Surgery: Theory, Technique and Tissue, 3rd ed. St Louis: Mosby-Yearbook Inc, 1999:63:500-507.

129. The Australian Graft Registry Registry Report 1990-1992. Aust NZ J Ophthalmol 1993;21:1-48.

130. Terry M. The evolution of lamellar grafting techniques over 25 years. Cornea 2000;19:611-6.

131. Azar D, Jain S, Sambursky R. A new surgical technique of microkeratome-assisted deep lamellar keratoplasty with a hinged flap. Arch Ophthalmol 2000;118:1112-5.

132. Jain S, Azar D. New lamellar keratoplasty techniques: posterior keratoplasty and deep lamellar keratoplasty. Curr Opin Ophthalmol 2001;12:262-8.

133. Melles G, Remeijer L, Geerards A, et al. The future of lamellar keratoplasty. Curr Opin Ophthalmol 1999;10:253-9.

134. Azar D, Jain S, Sambursky R, et al. Microkeratome assisted posterior keratoplasty. J Cataract and Refractive Surg 2001;27:353-6.

135. Melles G, Lander F, Dooren BV, et al. Preliminary clinical results of posterior lamellar keratoplasty through a sclerocorneal pocket incision. Ophthalmol 2000;107:1850-6.

136. Ray M, Sharma N, Vajpayee R. Indications and Outcome of Penetrating Keratoplasty. In: Vajpayee R, ed. Corneal Transplantation. New Delhi: Jaypee brothers, 2002.

137. Sutphin J. Indications and Contraindications for penetrating keratoplasty. In: Brightbill FS, ed. Corneal Surgery: Theory, Technique and Tissue, 3rd ed. St Loius: Mosby-Yearbook, 1999;32:247-258.

138. Eye Bank Association of America Statistical Reports 2002. Washington, DC: EBAA. 2002.

139. Dobbins K, Price F, Whitson W. Trends in indications for penetrating keratoplasty in the Midwestern United States. Cornea 2000;19:813-6.

140. Flowers C, Chang K, McLeod S, et al. Changing indications for penetrating keratoplasty, 1989-1993. Cornea 1995;14:583-8.

141. Cosar C, Sridhar M, Cohen E, et al. Indications for penetrating keratoplasty and associated procedures 1996-2000. Cornea 2002;21:148-51.

142. Brady S, Rapuano C, Arentsen J, et al. Clinical indications for and procedures associated with penetrating keratoplasty, 1983-1988. Am J Ophthalmol 1989;108:118-22.

143. Robin J, Gindi J, Koh K, et al. An update of the indications for penetrating keratoplasty, 1979 through 1983. Arch Ophthalmol 1986;104:87-9.

144. Price F, Whitson W, Collins K, et al. Five-year corneal graft survival. Arch Ophthalmol 1993;111:799-805.

145. Legeais J, Parc C, D'Hermies. Nineteen years of penetrating keratoplasty at the Hotel Dieu Hospital in Paris. Cornea 2001;20:603-6.

146. Cursiefen C, Kuchle M, Naumann G. Changing indications for penetrating keratoplasty : Histopathology of 1250 corneal buttons. Cornea 1998;17:468-70.

147. Morris R, Bates A. Changing indications for keratoplasty. Eye 1989;3:455-9.

148. Brooks A, Weiner J. Indications for penetrating keratoplasty: a Clinicopathologic review of 511 corneal specimens. Aus NZ J Ophthalmol 1987;15:277-81.

149. Williams K, Muehlberg S, Bartlett C, et al. Australian Graft Registry Report. Adelaide. 2000.

150. Dada T, Sharma N, Vajpayee R. Indications for paediatric keratoplasty in India. Cornea 1999;18:296-8.

151. Dandona L, Ragu K, Janarthanan M, et al. Indications for penetrating keratoplasty in India. Indian J Ophthalmol 1997;45:163-8.

152. Al-Yousuf N, Mavrikakis I, Mavrikakis E, et al. Penetrating keratoplasty: indications over a 10 year period. Br J Ophthalmol 2004;88:998-1001.

153. Chan C, Wong T, Yeong S, et al. Penetrating keratoplasty in the Singapore National Eye Centre and donor acquisition in the Singapore Eye Bank. Annals Acad Med, Singapore 1997;26:395-400.

154. Al-Towerki A, Gonnah E, Rajhi AA, et al. Changing indications for corneal transplantation at the King Khaled Eye Specialist Hospital (1983-2002). Cornea 2004;23:584-8.

155. Lois N, Kowal V, Cohen E, et al. Indications for penetrating keratoplasty and associated procedures, 1989-1995. Cornea 1997;16:623-9.

156. Maeno A, Naor J, Lee H, et al. Three decades of corneal transplantation: indications and patient characteristics. Cornea 2000;19:7-11.

157. Soong H, Farzo A, Katz D, et al. Lamellar corneal patch grafts in the management of corneal melting. Cornea 2000;19:126-34.

158. Taylor D, Stern A. Recontructive keratoplasty in the management of conditions leading to corneal destructions. Ophthalmol 1980;87:892-904.

159. Killingworth D, Stern G, Driebe W, et al. The results of therapeutic keratoplasty. Ophthalmol 1993;100:534-41.

160. Buxton J. Corneal surgery. In: Collins J, ed. Handbook of clinical ophthalmology. New York: Masson, 1982.

161. Vail A, Gore S, Bradley B, et al. Conclusions of the corneal transplant follow-up study. Br J Ophthalmol 1997;81:631-6.

162. Dandona L, Naduvilath T, Janarthanan M, et al. Survival analysis and visual outcome in a large series of corneal transplants in India. Br J Ophthalmol 1997;81:726-31.

163. Vail A, Gore S, Bradley B, et al. Corneal graft survival and visual outcome: a multicenter study. Ophthalmology 1994;101:120-7.

164. Bradley B, Vail A, Gore S, et al. Penetrating keratoplasty in the United Kingdom: an interim analysis of the corneal transplant follow-up study. Clinical transplants 1993:293-315.

165. Chan C, Wong T, Yeong S, et al. Penetrating keratoplasty in the Singapore National Eye Centre and donor cornea acquisition in the Singapore Eye Bank. Annals of the Academy of Medicine 1997;26:395-400.

166. Williams K, Muehlberg S, Lewis R, et al. How successful is corneal transplantation? A report from the Australian Corneal Graft Register. Eye 1995;9:219-27.

167. Gain P, Thuret G, Chiquet C, et al. Corneal procurement from very old donors: post organ culture cornea outcome and graft failure. Br J Ophthalmol 2000;86:404-11.

168. Muraine M, Sanchez C, Watt L, et al. Long-term results of penetrating keratoplasty. A 10-year-plus retrospective study. Graef Arch Clin Exp Ophthalmol 2003;241:571-6.

169. Teenan D, Sim K, Hawksworth N. Outcomes of corneal transplantation: a corneal surgeon vs the general ophthalmologist. Eye 2003;17:727-30.

170. Borderie V, Schee S, Touzeau O, et al. Donor organ cultured corneal tissue selection before penetrating keratoplasty. Br J Ophthalmol 1998;82:382-88.

171. Yorston D, Wood M, Foster A. Penetrating keratoplasty in Africa: graft survival and visual outcome. Br J Ophthalmol 1996;80:890-94.

172. Thompson R, Price M, Bowers P. Long-term Graft Survival after Penetrating Keratoplasty. Ophthalmol 2003;110:1396-402.

173. Ing J, Ing H, Nelson L, et al. Ten-year postoperative results of penetrating keratoplasty. Ophthalmol 1998;105:1855-65.

174. Price M, Thompson R, Price F. Risk factors for various causes of failure in initial corneal grafts. Arch Ophthalmol 2003;121:1087-93.

175. Maguire M, Stark W, Gottsch J, et al. The Collaborative Corneal Transplantation Studies Research Group. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. Ophthalmol 1994;101:1536-47.

176. Boisjoly H, Tourigny R, Bazin R, et al. Risk factors for corneal graft failure. Ophthalmol 1993;100:1728-35.

177. Volker-Dieben H, D'Amaro J, Alphen CK-V. Hierarchy of prognostic factors for corneal graft survival. Aus NZ J Ophthalmol 1987;15:11-8.

178. Abbot R, Forster R. Determinants of graft clarity in penetrating keratoplasty. Arch ophthalmol 1979;97:1071-5.

179. Williams K, Muehlberg S, Lewis R, et al. Influence of advanced recipient age and donor age on the outcome of corneal transplantation. Br J Ophthalmol 1997;81:835-9.

180. Palay D, Kangas T, Stulting R, et al. The effects of donor age on the outcome of penetrating keratoplasty in adults. Ophthalmol 1997;104:1576-9.

181. Vail A, Gore S, Bradley B, et al. Influence of donor and histocompatibility factors on corneal graft outcome. Transplant 1994;58:1210-6.

182. Anderson J, Elders N. The influence of donor age and post mortem time on corneal graft survival and thickness when employing banked donor material (A five-year follow-up). Acta Ophthalmol 1988;66:313-7.

183. Sit M, Weisbrod D, Noar J, et al. Corneal graft outcome study. Cornea 2001;20:129-33.

184. Claesson M, Armitage W, Fagerholm P, et al. Visual outcome in corneal grafts: a preliminary analysis of the Swedish Corneal Transplant Register. Br J Ophthalmol 2002;86:174-80.

185. Pineros O, Cohen E, Rapuano C, et al. Long-term results after penetrating keratoplasty for Fuchs' endothelial dystrophy. Arch Ophthalmol 1996;114:15-8.

186. Fishbaugh J. Visual acuity, residual astigmatism, and graft clarity following penetrating keratoplasty for keratoconus. Insight 1991;16:24-7.

187. Binder P. Surgical correction of astigmatism. In cornea, refractive surgery, and contact lens. Trans New Orleans Acad Ophthalmol 1987:7.

188. Stulting R, Sumers K, Cavanagh H, et al. Penetrating keratoplasty in children. Ophthalmol 1984;91:1222-30.

189. Dana M, Moyes A, Gomes J, et al. The indications and outcome in paediatric corneal tranplantation. Ophthalmol 1995;102:1129-38.

190. Cowden J. Penetrating keratoplasty in infants and children. Ophthalmol 1990;97:324-9.

191. Aasuri M, Prashant G, Gokhle N, et al. Penetrating keratoplasty in children. Cornea 2000;19:140-4.

192. Stulting R. Penetrating keratoplasty in children. In: Brightbill F, ed. Corneal surgery: theory, technique and tissue, 3 ed. St Louis: Mosby, 1999.

193. Vajpayee R, Ramu M, Panda A, et al. Oversized grafts in children. Ophthalmol 1999;106:829-32.

194. Dada T, Sharma N, Vajpayee R. Indications for paediatric corneal transplantation in India. Cornea 1999;18:296-8.

195. Legeais J, Jobin D, Pouliquen Y. Keratoplasties chez l'enfant. J Fr Ophthalmol 1990;13:116-20.

196. Erlich C, Rootman D, Morin J. Corneal transplantation in infants, children and young adults: experience of the Toronto Hospital for Sick Children 1979-1988. Can J Ophthalmol 1991;26:206-10.

197. Parmley V, Stonecipher K, Rowsey J, et al. Peters' anomaly: a review of 26 keratoplasties in infants. Ophthalmic Surg 1993;24:31-5.

198. Williams K, Roder D, Esterman A, et al. Factors predictive of corneal graft survival. Report from the Australian Graft Registry. Ophthalmol 1992;99:403-14.

199. Yang L, Lambert S, Lynn M, et al. Long-term results of corneal graft survival in infants and children with Peter's anomaly. Ophthalmol 1999;106:833-48.

200. Waring G, Laibson P. Keratoplasty in infants and children. Trans Am Acad Ophthalmol Otolaryngol 1977;83:283-96.

201. Musch D, Meyer R. Risk of endothelial rejection after bilateral penetrating keratoplasty. Ophthalmol 1989;96:1139-43.

202. Voolker-Dieben H, Alphen CK, Lansbergen Q, et al. Different influences on corneal graft survival in 539 transplants. Acta Ophthalmol 1982;60:190-202.

Section 2

The New Zealand National Eye Bank Studies

Chapter 5

The New Zealand National Eye Bank Study 1991-2003: A Review of the Source and Management of Corneal Tissue

5.1 Abstract

Purpose

To evaluate donor demographics and source, donor tissue processing and storage, biological contamination, and the utilization and distribution of corneal tissue procured by the New Zealand National Eye Bank.

Methods

As part of a prospective longitudinal study, the electronic records of the NZNEB for the 13 year period 1991-2003 were analyzed for each year with respect to donor demographics, donor source, donor cause of death, death to preservation interval, storage methods, endothelial assessment, biological contamination, corneal tissue utilization and distribution.

Results

During the study period 3221 corneas were retrieved from 1628 donors (69.8% male, 30.2% female), with the mean age of donors 59.4 years (SD = 18.3 years) and range 4 to 95 years. No significant correlation was found between donor age group (using 10 year intervals) and the proportion of corneas suitable for transplantation. Donors were procured from the Coroner's service (67.6%), public hospitals (23.5%) and multi-organ donors (7.1%). The most common causes of donor death were cardiovascular disease, trauma and cerebrovascular disease. Average storage duration increased from 3.5 to 11.8 days when organ culture replaced hypothermic storage in 1992. Biological contamination occurred in 5% of all donor corneas. The most common bacterial and fungal isolates were coagulase-negative staphylococci

and *Candida* sp respectively. A significant decrease in contamination rate over the years of the study was identified. Overall, 79.4% of corneal tissue procured was used for corneal transplantation (75.8% for penetrating keratoplasty, 2.1% for lamellar keratoplasty, and 1.5% for unspecified transplants), and 21.6% was discarded. Most common reasons for discarding tissue were biological contamination, abnormal serology, and failed endothelial assessment.

Conclusion

Analysis of the NZNEB database provides valuable information in relation to eye banking and corneal transplantation in New Zealand. Significant trends were identified in donor demographics, donor procurement source, improved donor tissue processing and storage, decreased biological contamination, and increased utilization of corneal tissue.

5.2 Introduction

The New Zealand National Eye Bank (NZNEB), founded in 1991, is the major supplier of donated ocular tissue for transplantation in New Zealand. Serving a population of four million, the NZNEB provides over 200 corneas per year for transplantation. The highest international standards are observed in all areas of NZNEB operation including donor selection and screening, tissue retrieval and storage, testing and evaluation, and transport to transplant centers.

A standard protocol of the NZNEB since 1991 is the maintenance of a comprehensive database, supported by New Zealand ophthalmic surgeons, in which prospective data are collected on all aspects of corneal donation and transplantation. In this study we have analyzed this database to evaluate the source and management of donor corneal tissue in New Zealand.

5.3 Methods

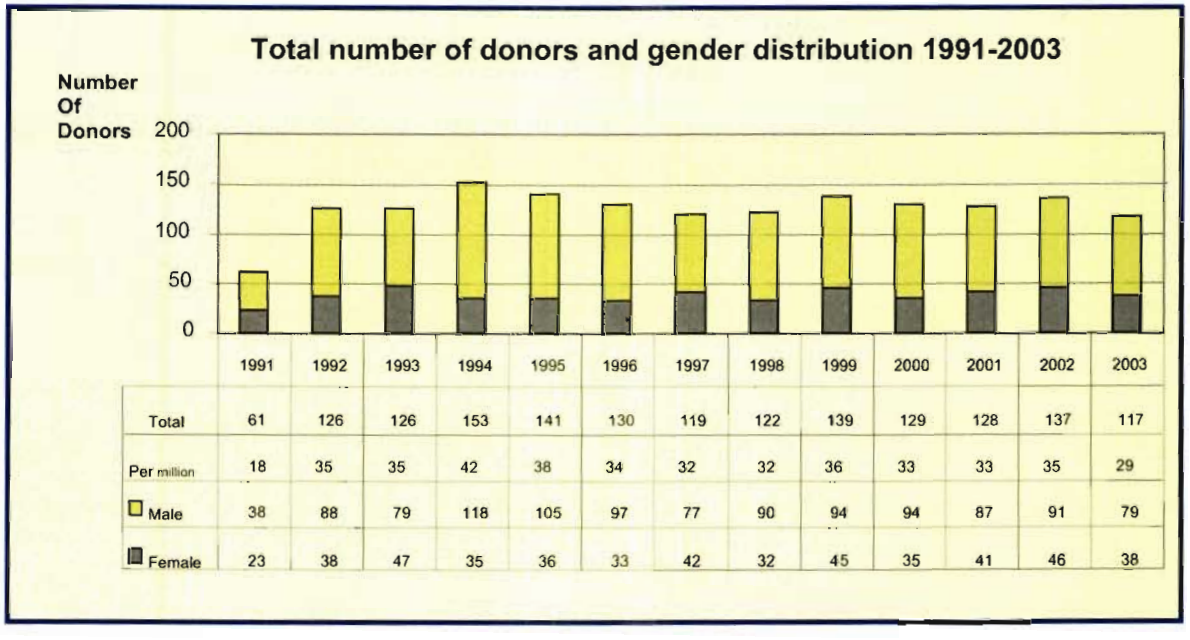
As part of a longitudinal, prospective study, the electronic records of the NZNEB for the 13 year period 1991-2003 were analyzed for each year with respect to donor demographics (gender, age, and ethnicity), donor procurement source, donor cause of death, death to preservation interval, storage methods, endothelial assessment, biological contamination, corneal tissue utilization and distribution. Statistical analysis of data was performed in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. Statistical methods used were linear regression

analysis to evaluate trends, χ^2-testing to compare proportions and the Student's *t* test to compare means between groups.

5.4 Results

5.4.1 Donor Demographics

There were 1628 donors during the 13-year study period with the average number per year being 125 (Standard deviation (SD) = 21.7). Donor numbers per year remained constant from 1992 onwards with no significant trends being identified (ß-coeff = 0.12, se = 0.45, p = 0.79). The gender distribution demonstrated a male preponderance in every year of the study, and overall 69.8% (n = 1137) of donors were male and 30.2% (n = 491) female. Donor numbers and gender distribution for each year of the study are shown in figure 5:1.



	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003
Total	61	126	126	153	141	130	119	122	139	129	128	137	117
Per million	18	35	35	42	38	34	32	32	36	33	33	35	29
Male	38	88	79	118	105	97	77	90	94	94	87	91	79
Female	23	38	47	35	36	33	42	32	45	35	41	46	38

Figure 5:1 ***Bar graph showing total number of donors, donors per million population, and gender distribution 1991-2003.***

The age distribution of donors during the study period is illustrated in figure 5:2. The largest proportion of donors was found within the 71 to 80 year age group (29.4%, n = 479) followed by the 61-70 year age group (24.0%, n = 391). The mean age of donors was 59.4 years (SD = 18.3), the median age was 65.0 years, and the age range was 4 to 95 years. An increase in mean age of donors over the years of the study was identified (ß-coeff = 0.62, se = 0.23, p=0.02) (figure 5:3). The proportion of donor corneas that were suitable for transplantation was analyzed for different donor age groups (using 10 year intervals). The results ranged from 73.2% for the 31-40 year age group to 84.0% in the 51-60 year age group. In the oldest age group (81-85 years), 79.5% of donor corneas were suitable for transplantation. Overall, no correlation was identified between donor age group and the proportion of corneas suitable for transplantation (ß-coeff = 0.57, se = 0.49, p = 0.29). Donors aged less than 10 years or greater than 85 years were generally not accepted due to the age criteria set by the NZNEB. The difference in the mean age of donor corneas used for transplantation (59.5 years) versus those that were not utilized (58.6 years) was not significant (p = 0.14).

Ethnicity data has been entered into the NZNEB database since 1993. The majority of donors were European Caucasian (89.0%, n = 1283) followed by Maori (1.6%, n = 23), Polynesian (1.1%, n =16), Indian (0.8%, n =12), Asian (0.4%, n = 6), with 6.6% (n = 94) either not recorded or unspecified.

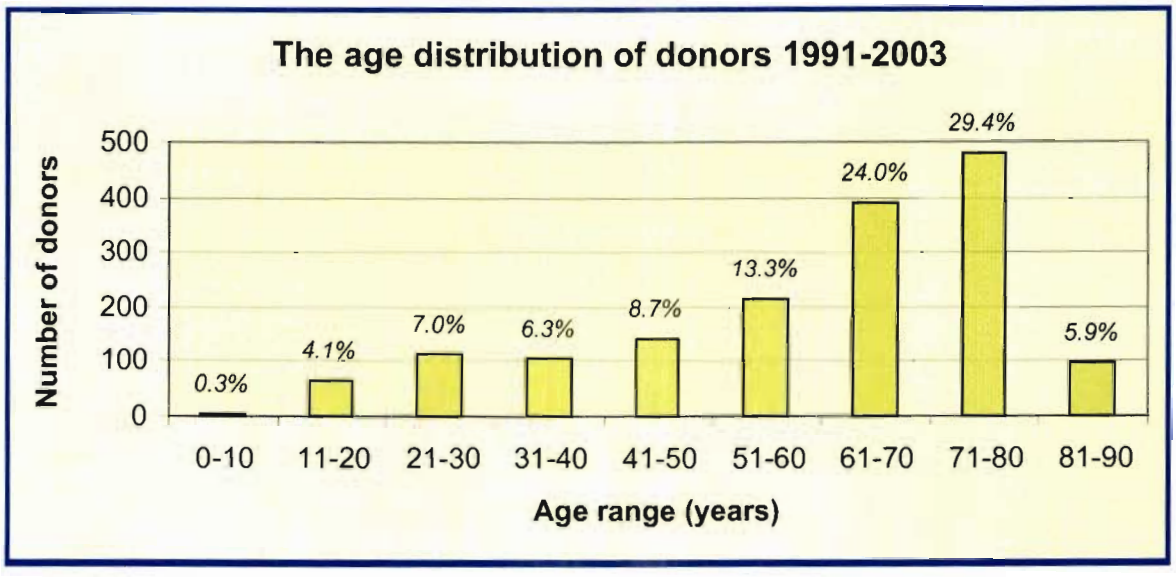


Figure 5:2 ***Bar graph showing age distribution of donors with corresponding percentage of total in the period 1991-2003.***

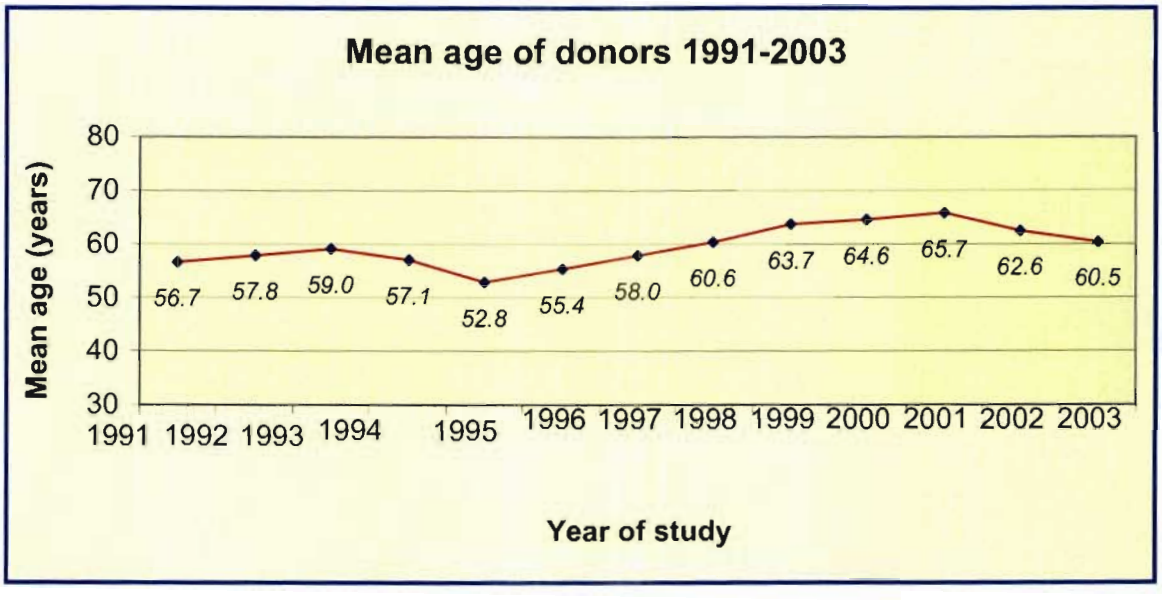


Figure 5:3 ***Line Graph showing mean age of donors for each year of the study***

5.4.2 Donor Source and Donor Cause of Death

The NZNEB procured corneal donors from the following sources: Auckland Coroner's service (67.6%, n = 1100), public hospitals (23.5%, n = 383), multi-organ donors (7.1%, n = 116), and private hospitals/ rest homes (1.8%, n = 24). As illustrated in figure 5:4, there has been a trend of fewer donors procured from the Coroner's service since 1994, but a corresponding increase in donors from public hospitals, and to a lesser extent, multi-organ donors.

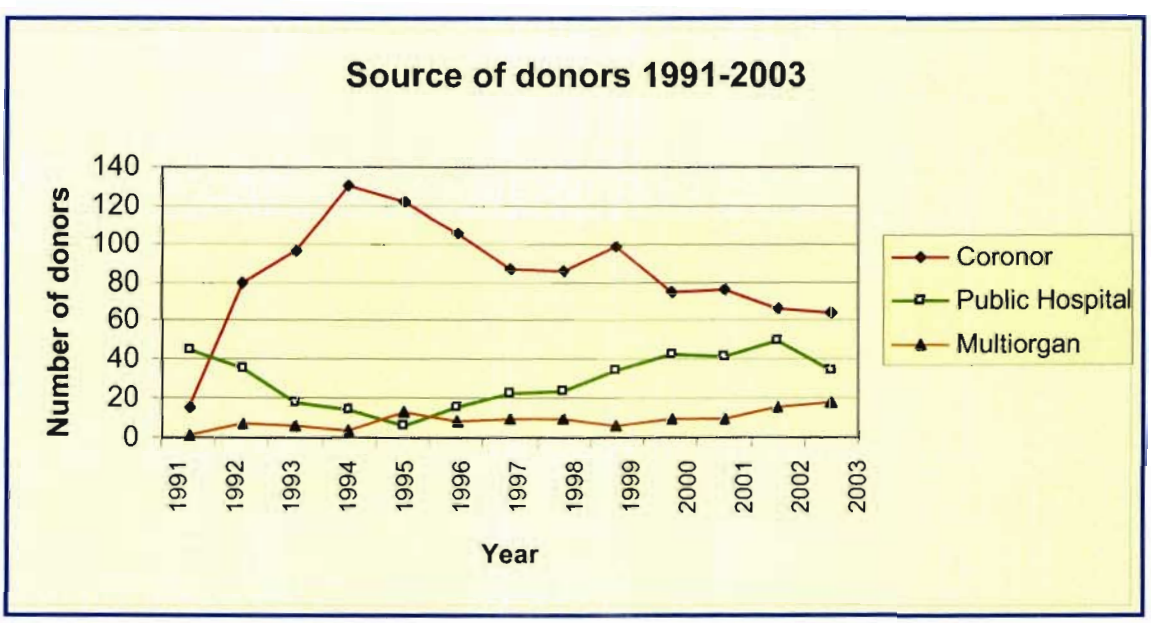


Figure 5:4 ***Line graph showing trends in donor procurement source 1991-2003.***

The most common cause of death of donors was cardiovascular disease (50.5%, n = 820), followed by trauma (12.5%, n = 203), cerebrovascular

disease (11.1%, n = 180), respiratory disease (7%, n = 115) and cancer (6.1%, n = 100). Table 5:1 presents the age, gender and percentage of corneas transplanted for each cause of death. Of note, there was a higher proportion of corneal tissue transplanted from donors who died from either cardiovascular disease or cerebrovascular disease compared with that from donors who died from other causes ($p < 0.05$).

Cause of death	% of donors	Mean age (SD)	% male	% transplanted
Cardiovascular disease	50.5	65.0 (14.5)	72.5	82.1
Trauma/Multiple injury	12.5	41.0 (21.5)	82.5	72.0
Cerebrovascular disease	11.1	58.9 (17.1)	55.4	83.4
Respiratory disease	7.0	62.8 (18.4)	61.0	74.6
Cancer	6.1	61.4 (16.0)	55.6	73.5
Asphyxiation	2.0	36.2 (19.4)	89.0	71.3
Poisoning	2.0	46.3 (18.3)	77.2	74.0
Other	8.8	57.6 (21.7)	70.0	71.0

Table 5:1 ***Showing cause of donor death, mean age, gender distribution, and percentage transplanted.***

Donor demographics also varied with donor source. Donors from the Coroner's service had a mean age of 58 years, with 80% male, and the most common cause of death was cardiovascular disease followed by trauma. Public hospital donors had a mean age of 68 years, with 72% male, and the most common cause of death was cardiovascular disease, followed by cerebrovascular disease and cancer. Multi-organ donors had a mean age of 41 years, with 55% male and the most common cause of death was cerebrovascular disease followed by trauma. No significant difference was found in the proportion of corneal tissue suitable for transplantation between the different donor sources.

5.4.3 Death to Preservation Interval

The death to preservation interval (DPI) is the time from donor death to preservation of donor corneal tissue. The NZNEB generally do not procure tissue if the DPI is greater than 24 hours, except in emergency circumstances or for use in tectonic procedures. The overall mean DPI during the study period was 15.2 hours (SD = 6.2) with the median 15.6 hours. Figure 5:5 illustrates the trend in DPI from 1991 to 2003. There was a rapid increase in mean DPI from 1991 (6.6 hours, SD = 3.0) to 1994 (14.5 hours, SD = 5.1), associated with the change in criteria from hypothermic to organ culture storage. From 1995 onwards the mean DPI remained relatively constant with a range of 14.8 to 17.7 hours. No difference was found in mean DPI between donor corneas that were suitable for transplantation (15.3 hours) versus those that were not (15.4 hours, p =0.46). The DPI in relation to donor source was assessed highlighting that the mean DPI for donor tissue from the Coroner's

service (16.6 hours) was significantly greater than that for public hospitals (12.1 hours) and multi-organ donors (12.7) ($p<0.001$).

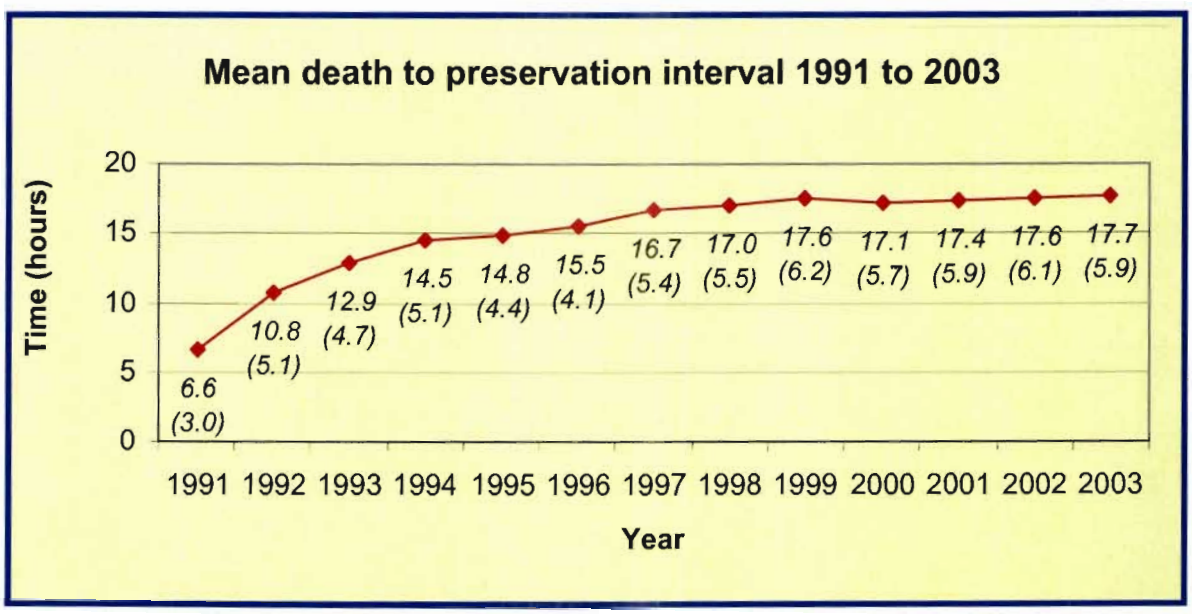


Figure 5:5 ***Line graph showing mean death to preservation interval for each year of the study (SD in brackets).***

5.4.4 Corneal Storage Methods

The NZNEB protocol for corneal storage changed from the use of Optisol (4°C) (Bausch and Lomb, Rochester, New York) to organ culture storage (34°C) during 1992. In 1991 all donor corneas were stored in Optisol. In 1992 30% were stored in Optisol and 70% stored in organ culture. From 1993 onwards all donor corneas were stored using organ culture. The mean storage duration with Optisol was 3.5 days (SD = 2.0), compared to 11.6 days (SD = 4.3) for organ culture. The mean storage duration for each year of the

study is presented in figure 5:6. The corneal utilization rate was significantly higher with organ culture storage (79%) compared to Optisol storage (65%, $p < 0.01$). The mean DPI for corneas stored in Optisol was significantly less than that for corneas stored with organ culture (7.1 vs. 15.0 hours, $p < 0.001$).

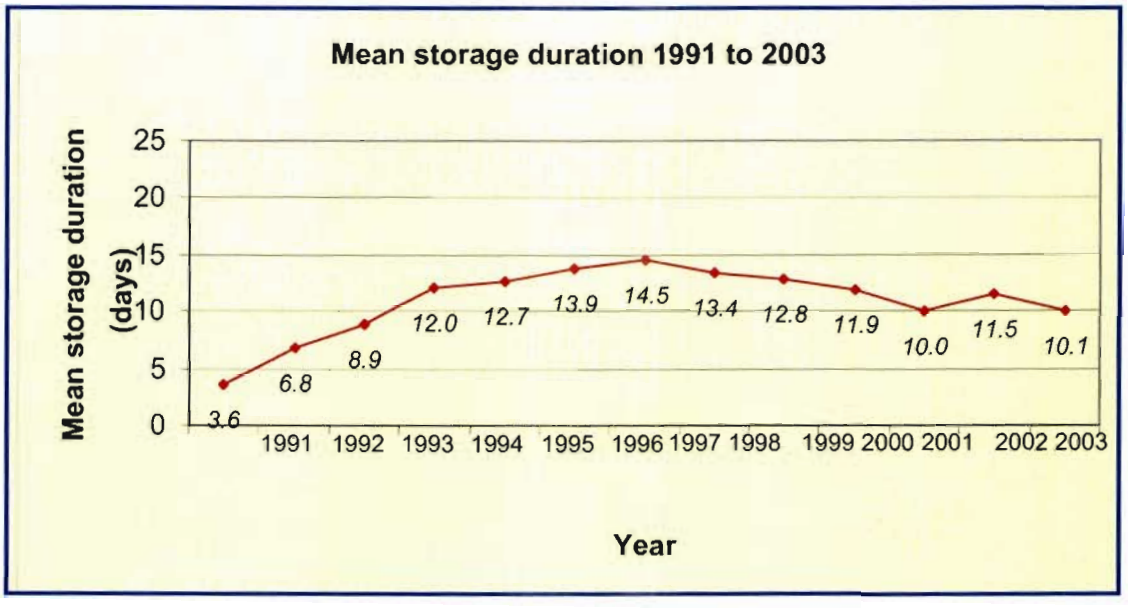


Figure 5:6 ***Line graph showing average storage duration for each year of the study.***

5.4.5 Endothelial Cell Count of Donor Tissue

The NZNEB uses light microscopy accompanied by intravital staining with trypan blue to assess the endothelial layer. An endothelial cell density greater than or equal to 2500 cells/mm² is the threshold required for a cornea to be

accepted for transplantation. Of all the donor corneas between 1993 and 2003, 2.6% (n = 84) had an endothelial cell density of less than 2500 cells/mm² and therefore were not transplanted. Based on donor age, only 0.8% of donors less than 50 years of age failed endothelial assessment, compared with 2.5% for the 51-60 year age group, 3.5% for the 61-70 year age group, 3.0 % for the 71-80 year age group, and 3.6% for the 81-85 year age group. Overall a significant correlation was found between increasing donor age and the rate of failed endothelial assessment (ß-coeff = 0.55, se = 0.12, $p < 0.05$). The mean age of donors whose tissue failed endothelial assessment was significantly higher than those whose tissue was suitable for transplantation (64.0 vs. 59.4 years, $p < 0.05$). A comparison was also made between phakic and pseudophakic donors, revealing that a significantly higher proportion of pseudophakic donors failed endothelial assessment compared to phakic donors (5.7% vs. 2.4%, $p < 0.05$).

Endothelial cell density of donor corneas that were transplanted is presented in figure 5:7. The mean endothelial cell density was 3024 cells/mm² (SD = 324 cells/ mm²). Corneal tissue from donors less than 20 years of age had a significantly higher endothelial cell density (mean = 3442 cells/ mm²) when compared to all other groups. All other age groups had endothelial cell densities ranging from of 3175 cells/ mm² (21-30 year age group) to 2917 cells/ mm² (81-85 year age group). A significant correlation was found between advancing donor age and lower endothelial cell density (ß-coeff = -4.7, se = 0.8, $p < 0.01$).

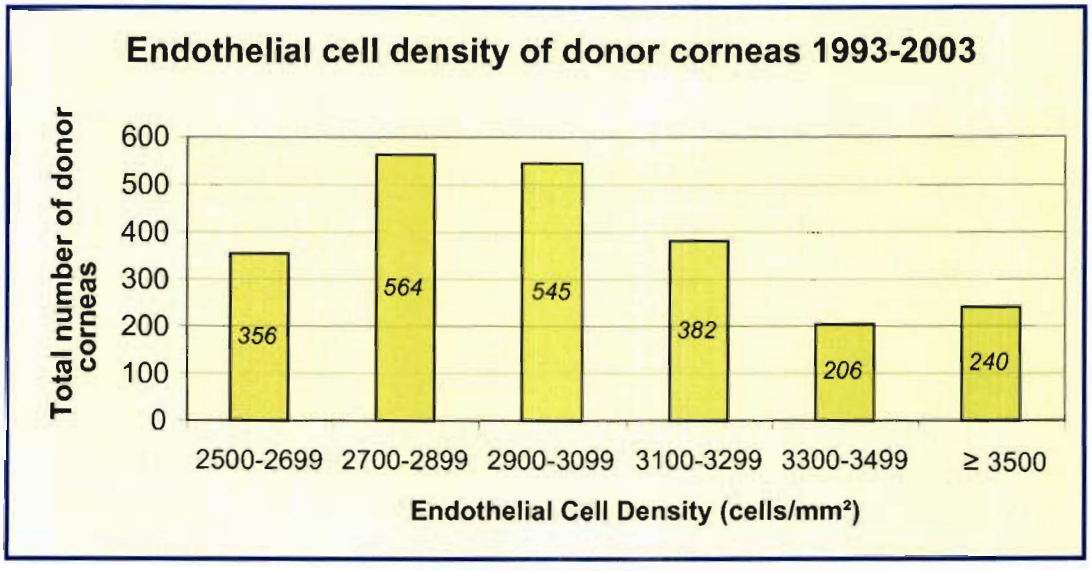


Figure 5:7 ***Bar graph showing the endothelial cell density of all donor corneas transplanted between 1993 and 2003.***

5.4.6 Biological Contamination of Donor Tissue

The NZNEB performs microbiological testing on the corneoscleral rim (CSR) prior to storage and on the organ culture medium (OCM) prior to transplantation on all donor tissue. Over the study period, the CSR testing showed significant bacterial growth in 1.7% (n = 54) of all donor corneas, and mycological growth in 0.3% (n = 11). The corresponding figures for OCM testing were 2.0% (n = 63) and 1.9% (n = 58) respectively. Overall, 5.0% (n=158) of all donor corneas were discarded due to biological contamination (1.1% from CSR result alone, 3.0% from OCM result alone, 0.9% from both CSR and OCM results). Over the years of the study there was a significant decrease (beta coeff = -0.73, se = 0.14, $p < 0.001$) in contamination rate, as illustrated in figure 5:8. Notably in 2002 only 0.7% (n=2) of corneas were discarded due to contamination, and in 2003 no donor tissue was discarded

due to contamination. There was no significant difference between the DPI of donor corneas that were discarded due to contamination versus those that were suitable for transplantation (14.9 vs. 15.1 hours, p = 0.29).

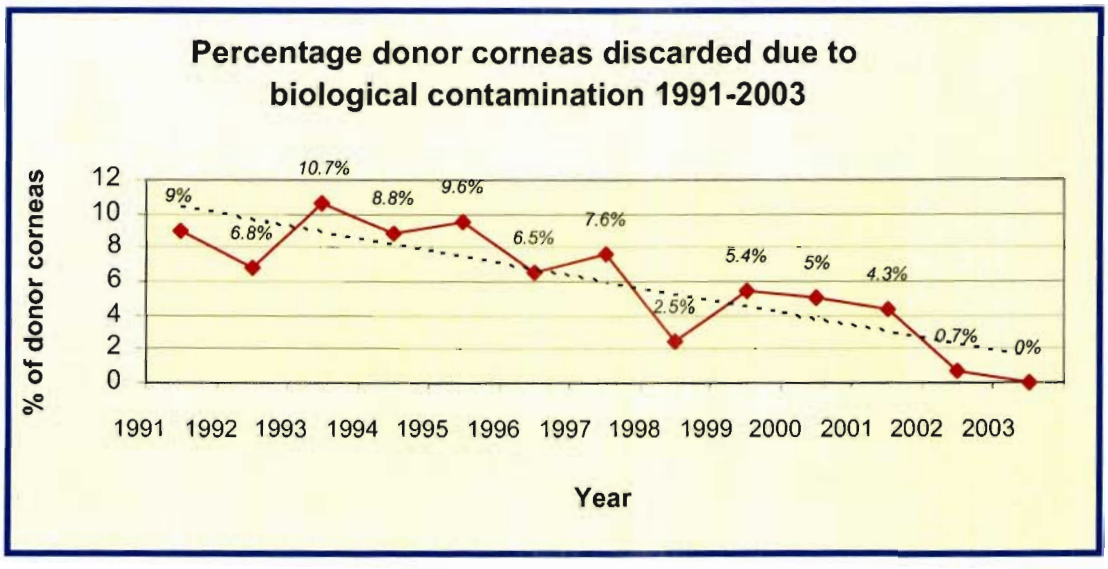


Figure 5:8 ***Line graph showing percentage of donor corneas discarded due to biological contamination for each year 1991- 2003.***

The most common bacterial isolates were: coagulase-negative staphylococci (31%), *Staphylococcus aureus* (14%), *Streptococcus* sp (14%), *Pseudomonas aeruginosa* (11%), *Corynebacterium* sp (7.5%), and *Enterobacteriaceae* (3.5%). The most common mycological isolates were: *Candida albicans* (26%), *Candida glabrata* (19%), *Cryptococcus* sp (5%), *Fusarium* sp (4.5%) and *Penicillium* sp (4.5%). Overall there were 16 different bacterial and 25 different mycological organisms isolated.

The most commonly isolated bacteria from the CSR testing were coagulase-negative staphylococci (27%), *Streptococcus* sp (20%) and *Pseudomonas aeruginosa* (13%), whilst for OCM testing they were coagulase-negative staphylococci (35%), *Staphylococcus aureus* (19%) and *Corynebacterium* (11.6%). The mycological isolates in both CSR and OCM were similar to the overall figures (as above).

5.4.7 Utilization and Distribution of Corneal Tissue

The NZNEB received a total of 3221 donor corneas during the study period, of which 79.1% (n = 2547) were transplanted and 20.9% (n = 674) were unsuitable for transplantation. The corneal utilization rate (proportion of donor corneas used for transplantation each year) has significantly increased over the years of the study (ß-coeff = 1.60, se = 0.38, $p < 0.01$), as illustrated in figure 5:9. In 2003 over 90% of all donor corneas were transplanted. The average number of corneal transplants per year was 204, with a significant increase in transplants per year identified over the years of the study (ß-coeff = 4.4, se = 1.3, $p < 0.01$). Overall, 2442 donor corneas were supplied for penetrating keratoplasty, 64 for lamellar keratoplasty, and 48 for unspecified transplants.

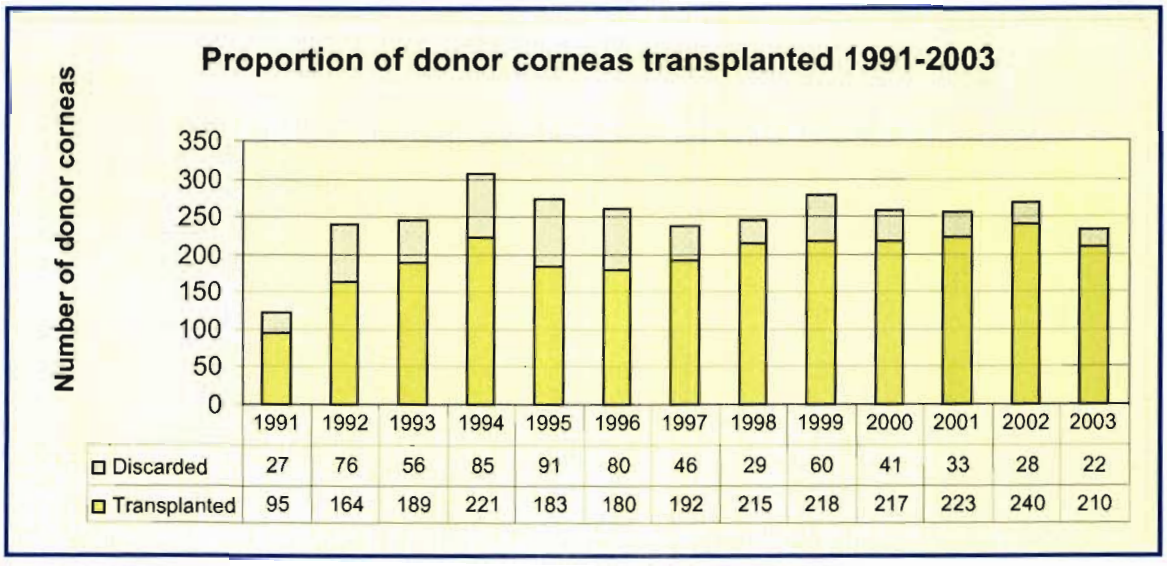


Figure 5:9 ***Bar graph showing number of donor corneas transplanted versus discarded for each year 1991-2003. The percentage transplanted is shown in each bar.***

Reasons why donor corneas were excluded from transplantation were analyzed; with the most common reason being biological contamination (5.0% of all donor corneas, n = 158), followed by abnormal serology (3.9%, n = 125) and failed endothelial assessment (2.6%, n = 84). Abnormal serology included Hepatitis B (3%, n = 98), Hepatitis C (0.8%, n = 25) and HIV (0.1%, n =2). There were 55 (1.7%) donor corneas that were deemed suitable and dispatched to the transplant centre but were not used. Less common reasons included contraindications in the donors past medical or ocular history (0.8%, n = 27), storage period greater than the 21 day maximum (0.9%, n = 30), and late harvesting of tissue (collected for emergency or tectonic procedures but not used) (0.7%, n = 27). Unfortunately a high percentage of reasons were classified as 'other' (3.4%) or not recorded (2.7%).

Over the study period the NZNEB supplied the vast majority of donor tissue for corneal transplantation in New Zealand. One center, Auckland, received the greatest number of donor corneas (41%), but corneal tissue was widely distributed throughout New Zealand to 12 other centres that received between 0.2% and 12% of all donor tissue. Only one center, Christchurch, received donor tissue from other sources during the entire study period (sourced local donor tissue and short-term storage). This center commenced using tissue from the NZNEB in 1996, and over the last 4 years, has subsequently received most of its corneal tissue from the NZNEB. From 1991-99 it was estimated that the NZNEB supplied at least 85% of donor tissue nationwide.[1] Using the same method (based on current usage of tissue supplied to Christchurch, plus unfilled demand in the first two years of the study, calculated from donor tissue utilization in year 3 and subsequent years), by extrapolation it is estimated that the NZNEB has supplied a minimum of 90% of all donor tissue between 1991 and 2003. It is further estimated that over the last four years, the NZNEB supplied at least 98% of donor tissue in New Zealand. Of all donor tissue, 67.6% was distributed to public hospitals and 31.7% to the private sector, with 0.7% unspecified. This 2:1 ratio of public to private distribution remained constant (beta coeff = 0.0025, p = 0.51) for each year of the study with the exception of 1991 where 84.1% went to public hospitals.

5.5 Discussion

The NZNEB is the major source of donor tissue for corneal transplantation in New Zealand supplying at least 90% of all tissue. New Zealand has a

population of approximately 4 million, served by 110 Ophthalmologists, distributed over a geographical area slightly greater than the United Kingdom. Over 200 corneal transplants are now performed each year in New Zealand and the NZNEB provides an essential service in the procurement of sufficient donor tissue to meet this demand. In addition, the NZNEB also ensures that the quality and safety of donor corneas is maintained according to established and internationally recognized standards and practices, and that corneal tissue is distributed in a fair and equitable way.[2] Indeed, currently all corneal transplants can usually be electively booked for surgery within a 2-4 month period with guarantee of tissue availability.

The age distribution of donors was comparable to published results from other eye banks[3], with the majority of donors being over 60 years of age (60%) and the greatest proportion between 70 to 80 years of age. There was an increase in the mean age of donors over the years of the study. We found no significant difference in the proportion of donor corneas that were suitable for transplantation between different age groups. Notably, even in the oldest age group (81-85 years), 79.5% of corneas were of sufficient standard to be transplanted. Several eye banks have reported lower rates of corneal utilization with advanced donor age: Moyes *et al* reported a 23% rate in donors aged 70-75 years[4], Armitage *et al* reported a 45% rate in donors 80 years and over[5], and Gain *et al* reported a 53% rate in donors 85-100 years[6], with contraindications in medical history the most commonly cited reason for non-utilization of donor tissue. The higher corneal utilization rate of the NZNEB may be explained by the thorough pre-screening of potential donors,

where those that do not meet donation criteria based on age, ocular or medical contraindications are generally excluded prior to the procurement of their tissue. This is made possible through the close relationship between the NZNEB and its donor sources, enabling the NZNEB to have greater control over what donor tissue is procured.

The high proportion of donor corneas suitable for transplantation that was identified in the 81-85 year age suggests that donor age alone is not indicative of poor tissue quality. Appropriately screened tissue from donors of advanced age may therefore be of use, particularly for recipients of similar age, although the NZNEB does not have a specified policy of age matching tissue. Previous studies have indicated that advanced donor age alone does not have an adverse effect on transplant survival or outcome.[4,7-13] None the less, decreased endothelial cell density and function with advanced age is well documented[14-19], therefore tissue from such donors needs a thorough endothelial assessment before approval for transplantation. Our study highlighted the expected correlation between advancing age and higher endothelial assessment failure rate (3.6% of corneas from donors over 80 years). The NZNEB minimum age of donation is 10 years, which is higher than most eye banks.[3] Reasons previously cited for not using infant or young corneas include increased technical difficulties during transplantation (due to its thinness and smaller diameter) and higher postoperative myopia due to the steeper curvature of the cornea.[2 14]

The male preponderance of donors to the NZNEB is similar to results published by other eye banks.[3] [20] Statistics published by the New Zealand Health Information Service[21] indicate that males die at a much younger age than females, with the highest proportion of male deaths in the 70-79 year age range (30%), compared to that of females with the highest proportion in the 80-89 year range (35%). The higher prevalence of male deaths in younger age groups is due to higher mortality from trauma and cardiovascular disease. This was reflected in our donor demographic data.

The major donor procurement source to the NZNEB was the Coroner's service followed by public hospitals and multi-organ donors. In recent years, the supply from the Coroner's service has decreased in contrast to that from public hospitals and multi-organ donors which has increased. The decreased supply from the Coroner's service reflects the decrease in the overall number of Coroner's cases requiring autopsy per year over the same time period. The increased supply from public hospitals is due to the efforts of one teaching hospital, Middlemore Hospital, Auckland, whose well organized donation program contributed 70% of donors in this group. This indicates the potential to increase donor numbers if similar programs are established in other hospitals. The increased supply from multi-organ donors is due to improved efforts from organ donor coordinators and intensivists around the country, although typically there are fewer than 40 multi-organ donors per year in New Zealand.

The most common donor cause of death was cardiovascular disease which accounted for 50% of all donor deaths. This was followed by trauma, cerebrovascular disease, respiratory disease and cancer with their contributions ranging from 12% to 6% each. Interestingly, these results differ from overall New Zealand mortality statistics[22], which were: cardiovascular disease (29%), followed by cancer (27%) and cerebrovascular disease (10%). Trauma accounted for only 3% of deaths in New Zealand, significantly lower than the percentage identified in donors. The causes of death for NZNEB donors were similar to that reported by other eye banks.[3 20] Interestingly, corneal tissue from donors that died of cardiovascular disease and cerebrovascular disease were more likely to be suitable for transplantation compared to those that died from other causes. A possible explanation is the high proportion of donors in these groups who died suddenly with less co-morbidity.

With the change from hypothermic to organ culture storage as standard NZNEB policy, the storage duration increased from a maximum of 7-10 days to 21 days. This has enabled the more efficient management of corneal tissue, which is of particular importance for the NZNEB, as it is the only supplier of tissue in New Zealand, where surgeons are distributed over a relatively large geographical area. Donor tissue can be supplied when required to ophthalmic surgeons, so that corneal transplantation can be performed as a planned elective procedure, with the postponement rate due to tissue unavailability significantly reduced. The reserve of tissue for emergency procedures has also increased. Increased storage duration also

allows additional time for improved microbiological and serological screening, and for tissue compatibility matching if required. There is significant variation in storage methods used by different eye banks around the world, with the UK and Europe preferring organ culture, and the United States and Australia preferring short term hypothermic storage.[3 19 23-25] Reasons cited for using hypothermic storage over organ culture include decreased cost and the lesser technical complexity required. It has also been suggested that there is decreased initial corneal swelling and increased preservation of endothelial viability with short-term storage.[26] However, several studies have clearly shown that endothelial cell density and morphology are well maintained even up to 35 days of storage with organ culture[14 27], and that postoperatively, survival and eventual corneal thickness were similar compared with short term storage media.[24]

Postoperative bacterial or fungal endophthalmitis is a serious complication of corneal transplantation and can have devastating effects on eventual outcome. The reported incidence of postoperative endophthalmitis ranges from 0.1% to 2%.[28-32] Studies have shown a correlation between postoperative endophthalmitis and infection of donor tissue.[31, 33] Therefore donor screening, microbiological screening, and decontamination of donor tissue is a priority of the NZNEB. Microbiological testing on the corneoscleral rim prior to storage and on the organ culture storage medium before transplantation is performed. During the study period, 5% of all donor corneas were discarded due to biological contamination. Reported figures from other eye banks in the literature range widely from 12-39% for hypothermic storage

and 0.7%-5% for organ culture storage.[28 30-32 34] Biological contamination has significantly decreased from 10% in 1991 to a current rate of less than 1%. This is possibly associated with improved facilities and equipment, in addition to more experienced and technically skilled eye bank staff. Of note, multi-organ donors had significantly lower contamination rates than donors from other sources. This may be related to the protective functions of the living eye and the increase in growth of normal flora associated with cadaveric eyes.[38] Variations in death to preservation interval did not influence NZNEB contamination rates.

The major bacterial contaminant in our study was coagulase-negative staphylococci, followed by *Staphylococcus aureus*, *Streptococcus* sp, *Pseudomonas aeruginosa*, and *Corynebacterium.* Coagulase-negative staphylococci were the major contaminant in both corneoscleral rim and culture medium testing. Of note was the particularly high prevalence of *Streptococci* sp in the corneoscleral rim cultures. Similar bacterial isolates have been reported in other studies, with coagulase-negative staphylococci the major contaminant in almost all cases.[31-32 34-40] This suggests most of the contamination is due to bacteria derived from normal ocular flora. The same studies report *Candida* sp to be the most common mycological organism isolated. In studies evaluating the organisms found in post penetrating keratoplasty endophthalmitis, *Streptococci* sp was the most common bacterial isolate reported, and *Candida* sp were the most common fungi reported[28 31 33 41 42] No cases of endophthalmitis due to contamination of donor tissue were reported to the NZNEB during the study period.

Of all the donor tissue procured by the NZNEB, 79% was used for corneal transplantation and 21% was deemed unsuitable. This corneal utilization rate is high when compared to published data from other eye banks with rates varying between 50-70%.[43-45] A possible reason for the higher utilization rate is the thorough pre-screening of potential donors (for age and medical contraindications) prior to procurement of tissue, as previously noted. The most common reasons for discarding tissue were microbiological contamination, abnormal serology, and failed endothelial assessment. Less common reasons included contraindications in medical or ocular history (not initially detected at prescreening), prolonged storage duration, and late harvesting (collected for emergency or tectonic procedures but not used). Advanced age, contraindications in the donor's medical and ocular history, and poor tissue quality were the most common reasons for exclusion of donor corneas reported by other eye banks.[5 17 43-45] The corneal utilization rate increased throughout each year of the study, and in the last few years nearly 90% of all tissue procured was used for transplantation. This reflects improvements in all areas of NZNEB operation, and in particular, corneal storage and decreased microbiological contamination.

5.6 Conclusion

The comprehensive database maintained by the NZNEB is an important part of the NZNEB operation, and analysis of this database provides valuable information in relation to all aspects of eye banking in New Zealand. Over the 13 year period analyzed, significant trends were identified in relation to donor

demographics, donor procurement source, improved donor tissue processing and storage, decreased biological contamination, and increased utilization of corneal tissue.

Chapter 6

Current Trends and Ethnicity Differences in Indications for Penetrating Keratoplasty in New Zealand

6.1 Abstract

Purpose

To identify current trends and ethnicity differences in indications for penetrating keratoplasty (PKP) in New Zealand.

Methods

As part of a prospective longitudinal study, PKP data collected by the New Zealand National Eye Bank (NZNEB) was analyzed for the 4-year period 2000 to 2003. A comparison with results from a previously published NZNEB study (in which data from 1991 to 1999 was analyzed) was performed.

Results

During the 4-year study period the NZNEB supplied donor tissue for 889 keratoplasties, of which 94% (n = 838) was for PKP. This accounted for at least 95% of all PKP's performed in New Zealand from 2000 to 2003. Keratoconus remained the leading indication for PKP (44%) in New Zealand, followed by aphakic or pseudophakic bullous keratopathy (14%), regraft (13%), Fuchs' dystrophy (7%) and viral keratitis (5%). There was a significant decrease compared to the earlier NZNEB study in the proportion of PKP's for aphakic or pseudophakic bullous keratopathy, and a significant increase for regraft and Fuchs' dystrophy. Keratoconus was the leading indication in all ethnicity groups, although the relative proportions differed significantly, ranging from 37% in the Caucasian European population to 58% in the Polynesian and 59% in the Maori

population. Polynesians (mean age 27 years, SD 7 years) and Maori (mean age 27 years, SD 9 years) were significantly younger at the time of PKP for keratoconus than Caucasian Europeans (mean age 34 years, SD 13 years) ($p < 0.001$).

Conclusion

The study findings suggest that keratoconus leading to PKP may have an increased prevalence, severity, and/ or more rapid disease progression in New Zealand, particularly amongst the Maori and Polynesian populations. A significant decreasing trend in the proportion of PKP's for aphakic or pseudophakic bullous keratopathy was identified. A significant increasing trend in PKPs for regraft and Fuchs' dystrophy was also identified. These trends were in concordance with those recognized in other published reports.

6.2 Introduction

In a previous study using the NZNEB database the indications for penetrating keratoplasty (PKP) in New Zealand were reported from 1991 to 1999.[1] Keratoconus was identified as the leading indication for PKP (46%), accounting for a higher proportion than that recognized in other published reports. A higher prevalence of keratoconus and more rapid disease progression to the stage of requiring PKP in New Zealand were considered possible explanations. There is also a strong clinical impression that keratoconus has a particularly high prevalence and severity in the Maori and Polynesian communities of New Zealand. However, this had not been previously confirmed, and up until 1999 the NZNEB did not include ethnicity data in the database.

In this study the NZNEB database was analyzed with respect to clinical indications for PKP in New Zealand. An emphasis was placed on identifying ethnicity differences and trends in clinical indications for PKP in New Zealand.

6.3 Methods

As part of a longitudinal, prospective study, the electronic records of the NZNEB were analyzed for the four-year period 2000 to 2003 with respect to age, gender, ethnicity, and indication for PKP. The indication for PKP was the clinical diagnosis given by the surgeon at the time of operation. When more than one clinical diagnosis was identified the priority scheme suggested by Brady et al[46]

was used, in particular, the diagnosis of regraft was given priority over all other diagnoses.

Statistical analysis was performed in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. The SPSS V 12 software package was used. Statistical methods were chi-square testing to compare proportions between groups and the Student's *t* test to compare means between groups.

6.4 Results

During the four-year study period the NZNEB supplied donor tissue for 889 keratoplasties. PKP was performed in 94% (n = 838), lamellar keratoplasty in 4% (n = 35), with 2% (n = 16) unspecified. The leading indications for PKP were keratoconus (44%, n = 365), pseudophakic or aphakic bullous keratopathy (14%, n = 114), regraft (13%, n = 108), Fuchs' endothelial dystrophy (7%, n = 60) and viral keratitis (5%, n = 44). Table 6:1 presents the indications for PKP in this study along with results from the earlier NZNEB study. Aphakic or pseudophakic bullous keratopathy was significantly less common as an indication for PKP in this study compared to the earlier study ($p = 0.007$). In contrast, regraft ($p = 0.002$) and Fuchs' endothelial dystrophy ($p = 0.006$) were significantly more common. Viral keratitis was less common as an indication for PKP in this study but the difference did not reach statistical significance.

Indication for PKP	1991-1999 Total number (%)	2000-2003 Total number (%)
Keratoconus	597 (45.6)	365 (43.6)
A/P bullous keratopathy	235 (17.9)	114 (13.6)
Regraft	114 (8.7)	108 (12.9)
Fuchs' endothelial dystrophy	58 (4.4)	60 (7.2)
Viral keratitis	96 (7.3)	48 (5.4)
Other infective keratitis	43 (3.3)	33 (3.9)
Trauma	72 (5.5)	33 (3.9)
Stromal dystrophies	53 (4.0)	27 (3.2)
Non-infective keratitis	16 (1.2)	16 (1.9)
Other	99 (7.7)	24 (3.0)
Not recorded	18 (1.3)	10 (1.3)
Total	**1308**	**838**

Table 6:1 ***Indications for PKP in New Zealand from 2000 to 2003***
(A/P = aphakic/pseudophakic)

The mean age of patients undergoing PKP was 48 years (Standard deviation (SD) = 22 years), median age 46 years, and age range 4 to 95 years. Age distribution (using stratified 10-year intervals) highlighted a bimodal pattern with peaks in the 21-30 year and 71-80 year age groups (figure 6:1). The mean age at

the time of PKP for the most common indications were; keratoconus, 32 years (SD = 12 years); aphakic or pseudophakic bullous keratopathy, 71 years (SD = 14 years); regraft, 55 years (SD = 18 years); Fuchs' endothelial dystrophy, 70 years (SD = 11 years); and viral keratitis, 54 years (SD 19 years).

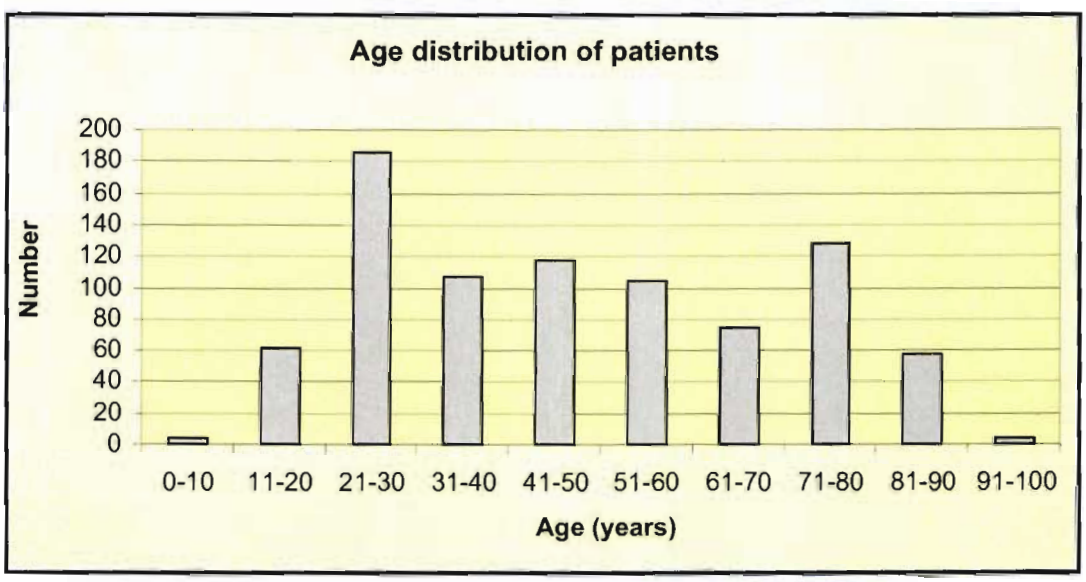


Figure 6:1 ***Total number of PKPs plotted against age group (10-year intervals) highlighting a bimodal distribution***

The gender distribution of patients having undergone PKP was 57% (n = 476) male and 43% (n = 362) female. The indications for PKP for each gender are presented in Table 6:2. Keratoconus was the most common indication for both males (47%, n = 225) and females (39%, n = 140). Using chi-squared testing, the indications identified that were significantly more common in males compared to

females were keratoconus ($p = 0.01$) and trauma ($p < 0.001$). Fuchs' endothelial dystrophy was the only indication significantly more common in females ($p < 0.001$). Aphakic or pseudophakic bullous keratopathy was more common in females but this did not reach statistical significance.

Indication for PKP	Male (%)	Female (%)
Keratoconus	225 (47.3)	140 (38.7)
Bullous keratopathy	52 (10.9)	62 (17.1)
Regraft	61 (12.8)	47 (13.0)
Fuchs' endothelial dystrophy	18 (3.8)	42 (11.6)
Viral keratitis	26 (5.5)	22 (6.0)
Other infective keratitis	17 (3.6)	16 (4.4)
Trauma	29 (6.0)	4 (1.1)
Stromal dystrophies	17 (3.6)	10 (2.8)
Non-infective keratitis	8 (1.7)	8 (2.2)
Other	16 (3.5)	8 (2.6)
Not recorded	7 (1.7)	3 (1.1)
Total	**476**	**362**

Table 6:2 ***The indications for PKP based on gender 2000-2003***

Males underwent PKP at a significantly younger age (mean 45 years, SD 21 years) than females (mean 53 years, SD 22 years) ($p = 0.001$). Males also underwent PKP for keratoconus at a significantly younger age (mean 31 years, SD 11 years) than females (mean 34 years, SD 13 years) ($p = 0.03$). Females underwent PKP for bullous keratopathy at a significantly older age (mean 75 years, SD 11 years) than males (mean 69 years, SD 14 years) ($p = 0.02$).

The ethnicity distribution of patients having undergone PKP is presented in Table 6:3. A comparison with the overall New Zealand ethnicity distribution is provided (using 2001 New Zealand consensus data[47]). The indications for PKP within each of these ethnic groups are presented in Table 6:4. Keratoconus was the most common indication for PKP in all ethnic groups, although the relative proportions differed significantly. In Caucasian Europeans the proportion of PKP's for keratoconus was 37% ($n = 198$), in Maori 59% ($n = 77$) and in Polynesians 58% ($n = 47$). The higher proportion of PKP's for keratoconus in the Maori and Polynesian populations compared to the Caucasian European population was statistically significant ($p < 0.001$). The mean age of Maori (27 years, SD 9 years) and Polynesians (27 years, SD 7 years) at the time of PKP for keratoconus was also significantly less than that of Caucasian Europeans (34 years, SD 13 years) ($p < 0.001$). Gender distribution was not significantly different between ethnic groups.

The proportion of PKP's for bullous keratopathy was significantly greater in the Caucasian European population compared to both the Maori ($p < 0.001$) and Polynesian ($p = 0.01$) populations. Fuchs' endothelial dystrophy also accounted for a significantly greater proportion of PKP's in the Caucasian European population (11%, n = 56) compared to all other ethnic groups.

Ethnicity	% of NZ population*	Number of PKP's (% of total)	Mean age (SD)	% Female
European	76.0	530 (63.2)	53.1 (21.5)	45.4
Maori	13.7	130 (15.5)	36.9 (17.3)	38.1
Polynesian	6.0	80 (9.5)	37.2 (19.6)	42.9
Indian	1.6	28 (3.3)	41.2 (17.6)	36.7
Asian	2.7	17 (2.0)	48.9 (18.9)	50.0

Table 6:3 ***The age and gender distribution of each ethnic group 2000-2003 (*based on 2001 consensus population data from Statistics New Zealand)***

The number of PKP's performed annually per 100,000 population of each ethnic group was analyzed (using 2001 New Zealand consensus data[47]), with the results presented in Table 6:5. The highest incidence was identified in the Indian population (11.8 PKP's per 100,000) and the lowest in the Caucasian European population (4.7 PKP's per 100,000). An identical analysis was conducted for the

number of PKP's performed for keratoconus only (Table 6:5). The highest incidence was identified in the Polynesian population (4.9 PKP's per 100,000) and the lowest in the Caucasian European population (1.6 PKP's per 100,000).

Indication for PKP	European (%)	Maori (%)	Polynesian (%)	Indian (%)	Asian (%)
Keratoconus	198 (37.4)	77 (59.2)	47 (58.4)	12 (42.9)	7 (41.2)
Bullous keratopathy	90 (17.0)	10 (7.7)	7 (8.8)	3 (10.7)	2 (11.8)
Regraft	74 (14.0)	16 (12.3)	5 (6.3)	3 (10.7)	1 (5.9)
Fuchs' dystrophy	56 (10.6)	0 (0)	0 (0)	0 (0)	1 (5.9)
Viral keratitis	32 (6.0)	9 (6.9)	3 (3.8)	3 (10.7)	1 (5.9)
Other infective keratitis	14 (2.6)	7 (5.4)	8 (10.0)	2 (7.1)	1 (5.9)
Trauma	21 (4.0)	5 (3.8)	5 (6.3)	1 (3.5)	1 (5.9)
Stromal dystrophies	21 (4.0)	2 (1.5)	1 (1.3)	2 (7.1)	2 (11.8)
Non-infective keratitis	10 (1.9)	2 (1.5)	1 (1.3)	0 (0)	1 (5.9)
Other	10 (1.9)	2 (1.5)	2 (2.5)	1 (3.5)	0 (0)
Not recorded	4 (0.7)	0 (0)	1 (1.3)	1 (3.5)	0 (0)
Total	**530**	**130**	**80**	**28**	**17**

Table 6:4 ***The indications for PKP for each ethnic group 2000-2003***

Ethnicity	Total number of PKP's*	Number of PKP's for keratoconus *
Caucasian European	4.7	1.6
Maori	6.3	3.6
Polynesian	8.6	4.9
Indian	11.8	4.7
Asian	6.2	1.9
Overall	5.9	2.5

Table 6:5 ***The annual number of PKPs performed per 100,000 population of each ethnic group 2000-2003***

6.5 Discussion

New Zealand is a diverse multicultural society with a population of approximately 4 million, distributed over a geographical area slightly greater than the United Kingdom. The largest ethnic group is comprised of the Caucasian European population, followed by the indigenous Maori population. New Zealand has many other minority ethnic groups as a result of significant recent migration from the Pacific Islands and Asia, amongst other places.

In concordance with the initial NZNEB study (1991 to 1999),[1] keratoconus remains the most common indication for penetrating keratoplasty (PKP) in New Zealand, accounting for a considerably greater proportion (44%) than the majority of other published reports (3% to 37%).[48-61] Only Claesson et al[62], in a recent study of a Palestinian population identified a higher proportion (51%). In the initial NZNEB study the authors postulated an unusually high prevalence of keratoconus in New Zealand, particularly in Maori and Polynesian communities, as a possible explanation. Another explanation was that there may be more rapid disease progression to the stage requiring PKP in New Zealand compared to other populations. Unfortunately no data exists on the prevalence or severity of disease in New Zealand to confirm this. The possibility that other indications may be disproportionately low in New Zealand was considered unlikely following comparison with other published reports.[1]

The current study identified keratoconus as the most common indication for PKP in all ethnic groups of New Zealand. The incidence of PKP for keratoconus was particularly high in the Maori and Polynesian communities of New Zealand. Maori and Polynesian patients were also significantly younger at the time of PKP for keratoconus. This suggests that there may be a higher prevalence, disease severity, and/or more rapid progression of keratoconus in New Zealand, particularly in the Maori and Polynesian populations. Other studies have identified similar differences for ethnic groups living in the same geographical area.[63-65] Pearson et al[63] in a study of a UK population identified a four-fold

greater incidence of keratoconus and a significantly younger age at operation for Indians compared to Caucasian Europeans. The Indian population of New Zealand also has a high annual incidence of PKP for keratoconus.

Asthma and atopic disease have a high prevalence in New Zealand, and both have been previously recognized as predisposing factors for keratoconus.[66-68] Owens et al[69] in a recent study examining the characteristics of patients with keratoconus in New Zealand confirmed a high prevalence of allergy, eye rubbing and asthma in this group. Maori and Polynesian patients were also noted to have more severe and less well-controlled asthma compared to the general population.[70] A high familial rate of keratoconus, particularly amongst Maori and Polynesian communities, may also be an important contributory factor for a higher prevalence of keratoconus in New Zealand. Owens et al[69], identified a familial rate of 24% in New Zealand, higher than that identified in other published reports (10-20%).[71-73]

Pseudophakic or aphakic bullous keratopathy remains the second most common indication for PKP in New Zealand, with a decreasing trend identified. Other recently published reports,[49, 53, 58, 74, 75] including that of the Eye Bank Association of America,[76] have also identified a decreasing trend in the proportion of PKP's performed for aphakic or pseudophakic bullous keratopathy. Improvements in cataract surgery technique, intraocular lens technology, use of viscoelastics, and

a reduction in anterior chamber intraocular lens use have all been cited as reasons for the decreasing trend.[49, 53, 58, 74, 75, 77]

Regraft remains the third most common indication for PKP in New Zealand, with an increasing trend identified. It is likely that the gradually expanding pool of PKP recipients has led to a corresponding increase in the number of regraft procedures being performed in New Zealand. Other published reports have also identified an increasing trend in the number of regrafts being performed.[48-51, 56, 74, 75, 78]

Fuchs' endothelial dystrophy is now the fourth most common indication for PKP, an increase from sixth in the earlier NZNEB study. The proportion of PKP's for Fuchs' endothelial dystrophy varies significantly in the literature with a range from 2 to 23% reported.[48-53, 56, 74, 75] Although PKP for Fuchs' endothelial dystrophy appears to be increasing in New Zealand, no similar trends were identified in other published reports. PKP for Fuchs' endothelial dystrophy was significantly more common in females, confirming the findings of the initial NZNEB study and in concordance with other published reports.[50, 74] Fuchs' endothelial dystrophy is more common in females,[79] and because of their greater life expectancy, more likely to manifest as corneal oedema.[1] Interestingly, almost all PKP's for Fuchs' dystrophy were performed in Caucasian Europeans. This may be related to the age structure of different ethnic groups in New Zealand, with only 3% of Maori,

Polynesian, Indian and Asians over 65 years of age, compared to 13% for Caucasian Europeans.[77]

Viral keratitis has decreased from fourth in the initial study to now being the fifth most common indication for PKP. A gradually decreasing trend from 1994 onwards was identified in the earlier NZNEB study and it appears that this is continuing. This is in concordance with other published reports.[46, 49, 51, 53, 59] The most likely reason for this is the significant advancement in the medical management of herpetic eye disease.[80, 81] Maori were over-represented in the ethnicity distribution of PKP for viral keratitis. This possibly reflects poorer medical management in this group rather than a greater prevalence of disease.

6.6 Conclusion

Keratoconus remains the most common indication for PKP in New Zealand, accounting for a significantly greater proportion than that identified in other published reports. Keratoconus was the most common indication in all ethnic groups, and was particularly common in the Maori and Polynesian populations. A greater prevalence of atopy and asthma, and a high familial rate are likely to be important contributory factors to a higher prevalence of keratoconus in New Zealand, however, this needs to be confirmed with further investigation.

Significant trends were identified in relation to other indications for PKP in New Zealand. In particular, there was a decrease in the proportion of PKP's for

pseudophakic or aphakic bullous keratopathy, and an increase for regraft and Fuchs' dystrophy. These trends are likely to continue, resulting in a significant change in indications for PKP in the future.

Chapter 7

The New Zealand National Eye Bank: Assessment of Survival and Visual Outcome one year following Corneal Transplantation 1993-2002

7.1 Abstract

Purpose

To identify potential donor, recipient, surgical and post-operative factors that may influence survival and visual outcome of penetrating keratoplasty (PKP).

Methods

As part of a prospective longitudinal study, the electronic records of the New Zealand National Eye Bank (NZNEB) were analyzed for the 10-year period 1993-2002. Both univariate and multivariate analyses were performed to identify factors significantly associated with decreased PKP survival and poor visual outcome.

Results

During the study period the NZNEB supplied 1820 corneas for PKP, of which 1629 (90%) had one-year follow-up data available. The overall one-year survival rate was 87% (n = 1429). The leading cause of PKP failure was irreversible rejection (7%, n = 114) followed by presumed primary tissue failure (1%, n = 17). Factors identified as independent risk factors for decreased PKP survival in multivariate analysis were: one or more episodes of reversible rejection; active inflammation at PKP; pre-existing corneal vascularisation; intra-operative complications (not further specified); small graft size (≤ 7.25 mm); large graft size (≥ 8.5 mm); pre-operative glaucoma; and a pre-operative diagnosis of regraft or trauma. Factors identified that were predictive of poor visual outcome in

multivariate analysis were advancing recipient age; active inflammation at PKP; pre-existing vascularisation; pre-operative glaucoma; one or more episodes of reversible rejection; and a pre-operative diagnosis of bullous keratopathy, trauma or non-infective keratitis. Keratoconus and Fuchs' endothelial dystrophy were identified as the pre-operative diagnoses with the most successful survival and visual outcome following PKP.

Conclusion

The large and comprehensive New Zealand National Eye Bank database has enabled detailed statistical analysis of a large series of PKPs to be performed, with several independent risk factors identified that significantly influenced survival and visual outcome. This information will be invaluable to patients and surgeons with respect to determining prognosis and clinical decision making.

7.2 Introduction

In this study the New Zealand National Eye Bank (NZNEB) database was analyzed with respect to survival and visual outcome one-year post-operatively for all penetrating keratoplasties (PKP) performed between 1993 and 2002. The purpose of the study was to identify potential donor, recipient, surgical and post-operative factors that may effect survival and visual outcome following PKP. A better knowledge and understanding of such risk factors will be invaluable to both patients and surgeons with regards to determining prognosis and clinical decision making.

7.3 Methods

As part of a longitudinal, prospective study, highlighted in earlier chapters, the electronic records of the NZNEB were analyzed for the 10-year period 1993-2002 with respect to donor, recipient, surgical and post-operative factors influencing PKP survival and visual outcome one-year post-operatively.

As previously note, data are entered into the computerized NZNEB database in a prospective manner by eye bank staff. Donor information is entered at the time of tissue procurement and includes demographic data, donor source and cause of death, death-to-preservation interval, endothelial assessment, and storage duration. Recipient and surgical information is collected from surgeons in the form of a questionnaire completed at the time of operation. Recipient information collected includes demographic data, pre-operative diagnosis, past ocular

history, and associated ocular conditions. Surgical information collected includes graft size, additional operative procedures, suture details, and intra-operative complications. Follow-up data are collected from surgeons at one-year post-operatively by way of a mailed questionnaire sent out at the appropriate time point. Data collected include PKP survival (defined as a clear corneal transplant), visual outcome, suture adjustment or removal, episodes of reversible rejection and post-operative complications. Missing data were routinely sought from surgeons by way of follow-up letters.

Statistical analysis was performed using SPSS software (V. 12) in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. Both univariate and multivariate analyses were performed. Statistical methods were the Fisher's exact test and chi-square testing to compare proportions between groups, Student's *t* test to compare means between groups, and binary logistic regression modeling to identify variables independently associated with decreased PKP survival and poor visual outcome. The level of statistical significance was $P < 0.05$ unless stated otherwise. Visual acuity was converted to a logMAR scale for the purposes of statistical analysis.

7.4 Results

7.4.1 Overall PKP survival outcome

During the study period 1820 PKPs were performed, of which 1629 (89.5%) had one-year follow-up data available. There were 182 (10.0%) patients lost to follow-

up and 9 (0.5%) that died during the follow-up period. Table 7:1 summarizes patient demographic data as categorized by the pre-operative diagnosis at the time of PKP. The overall one-year survival rate was 87.3% (n = 1429), with the remaining 12.7% (n = 200) of PKPs failing. Reasons for PKP failure are presented in Table 7:2, with irreversible rejection (n = 114, 7.0%) the leading cause, followed by presumed primary tissue failure (n = 17, 1.0%) and corneal vascularisation (n = 14, 0.9%).

Pre-operative diagnosis	Total number (%)	Mean age (SD)	Age range	% Female
Keratoconus	735 (45.1)	31.9 (12.6)	6-86	40.3
Bullous keratopathy	276 (16.9)	72.3 (12.9)	11-95	56.0
Viral keratitis	120 (7.4)	54.5 (19.9)	7-96	46.2
Regraft	115 (7.1)	52.3 (17.6)	9-94	42.7
Fuchs' endothelial dystrophy	81 (5.0)	70.9 (8.2)	45-86	70.1
Trauma	78 (4.8)	42.9 (19.4)	5-94	18.4
Stromal dystrophy	65 (4.0)	57.3 (20.9)	3-93	34.1
Infection (non-herpetic)	42 (2.6)	55.3 (19.7)	22-92	60.7
Non-infective keratitis	25 (1.5)	66.6 (17.5)	27-89	61.0
Not specified	92 (5.6)	54.7 (20.3)	11-88	52.3

Table 7:1 ***A summary of patient demographic data based on the pre-operative diagnosis at the time of PKP***

Reason for PKP failure	Total number (%)
Irreversible rejection	114 (57.0)
Presumed primary tissue failure	17 (8.5)
Vascularisation	14 (7.0)
Trauma	11 (5.5)
Glaucoma	9 (4.5)
Corneal melt	5 (2.5)
Hypotony	3 (1.5)
Other	17 (8.5)
Unknown	10 (5.0)
Total	**200**

Table 7:2 ***Reported causes for PKP failure at one year post surgery***

7.4.2 Donor factors influencing PKP survival outcome

There was no significant difference in survival rate between donor tissue procured from the Coroner's service (88.6%, 1187/1340), public hospitals (86.5%, 206/238), or multi-organ donors (92.9%, 73/78) ($p = 0.72$). No particular donor cause of death was associated with a reduced survival rate (range 84.0-90.6%, $p = 0.27$). Death-to-preservation interval (DPI) was analyzed by stratification into 5-hour intervals. No significant difference in survival rate between the intervals was identified, with survival rate ranging from 85.2%

(289/330) in the 21-25 hour interval to 92.3% (36/39) in the 0-5 hour interval (p = 0.47), and was 91.7% (33/36) for DPI longer than 25 hours.

An endothelial cell density (ECD) greater than or equal to 2500 cells/mm² is the threshold required for a cornea to be accepted for PKP according to the NZNEB protocol. Mean donor ECD was not significantly different between PKPs that survived (3020 cells/mm², SD 334 cells/mm²) and those that failed (3013 cells/mm², SD 330 cells/mm²) (p = 0.77). Further analysis of donor ECD by categorization into 300 cells/mm² intervals also failed to identify an association between ECD and survival rate (range 87.1% - 90.7%, p =0.94). Within the lowest ECD range accepted for PKP (2500-2800 cells/mm²) the survival rate was 87.7% (388/445). The effect of donor lens status on survival rate was analyzed highlighting no significant difference in survival rate between phakic (88.6%, 1374/1551), pseudophakic (81.8%, 31/38) or aphakic (91.6%, 11/12) donors (p = 0.80).

All donor tissue was stored using warm organ culture storage at 34°C, with the maximum storage duration 25 days as per NZNEB protocol. No difference in mean storage duration was identified between PKPs that survived (12 days, SD 4.3 days) and those that failed (11.8 days, SD 4.1 days) (p = 0.54). Storage duration was further analyzed by stratification into 5-day intervals, with no significant difference in survival rate identified between intervals (0-5 days,

89.7%; 6-10 days, 86.1%; 11-15 days, 87.7%; 16-20 days, 90.2%; 21-25 days 84.2%) ($p = 0.39$).

Donor age was not significantly associated with survival rate in this study. Although mean donor age of PKPs that survived (59.3 years, SD 17.5 years) was significantly less than those that failed (63.0 years, SD 18.0 years) ($p = 0.02$), it was identified that donor tissue used for keratoconus was significantly younger (mean age 54.8 years, SD 19.3 years) when compared to that for 'other pre-operative diagnoses combined' (mean age 64.1 years, SD 16.2 years) ($p < 0.01$). Separate analysis of keratoconus and 'other pre-operative diagnoses combined' failed to identify any significant association between donor age and survival rate ($p = 0.50$ and $p = 0.10$ respectively). Donor age was further analyzed by stratification into 10-year intervals. The highest survival rate was 93.1% for donor age 21-30 years and the lowest 85.7% for donor age 81-85 years (upper age limit acceptable for keratoplasty according to NZNEB protocol). The difference in survival rate between donor age 81-85 years and donor age less than 81 years (88.0%) did not reach statistical significance ($p = 0.20$).

7.4.3 Recipient factors influencing PKP survival outcome

A significant difference in survival rate between pre-operative diagnoses was identified (Table 7:3). Fuchs' endothelial dystrophy (95.1%, $p < 0.01$) and keratoconus (94.6%, $p < 0.01$) had significantly higher survival rates compared to the other pre-operative diagnoses. In comparison, PKP for trauma (76.9%, $p <$

0.01) and infection (non-herpetic) (69.0%, $p < 0.01$) had a significantly lower survival rate compared to other pre-operative diagnoses. Regraft procedures also had a significantly lower survival rate (70.4%, $p < 0.01$), the survival rate for first grafts being 88.9% (1168/1313), decreasing to 85.7% (234/273) for second grafts, 62.1% (18/29) for third grafts, and 64.3% (9/14) for fourth grafts. No significant difference in survival rate between phakic (88.0%, 1180/1370), aphakic (86.4%, 13/15) and pseudophakic (86.1%, 41/49) recipients was identified ($p = 0.64$).

Pre-operative diagnosis	One-year survival rate
Fuchs' endothelial dystrophy	95.1% (77/81)
Keratoconus	94.6% (694/735)
Stromal dystrophy	87.7% (57/65)
Bullous keratopathy	85.1% (235/276)
Viral keratitis	85.0% (102/120)
Unspecified	81.6% (74/92)
Non-infective keratitis	80.0% (20/25)
Trauma	76.9% (60/78)
Regrafts	70.4% (81/115)
Infection (non-herpetic)	69.0% (29/42)

Table 7:3 ***One-year survival rates based on pre-operative diagnosis***

Pre-existing vascularisation of the recipient cornea was significantly associated with decreased survival rate ($p < 0.001$). Recipients with pre-existing corneal vascularisation had a survival rate of 78.1% (373/478), compared to 92.9% (1056/1137) in those with no vascularisation. One quadrant of pre-existing vascularisation was associated with a survival rate of 81.4% (118/145), 2 quadrants 80.5% (99/123), 3 quadrants 75.0% (54/72), and 4 quadrants 67.6% (75/111). There was no statistically significant difference in survival rate between 1 and 2 quadrants ($p = 0.85$), 2 and 3 quadrants ($p = 0.36$) or 3 and 4 quadrants ($p = 0.20$) of pre-existing vascularisation. However, a significant difference in survival rate was identified when 4 quadrants was compared with either 1 ($p = 0.01$) or 2 quadrants ($p = 0.02$) of pre-existing vascularisation. Pre-existing corneal vascularisation was most commonly reported in viral keratitis cases (75.0%, 90/120), followed by of regraft cases (59.1%, 68/115), infection (non-herpetic) (54.0%, 23/42) and trauma cases (44.9%, 38/78).

Active anterior segment inflammation at the time of PKP was associated with a significantly lower survival rate ($p < 0.001$). Recipients with active inflammation had a survival rate of 68.6% (131/191) compared to 91.2% (1271/1394) in cases with no inflammation. Recipients with pre-operative glaucoma also had a lower survival rate (76.8%, 149/194) compared to those in whom elevated IOP had never been recorded (90.6%, 1208/1333) ($p < 0.001$).

Recipient gender and age were not significantly associated with a decreased survival rate in this study. Although the mean recipient age of PKPs that survived (46.8 years, SD 18.2 years) was significantly less than those that failed (54.3 years, SD 19.4 years) ($p = 0.01$), significant confounding by differences in pre-operative diagnosis between recipient age groups was identified. Analysis of each pre-operative diagnosis separately failed to identify any significant association between recipient age and survival rate (range $p = 0.19$ for regraft to $p = 0.88$ for Fuchs' dystrophy).

7.4.4 Surgical factors influencing PKP survival outcome

There was a significant association identified between graft diameter size and survival rate. The survival rate for different graft sizes is presented in Table 7:4. The survival rate was significantly decreased for graft sizes equal to or less than 7.25 mm ($p = 0.01$) and for graft sizes greater than or equal to 8.50 mm ($p < 0.01$), when compared to the 'medium' graft size range (7.50 mm to 8.25 mm). Table 7:5 presents the distribution of pre-operative diagnoses for each graft size range.

Graft size (mm)	Survival rate (%)
< 7.00	68.4 (13/19)
7.00	75.0 (27/36)
7.25	81.4 (92/113)
7.50	88.4 (129/146)
7.75	93.0 (371/399)
8.00	85.7 (197/230)
8.25	91.2 (426/467)
8.50	80.3 (53/66)
8.75	78.4 (80/102)
> 8.75	56.7 (17/30)

Table 7:4 ***Survival rate for different graft diameter sizes***

Pre-operative diagnosis	Number per graft size (%)		
	≤ 7.25mm	7.5-8.25mm	≥ 8.50 mm
Bullous keratopathy	25 (16.7)	213 (18.0)	38 (20.4)
Fuchs' dystrophy	4 (2.7)	72 (7.0)	4 (2.2)
Infection (non-herpetic)	6 (4.0)	12 (1.0)	8 (4.3)
Keratoconus	46 (30.9)	604 (51.1)	85 (45.7)
Non-infective keratitis	5 (3.4)	10 (0.8)	10 (5.4)
Stromal dystrophy	21 (14.9)	31 (2.6)	11 (5.9)
Regraft	12 (8.1)	90 (7.6)	13 (7.0)
Trauma	9 (6.1)	60 (5.1)	8 (4.3)
Viral keratitis	21 (14.1)	88 (7.4)	10 (5.4)

Table 7:5 ***The distribution of pre-operative diagnoses for different graft size ranges***

The performance of an additional operative procedure at the time of PKP was associated with a significant decrease in survival rate ($p < 0.01$). The survival rate reduced from 90.2% (1114/1235) if "PKP only" was performed to 82.5% (311/377) if there were one or more additional procedures. The most common additional procedures and the corresponding survival rates were: extracapsular cataract extraction and posterior chamber intraocular lens insertion (ECCE + PCIOL) (85.0%, 96/113); anterior vitrectomy (70.6%, 72/102); exchange of IOL (80.7%, 25/31); and removal of IOL (83.3%, 25/30). Of these, anterior vitrectomy ($p < 0.01$) resulted in a significant decrease in survival rate, whereas, the observed decrease in survival rate associated with ECCE + PCIOL ($p = 0.07$), exchange of IOL ($p = 0.08$) and removal of IOL ($p = 0.2$) failed to reach statistical significance.

The presence of operative complications (not further specified) significantly decreased the survival rate from 89.9% (1323/1472) to 78.0% (64/82) ($p < 0.001$). No significant difference in survival was identified between suture methods used: continuous 85.3% (388/455); interrupted 89.0% (389/437); and combined continuous/ interrupted 87.6% (632/723). Furthermore, no difference was identified between suture type ($p = 0.14$) or suture gauge ($p = 0.9$) used.

7.4.5 Post-operative factors influencing PKP survival outcome

The occurrence of one or more episodes of reversible rejection significantly decreased survival rate ($p < 0.001$). The survival rate was 93.6% (1163/1243) if

no episodes of reversible rejection occurred, 68.8% (172/250) if there was one episode, 67.3% (33/49) if there were two episodes, and 61.4% (27/44) in cases of three or more episodes.

Other post-operative complications (excluding reversible rejection and PKP failure) were reported in 12.0% (196/1629) of PKPs. The survival rate was 88.7% (1269/1431) in the absence of complications and significantly decreased to 81.7% (160/196) in cases where one or more complications were reported ($p < 0.01$). The most common complications reported and the corresponding one-year survival rates are presented in Table 7:6. Uveitis, suture-related problems, glaucoma, and persistent epithelial defect were the most frequently recorded complications. Only post-operative glaucoma resulted in a statistically significant reduction in survival rate ($p = 0.02$).

Suture status one-year post-operative was classified as 'all remaining', 'all removed', or 'some removed', with no difference in survival rate identified between the groups (88.1%, 92.2%, and 91.0% respectively, $p = 0.07$). There was no significant difference in survival rate ($p = 0.10$) between different post-operative antibiotic-steroid combinations used. A survival rate of 89.4% (884/987) was identified for a regimen of Maxitrol (Dexamethasone 0.1%, Neomycin 0.35%) compared to 85.7% (282/329) for Prednisone acetate 1% plus Chloramphenicol 0.5%.

Complication	No. of keratoplasties	No. survived (%)	p-value
Uveitis	31	25 (80.6%)	0.08
Suture-related	27	24 (88.8%)	0.24
Glaucoma	26	19 (73.1%)	0.02
Persistent epithelial defect	18	14 (77.8%)	0.08
Infection	13	11 (84.6%)	0.27
Wound leak	13	12 (92.3%)	0.70
Trauma	6	5 (83.3%)	0.60
Irido-corneal adhesion	4	4 (100%)	-
Shallow AC	3	3 (100%)	-
Hyphema	3	1 (33.3%)	-
Retinal detachment	3	1 (33.3%)	-

Table 7:6 ***Most commonly reported post-operative complications (statistical significance compares the specified complication compared to PKPs without complications)***

7.4.6 Multivariate analysis of PKP survival outcome

Factors identified as significantly associated with a decreased PKP survival rate in univariate analysis were further analyzed by multi-factorial modeling using binary logistic regression. This enabled confounding variables to be accounted for and the identification of independent risk factors associated with decreased PKP survival. The results of this analysis are presented in Table 7:7.

Factors identified as independent risk factors in order of significance were: one or more episodes of reversible rejection; active anterior segment inflammation at the time of PKP; pre-existing corneal vascularisation; intra-operative complications (not further specified); small diameter graft size (≤ 7.25 mm); large diameter graft size (≥ 8.5 mm); and pre-operative glaucoma.

Pre-operative diagnoses that were identified as independent risk factors were regraft and trauma. The remaining pre-operative diagnoses along with donor age, recipient age, additional surgical procedures and post-operative glaucoma were not identified as significant independent risk factors in multivariate analysis.

Variable	Odds ratio	Significance	CI (95%)
Donor age	1.01	0.79	0.99-1.02
Recipient age	0.99	0.52	0.98-1.01
Indication			
Bullous keratopathy	1.54	0.25	0.74-3.18
Fuchs' endothelial dystrophy	1.13	0.84	0.34-3.81
Infection (non-herpetic)	1.68	0.28	0.63-4.51
Keratoconus	0.85	0.67	0.40-1.80
Non-infective keratitis	2.79	0.08	0.88-8.90
Stromal dystrophy	1.29	0.62	0.48-3.45
Regraft	2.90	0.004	1.39-6.00
Trauma	2.85	0.015	1.22-6.69
Viral keratitis	1.35	0.78	0.65-3.68
Active inflammation	2.41	< 0.001	1.55-3.74
Corneal vascularisation	1.75	0.005	1.19-2.58
Pre-operative glaucoma	1.65	0.04	1.10-2.30
Small graft size	2.41	0.01	1.50-3.89
Large graft size	1.88	0.03	1.18-2.60
Anterior vitrectomy	1.74	0.15	0.82-3.72
Operative complications	1.87	0.04	1.03-3.40
≥ 1 reversible rejection episodes	5.43	< 0.001	3.80-7.76
Post-operative glaucoma	2.22	0.14	0.77-6.46

Table 7:7 ***Multivariate analysis of risk factors influencing PKP survival outcome***

7.4.7 Visual outcome

Keratoplasty was performed to improve visual function in 87.5% (n = 1430) of cases, for structural reasons in 6.8% (n = 112), for pain in 4.6% (n = 76), and for a combination of reasons in 1.1% (n = 17). Visual outcome (in terms of best-corrected Snellen visual acuity (BCSVA) at one-year post-operatively) for all surviving PKPs is presented in figure 7:1. The most frequently recorded BCSVA was 6/9 (27.8%) followed by 6/12 (15.3%) and 6/6 (11.9%), with the mean BCSVA overall 6/15 (logMAR 0.40). The mean BCSVA for each pre-operative diagnosis is presented in Table 7:8. Keratoconus had the most favourable visual outcome (mean BCSVA 6/10) followed by Fuchs' endothelial dystrophy (6/15) and viral keratitis (6/18). Bullous keratopathy (6/40) and non-infective keratitis (6/60) had the poorest visual outcome. Unfortunately pre-operative visual acuity was not recorded in the database preventing analysis of improvement in visual acuity. Post-operative astigmatism was insufficiently reported to allow analysis.

Table 7:9 presents the results of a multivariate analysis performed to identify independent risk factors associated with poor visual outcome (defined as BCSVA of 6/36 or worse). Advancing recipient age, active anterior segment inflammation at the time of keratoplasty, pre-operative glaucoma, pre-existing corneal vascularisation and one or more episodes of reversible rejection were all significantly associated with poor visual outcome. Bullous keratopathy, non-infective keratitis, and trauma were the pre-operative diagnoses identified as significant independent risk factors for poor visual outcome.

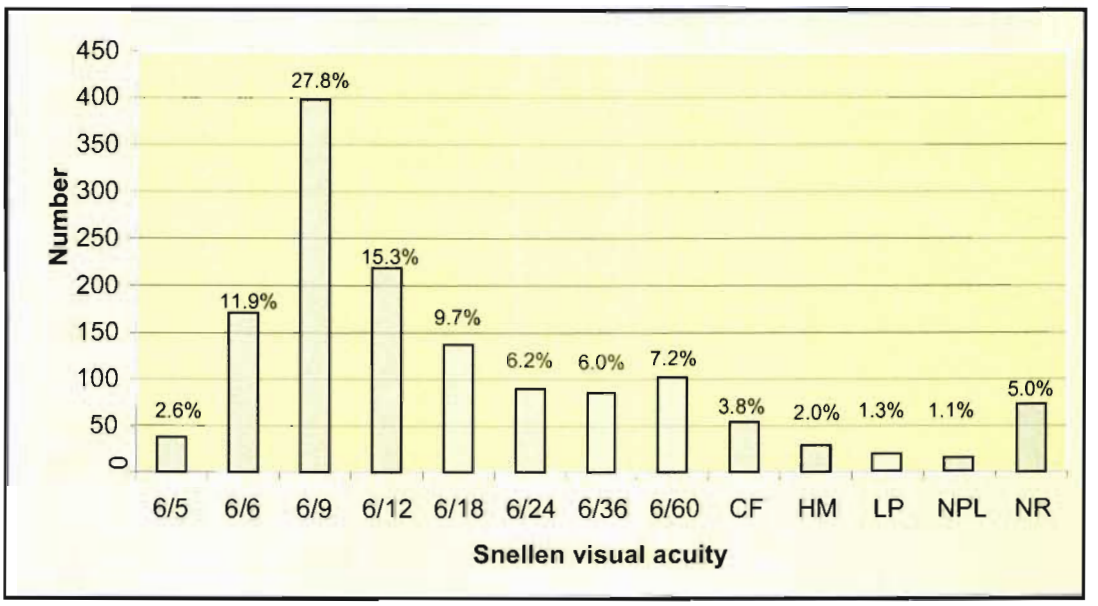


Figure 7:1 ***Distribution of best corrected Snellen visual acuity one-year post-operatively. (CF = counting fingers; HM = hand movements; LP = light perception; NPL = no perception of light; NR = not recorded)***

Pre-operative diagnosis	Mean BCVA	
	logMAR (SD)	Snellen VA
Keratoconus	0.25 (0.20)	6/10
Fuchs' dystrophy	0.40 (0.46)	6/15
Viral keratitis	0.46 (0.45)	6/18
Stromal dystrophy	0.47 (0.48)	6/18
Regraft	0.50 (0.55)	6/20
Infection (non-herpetic)	0.60 (0.59)	6/24
Trauma	0.72 (0.70)	6/30
Bullous keratopathy	0.80 (0.61)	6/40
Non-infective keratitis	0.99 (0.73)	6/60
Overall	**0.40 (0.49)**	**6/15**

Table 7:8 ***Mean post-operative best corrected visual acuity for each indication***

Variable	Odds ratio	Significance	CI (95%)
Donor age	1.00	0.87	0.99-1.01
Recipient age	1.08	0.01	1.06-1.11
Indication			
Bullous keratopathy	2.5	0.01	1.40-4.55
Fuchs' endothelial dystrophy	0.69	0.35	0.31-1.50
Infection	1.90	0.16	0.77-4.69
Keratoconus	0.58	0.10	0.32-1.10
Non-infective keratitis	4.05	0.01	1.40-11.80
Stromal dystrophy	1.05	0.91	0.48-2.30
Regraft	1.42	0.29	0.74-2.72
Trauma	2.34	0.02	1.17-4.83
Viral keratitis	1.15	0.67	0.61-2.20
Active inflammation	2.23	0.01	1.52-3.27
Corneal vascularisation	1.45	0.02	1.06-1.97
Pre-operative glaucoma	1.64	0.01	1.11-2.42
Small graft diameter size	0.81	0.20	0.49-1.20
Large graft diameter size	1.23	0.33	0.82-1.84
Additional lens surgery	1.18	0.50	0.74-1.90
Anterior vitrectomy	1.90	0.08	0.92-3.98
Operative complications	1.35	0.28	0.79-2.30
≥ 1 reversible rejection episodes	2.00	0.01	1.46-2.74
Post-operative complications	0.90	0.71	0.55-1.50

Table 7:9 ***Multivariate analysis of the risk factors associated with visual acuity of 6/36 or worse***

7.5 Discussion

The overall one-year survival rate identified in this study (87%) was comparable to that of other published reports, with one-year survival rates in the literature typically ranging from 80% to 91%.[60, 82-91] Irreversible rejection followed by endothelial failure and vascularisation were identified as the most common reasons for PKP failure in this study. Other published reports also cite glaucoma and infection as common reasons for PKP failure.[60, 88, 92, 93] Overall visual outcome was also similar to that of other published reports, with 60% of recipients achieving a one-year post-operative BCSVA of 6/18 or better compared with the range of 48%-70% reported in the literature.[60, 82, 89, 91, 94, 95]

Donor factors (donor age, donor source, donor cause of death, death to preservation interval, endothelial cell density, donor lens status, and storage duration) were not significantly associated with decreased keratoplasty survival or poorer visual outcome in this study. The results of this large New Zealand based study tend confirm trends that have previously been reported with respect to donor-related risk factors. [87, 90, 96-102]

Pre-operative diagnosis, as and indication for PKP, was significantly associated with survival rate in this study. The results of this study were consistent with those of earlier reports, with the highest survival rates identified in keratoconus followed by Fuchs' endothelial dystrophy, and the lowest associated with trauma and regraft.[60, 82, 88, 91-93, 104-109] For regraft, the survival rate further decreased

according to the number of previous grafts, also in concordance with the literature.[60, 82-84, 92, 96, 97, 107, 108, 110] Reasons cited for poor survival outcome in regraft and trauma included the presence of high-risk pre-operative conditions, the need for additional surgical procedures, and the increased incidence of post-operative complications.[107, 108, 111, 112]

Keratoconus and Fuchs' endothelial dystrophy were identified as the pre-operative diagnoses associated with the most successful visual outcome in this study. In contrast, non-infective keratitis, bullous keratopathy and trauma were identified as independent risk factors for poor visual outcome. Other published studies reported similar results with keratoconus having the best visual outcome overall, followed by Fuchs' endothelial dystrophy and viral keratitis.[60, 82, 84, 94, 95, 108, 113, 114] In these studies, bullous keratopathy, trauma and regraft were recognized as having the least successful visual outcome.

As has been reported in previous studies,[60, 82-84, 92, 93, 115, 116] pre-existing vascularisation of the recipient cornea was identified as a significant independent risk factor for decreased keratoplasty survival. Furthermore, the decrease in survival rate was proportional to the number of quadrants of pre-existing vascularisation, with four quadrants of vascularisation resulting in a significantly lower survival rate than one or two quadrants. This has also been recognized in other published reports.[60, 92] The results of this study support the conclusion that pre-existing vascularisation increases the risk of an immunological response

against the corneal graft, consequently leading to a higher rate of irreversible rejection.[93, 107]

The presence of active anterior segment inflammation at the time of PKP was identified as one of the most significant independent risk factors for decreased keratoplasty survival in this study. Two of the largest corneal transplant studies, the Australian Graft Registry[60] and the Corneal Transplant Follow-up Study,[82] also identified a similar strong association between active anterior segment inflammation at the time of PKP and a decreased survival rate. This study also confirmed that a history of pre-operative glaucoma is a significant independent risk factor for decreased PKP survival.[60, 82, 90, 93, 96, 97,119] Price et al[93] identified pre-operative glaucoma as an independent predictor of endothelial failure, and suggested that high IOP leads to increased risk of endothelial decompensation, possibly through direct damage to endothelial cells.

After accounting for the differences in pre-operative diagnosis between age groups, this study identified no significant decrease in survival rate with advancing recipient age. Again this is in concordance with other studies that report no association or only a marginal association between advancing recipient age and survival rate.[60, 82, 90, 93, 98, 104, 116, 120] Although there are conflicting reports in the literature regarding the influence of recipient gender on survival rate, with some studies reporting male gender to be a significant risk factor for

decreased PKP survival, this study identified no relationship between recipient gender and survival rate.[60, 93, 103, 104]

Recipient factors that were identified as independently associated with poor visual outcome were pre-existing vascularisation, active anterior segment inflammation at the time of keratoplasty, a history of pre-operative glaucoma, and advancing recipient age. Of these, pre-operative glaucoma, pre-existing vascularisation and advancing recipient age have been previously recognized as predictors of poor visual outcome.[82, 84, 91, 98, 114, 121, 122] Poor pre-operative visual acuity was also reported as a significant predictor of adverse visual outcome, unfortunately this data was unavailable for analysis in this study.[82, 122]

Both small and large graft diameter sizes were identified as independent risk factors for decreased PKP survival in multivariate analysis. There are conflicting reports in the literature regarding the influence of both small and large graft size on survival rate.[60, 82, 90, 93, 94, 107, 116, 122] Several large studies have identified small graft size to be significantly associated with decreased PKP survival.[60, 82, 90, 93, 97] Price et al[93] cited higher rates of endothelial failure due to a reduced number of transplanted intact endothelial cells as a possible reason for this association. Increased risk of rejection failure with smaller graft sizes has also been reported.[60, 93, 97] Several studies, including the preceding, have also identified large graft size to be significantly associated with decreased keratoplasty survival, with a higher rate of endothelial rejection cited as the reason for this

association.[60, 82, 90, 93, 94, 122] Other published studies however reported no association between graft size and survival rate.[107, 116]

In the current study, the performance of an anterior vitrectomy at the time of PKP was associated with a significant decrease in survival rate at the univariate level. However, in multivariate analysis the relationship was no longer apparent. A possible explanation is the association between identified between performance of an anterior vitrectomy and pre-operative diagnoses with lower survival rates (trauma, regraft, and bullous keratopathy). In the literature, a significant association between vitreous surgery and decreased PKP survival has been frequently reported, both at the univariate and multivariate level.[60, 82, 89, 96, 104, 107] In concordance with other published reports, the performance of additional lens surgery was not significantly associated with decreased PKP survival in this study.[60, 82, 92, 96, 104, 124, 125]

This study confirmed the association previously recognized between post-operative episodes of reversible rejection and decreased PKP survival.[60, 104, 126-129] Indeed, only one episode of reversible rejection was required to significantly decrease survival rate. The survival rate further decreased with increasing episodes of reversible rejection. In multivariate analysis, the occurrence of one or more episodes of reversible rejection was recognized as the most significant factor predictive of poor keratoplasty survival. A significant decrease in survival rate was identified in the presence of post-operative glaucoma at univariate level

only. Several other studies have reported post-operative glaucoma as a significant independent risk factor for decreased PKP survival.[60, 112, 119] In the current study, the only post-operative factor identified to be independently associated with poor visual outcome was the occurrence of one or more episodes of reversible rejection.

7.7 Conclusion

The large and comprehensive New Zealand National Eye Bank database has enabled detailed statistical analysis of this large series of PKPs performed in New Zealand. Several independent risk factors were identified that significantly influenced PKP survival and visual outcome. Factors identified that were predictive of poor keratoplasty survival were active inflammation at keratoplasty, pre-existing vascularisation, pre-operative glaucoma, small or large graft size, intra-operative complications, episodes of reversible rejection and a pre-operative diagnosis of regraft, trauma or infection.

Factors identified that were predictive of poor visual outcome were advancing recipient age, active inflammation at keratoplasty, pre-existing vascularisation, pre-operative glaucoma, one or more episodes of reversible rejection, and a pre-operative diagnosis of bullous keratopathy, trauma or non-infective keratitis.

Chapter 8

The Indications and Outcome of Paediatric Corneal Transplantation in New Zealand: 1991-2003

8.1 Abstract

Purpose

To evaluate patient characteristics, indications, surgical details, and outcome of paediatric keratoplasty in New Zealand.

Methods

As part of a prospective longitudinal study, paediatric keratoplasty data collected by the New Zealand National Eye Bank was analyzed for the 13-year period 1991-2003.

Results

During the study period the NZNEB supplied 2547 corneas for keratoplasty, of which 65 (3%) were used for paediatric patients (14 years or younger). The 65 keratoplasties were performed in 58 eyes of 52 patients (66% male, 34% female, mean age 10.6 years, SD = 4.3 years). Indications were classified into three groups: congenital (16%, n = 9), acquired non-traumatic (74%, n = 43), and acquired traumatic (10%, n = 6). Peters' anomaly (7% of total), keratoconus (67%) and penetrating trauma (8%) were the most common indications in each group respectively. Eighty-two percent of keratoplasties with known outcome survived (clear graft) one year post-operatively, 16% failed, and one patient died. Keratoplasty for congential indications had a lower one-year survival rate (78%) compared to acquired non-traumatic (85%) and traumatic (100%) indications, although the difference was not statistically significant (p = 0.65). Thirty-eight

percent of patients with known outcome had a one-year post-operative best-corrected Snellen visual acuity (BCSVA) of 6/9 or better, and 60% had a BCSVA of 6/18 or better. Visual outcome was significantly better for acquired compared to congenital indications ($p = 0.03$).

Conclusion

Analysis of the NZNEB database provided valuable information in relation to paediatric keratoplasty in New Zealand. In particular, this study highlighted an unusually high prevalence of keratoconus as an indication for keratoplasty. In addition, a high one-year survival rate and good visual outcome was identified, especially in cases of keratoplasty for acquired conditions.

8.2 Introduction

Paediatric keratoplasty is a difficult undertaking which presents a wide range of challenges pre-operatively, intra-operatively and post-operatively.[130-136] The presence of amblyopia, associated ocular pathology, and greater severity of disease may significantly limit visual outcome.[130-136] The surgical procedure is technically more complex due to the decreased rigidity and increased elasticity of the infant cornea and sclera, the smaller size of the infant eye, the increased intra-operative fibrin formation and the positive vitreous pressure.[130-136] Post-operative follow-up and management may be more complicated, and graft rejection is often difficult to detect and treat.[130-136]

In this component of the inter-related studies highlighted in preceding chapters, the NZNEB database was analyzed for the 13-year period 1991 to 2003 with respect to patient characteristics, indications, surgical details and outcome of paediatric keratoplasty.

8.3 Methods

As part of a longitudinal, prospective study, the electronic records of the NZNEB were analyzed for the 13-year period 1991-2003 with respect to demographics of recipients, indications, donor information, surgical details, and outcome of paediatric keratoplasty.

Data are entered into the computerized NZNEB database in a prospective manner by eye bank staff. Donor information is entered at the time of donor tissue procurement. Recipient and surgical information is collected from surgeons at the time of operation. Follow-up data is obtained at one and two years post-operatively and is collected from surgeons by way of a mailed questionnaire sent out at the appropriate time point.

Statistical analysis was performed in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. The SPSS V 12 software package was used. Statistical methods were the Fishers exact test (when total number of observations were less than 20) and chi-square testing to compare proportions between groups, the Student's *t* test to compare means between groups, and logistic regression modeling to identify factors associated with decreased keratoplasty survival. The level of statistical significance was $P < 0.05$ unless stated otherwise. Visual acuity was converted to a logMAR scale for the purposes of statistical analysis.

8.4 Results

8.4.1 Patient demographics

During the 13-year study period the NZNEB supplied 2547 corneas for keratoplasty, of which 65 (3%) were used for patients within the paediatric age group (14 years or younger). The 65 keratoplasties were performed in 58 eyes of 52 patients. The mean age of patients at the time of operation was 10.6 years

(standard deviation (SD) = 4.3 years), median age 12.0 years, and range 2 weeks to 14.0 years. The gender distribution was 66% (n = 34) male and 34% (n = 18) female.

8.4.2 Indications

The indications for paediatric keratoplasty are presented in Table 8:1. The diagnostic classification system developed by Stulting et al[130] was used to facilitate comparison with other published reports. Indications were classified into three groups: congenital, acquired non-traumatic and acquired traumatic conditions. The congenital group accounted for 16% (n = 9) of keratoplasties, the acquired non-traumatic group 74% (n = 43), and the acquired traumatic group 10% (n = 6). Peters' anomaly (7% of total, n = 4) was the most common indication in the congenital group, keratoconus (67% of total, n = 39) in the acquired non-traumatic group, and penetrating trauma (9% of total, n = 5) in the acquired traumatic group. There were seven regraft procedures performed during the study period with original indications being keratoconus (n=3), viral keratitis (n = 1), Peters' anomaly (n = 2), and penetrating trauma (n = 1). Keratoplasty was performed to improve visual acuity in 86% (n = 56) of cases, for tectonic reasons in 6% (n = 4), and for a combination of reasons in 8% (n = 5).

Preoperative Diagnosis	Number of eyes	% of total
Congenital	**9**	**15.5**
Peters' anomaly	4	6.9
CHED	2	3.4
Limbal dermoid	1	1.7
Congenital corneal opacity not otherwise specified	2	3.4
Acquired non-traumatic	**43**	**74.1**
Keratoconus	39	67.2
Viral keratitis	4	6.9
Acquired traumatic	**6**	**10.3**
Penetrating trauma	5	8.6
Aphakic corneal oedema	1	1.7

Table 8:1 ***Indications for paediatric keratoplasty (CHED = congenital hereditary endothelial dystrophy)***

The age and gender distribution for each diagnostic group are presented in Table 8:2. There was no significant association between pre-operative diagnosis and gender identified (congenital, $p = 0.16$; acquired non-traumatic, $p = 0.26$; acquired traumatic, $p = 0.43$; regraft, $p = 0.41$, *X^2-test*). The indications for different age groups (using stratified 5-year intervals) are presented in Table 8:3.

Peters' anomaly was the leading indication in the less than five years age group and keratoconus in the remaining two groups.

Diagnosis group	Mean age (SD) (years)	Median age (years)	Age range	Male (%)
Congenital	3.0 (3.2)	2	2 wk -10 yrs	44%
Acquired non-traumatic	12.4 (2.2)	13.0	6 -14 yrs	64%
Acquired traumatic	10.8 (3.6)	12	5 - 14 yrs	80%
Regraft	9.0 (4.9)	8.0	5 mths -14 yrs	71%

Table 8:2 ***Age and gender distribution for each diagnostic group***

Age Group	Most common diagnoses	Number of eyes
Less than 5 years	Peters' anomaly	3
	CHED	2
	Congenital corneal opacity not otherwise specified	2
Aged 5 to 9 years	Keratoconus	5
	Penetrating trauma	2
	Viral keratitis	2
	Peters anomaly	1
Aged 10 to 14 years	Keratoconus	34
	Penetrating trauma	3
	Viral keratitis	2
	Aphakic corneal oedema	1
	Limbal dermoid	1

Table 8:3 ***Indications for keratoplasty for different age groups (CHED = congenital hereditary endothelial dystrophy)***

8.4.4 Surgical details

Keratoplasty was performed by 22 different surgeons in 10 centres throughout New Zealand. Penetrating keratoplasty was performed in 62 cases and lamellar keratoplasty in the remaining three cases. The indications for lamellar keratoplasty were limbal dermoid, Peters' anomaly, and congenital corneal

opacification not otherwise specified. Donor information was available for all corneal tissue used with mean donor age 44.4 years (SD = 18.0 years), median age 46.5 years, and range 10 to 77 years. Mean endothelial cell density (ECD) was 3074 cells/mm² (SD = 386 cells/mm²), median ECD was 3065 cells/mm², and ECD range was 2578 to 4210 cells/mm². The donor cornea button was sutured to the recipient corneal rim with 10-0 nylon in 95% (n = 62) and a combination of nylon and prolene in 5% (n = 3). An interrupted suture technique was used in 40% (n = 26), a single continuous suture in 26% (n = 17), and a combined interrupted/ continuous technique in 34% (n = 22).

Reported pre-operative ocular conditions included corneal vascularisation in 19% (n = 12), previous intraocular surgery in 14% (n = 9), a history of elevated intraocular pressure in 6% (n = 4), and active ocular inflammation at the time of operation in 9% (n = 6). Additional operative procedures performed were anterior vitrectomy in 5% (n = 3), cataract extraction and intraocular lens insertion in 3% (n = 2), iridectomy in 2% (n = 1), and iridectomy plus synechiolysis in 2% (n = 1). No significant intraoperative complications were reported. Early post-operative complications (within 3 weeks) included wound leak in 5% (n=3), wound infection in 2% (n=1), corneal ulcer in 3% (n=2), and early graft rejection in 2% (n=1). Postoperative medical management consisted of Maxitrol (Dexamethasone 0.1%, Neomycin 0.35%) in 60% (n = 39), Prednisone acetate 1% plus Chloramphenicol 0.5% in 32% (n =21), with other antibiotic-steroid combinations in 8% (n =5).

8.4.5 Outcome

Outcome was evaluated one-year post-operatively with follow-up data available for keratoplasties performed from 1991 to 2001. There were 58 keratoplasties during this interval with follow-up data available for 88% (n = 51). The remaining 12% (n = 7) were lost to follow-up. The survival rate was determined by analyzing the percentage of keratoplasties that were surviving (clear graft) at one-year post-operatively. Eighty-two percent (n = 42) of keratoplasties survived, 16% (n = 8) failed, and one patient died. Survival rates for different diagnostic groups were: congenital, 78%; acquired non-traumatic, 85%; acquired traumatic, 100%; and regraft procedures, 80%. Survival rates for different age groups were: less than 5 years, 82%; 5 to 9 years, 78%; and 10 to 14 years, 83%. The differences between diagnostic groups ($p = 0.65$) and age groups ($p = 0.51$) were not statistically significant. There was no statistical difference in survival rate based on suture method ($p = 0.50$) or type of post-operative medication used ($p = 0.91$).

The most common reason for keratoplasty failure was irreversible rejection (10% of total, n = 5), followed by presumed primary tissue failure (defined as failure of the graft to clear) (4%, n = 2) and trauma (2%, n = 1). Episodes of reversible rejection were reported in 22% (n = 9) of cases that survived one-year post-operatively. Logistic regression analysis was performed in an attempt to identify factors which may be associated with decreased keratoplasty survival. Factors included were pre-existing corneal vascularisation, pre-operative glaucoma, active inflammation at keratoplasty, small or large diameter graft size, additional

intra-operative procedures, immediate post-operative complications, and episodes of reversible rejection. However, no individual factor was identified from this analysis which resulted in a statistically significant decrease in keratoplasty survival.

Best-corrected Snellen visual acuity (BCSVA) was reported in 90% (n = 38) of cases that survived one-year post-operatively (Table 8:4). Thirty-eight percent (n = 19) had a BCSVA of 6/9 (20/30) or better and 60% (n =30) had a BCSVA of 6/18 (20/60) or better. Spectacles (n = 14) or contact lens (n = 2) were provided in 38% of cases. Visual outcome for each diagnostic group is presented in Table 5. Visual outcome was significantly better for acquired (mean logMAR 0.2, 6/10) compared to congenital indications (mean log MAR 1.1, 6/75) (p = 0.03). Unfortunately pre-operative visual acuity was not available for analysis as this was not recorded in the NZNEB database.

Outcome and visual acuity	Number of corneas (%)
Survived	**42 (82.0)**
> 6/6	1 (2.0)
6/6- 6/9	17 (33.3)
6/12 - 6/18	12 (23.5)
6/36- 6/60	4 (7.8)
< 6/60	4 (7.8)
Not tested	4 (7.8)
Failed	**8 (16.0)**
Patient Died	**1 (2.0)**

Table 8:4 ***Outcome of paediatric keratoplasty one-year post-operatively***

Diagnostic group	≥ 6/9	6/12-6/18	6/36-6/60	< 6/60	VA not reported	Failed
	Number of eyes					
Congenital	1	-	2	2	2	2
Acquired nontraumatic	13	11	2	-	2	5
Acquired traumatic	2	1	-	-	-	0
Regraft	2	-	-	2	-	1

Table 8:5 ***Keratoplasty outcome for each diagnostic group and for regraft procedures***

8.5 Discussion

As previously noted, New Zealand is a multi-cultural society with a population of approximately 4 million, served by 110 Ophthalmologists, distributed over a geographical area slightly greater than the United Kingdom. Over 200 keratoplasties are performed annually in New Zealand and the NZNEB was established in 1991 to support this demand. Over the 13-year study period it was estimated that the NZNEB supplied at least 90% of all donated ocular tissue. Therefore, in relation to paediatric keratoplasty, analysis of the NZNEB database provides an accurate representation of corneal disease and keratoplasty in New Zealand.

The indications for paediatric keratoplasty vary significantly in the literature. Table 8:6 provides a comparison between this study and the major studies published over the last two decades. Most studies used the age criteria of 14 years or younger. The proportion of keratoplasties performed for congenital indications ranged from 14-64%, for acquired non-traumatic 19-80%, and for acquired traumatic 6-29%.[134-137, 140] In this study, the proportion of keratoplasties performed for acquired non-traumatic indications (74%) was significantly greater than that for congenital (16%) and acquired traumatic (10%) indications. This is in contrast to the majority of published reports, in which congenital indications contribute a significantly greater proportion.[130-133, 136]

Study	Age criteria	Number of eyes*	Congenital	Acquired non-traumatic	Acquired traumatic
Stulting et al[130]	< 15years	107	45 (42%)	31 (29%)	31 (29%)
Cowden et al[132]	< 15 years	57	25 (44%)	16 (28%)	16 (28%)
Aasuri et al[133]	< 15 years	154	47 (31%)	85 (55%)	22 (14%)
Dana et al[131]	< 12 years	131	84 (64%)	25 (19%)	22 (17%)
Dada et al[136]	< 13 years	370	51 (14%)	296 (80%)	23 (6%)
Current study	< 15 years	58	9 (16%)	43 (74%)	6 (10%)

Table 8:6 ***Indications for paediatric keratoplasty reported in the literature.***
(* Excludes regraft procedures)

Keratoconus was the most common acquired non-traumatic indication in this study, accounting for 67% of all keratoplasties. This was notably higher than other published reports where keratoconus accounted for only 0-11% of paediatric keratoplasties, with post-infectious corneal scarring the most common acquired non-traumatic indication reported in the literature. [130-133, 136] Similar to previous studies, [130-133, 136] the most common congenital indication identified in this study was Peters' anomaly followed by congenital hereditary endothelial dystrophy, and the most common indication in the acquired traumatic diagnostic group was penetrating trauma.

The notably high prevalence of keratoconus as an indication for paediatric keratoplasty reflects that which was identified by Edwards et al[1], who were the

first to report that keratoconus was the leading indication (45%) for keratoplasty in the adult population in New Zealand, accounting for a significantly higher proportion of keratoplasties compared to other published reports. Ethnic differences in keratoconus prevalence, severity, and rate of disease progression have been well recognized,[137-139] and keratoconus is thought to be particularly prevalent in Maori and Pacific Island communities, which constitute a large proportion of the New Zealand population. Edwards et al[1] postulated that this high prevalence, and possibly more rapid disease progression and severity, has led to the uniquely high prevalence of keratoconus as an indication for keratoplasty in New Zealand. Similarly, this may explain the high prevalence of keratoconus identified in this study. Over the last six years the NZNEB database has incorporated recipient ethnicity data to further investigate the relationship between ethnicity and keratoconus in New Zealand.

Survival rates published by the foremost studies of paediatric keratoplasty are presented in Table 8:7. Mean follow-up generally ranged from one to two years and the reported survival rates ranged from 46 to 80%.[130-133, 140, 141] Keratoplasty performed for congenital indications had a lower survival rate compared to acquired non-traumatic and acquired traumatic indications.[130-133, 140, 141] The overall survival rate of 82% in this study was high when compared to other published reports.[130-133, 140, 141] This may be due to the longer follow-up period at which survival rates were reported in some of the other studies.[132, 133, 140, 141] Another contributing factor may be the high proportion of keratoplasties

performed for acquired non-traumatic indications. In concurrence with other published reports, a higher survival rate for acquired compared to congenital indications was identified in this study,[132, 133, 140, 141] although this did not reach statistical significance, possibly due to the small size of the congenital group. Of particular note, keratoplasty performed for keratoconus had an excellent prognosis with a one-year survival rate of 90%.

		Survival Rate (%)			
Study	**Mean follow-up period (years)**	**Congenital**	**Acquired non-traumatic**	**Acquired traumatic**	**Overall**
Stulting et al[130] 1984	1	60	73	70	66
Dana et al[131] 1995	1	80	76	84	80
Aasuri et al[133] 2000	1.3	64	71	55	66
Legeais et al[140] 1990	2.1	38	79	71	72
Erlich et al[141] 1991	1.7	29	40	71	46
Cowden et al[132] 1990	1-10*	56	50	56	54
Current study	1	78	85	100	82

Table 8:7 ***Summary of published survival rates in paediatric keratoplasty***

In other published reports, several factors have been identified which increase the risk of failure in paediatric keratoplasty.[130-133, 142-147] Performance of an additional surgical procedure at the time of keratoplasty was most significantly associated with a decreased survival rate, with other factors reported including pre-operative glaucoma, associated ocular conditions, and corneal vascularisation.[130-133, 142-147] In this study, no factor was independently associated with a statistically significant increase in failure rate. However, the relatively small number of subjects limited this analysis. The influence of age alone on paediatric keratoplasty survival has been evaluated with conflicting reports in the literature. Aasuri et al[133] identified a correlation between age under 5 years and allograft rejection, and commented that this may be due to a more active immune system in younger patients. Other studies, including this one, did not identify such an association.[130, 131]

Poor visual outcome in a surviving keratoplasty (clear graft) is well recognized within the paediatric age group and is most commonly a result of amblyopia, non-corneal ocular abnormalities, and post-operative astigmatism.[130-133, 136, 137] In concordance with other published reports, this study identified a poorer visual outcome for congential compared to acquired indications.[130-133, 136, 137] A higher prevalence of amblyopia and associated ocular abnormalities in the congenital group has been cited as the reason for the less successful visual outcome in this group.[130-133, 136, 137] We suspect that this may also be the case in this study. Important considerations therefore are the timing of keratoplasty which should

not be delayed unnecessarily and the high priority of post-operative amblyopia management in at-risk patients.

8.6 Conclusions

Analysis of the New Zealand National Eye Bank database has provided valuable information in relation to paediatric keratoplasty in New Zealand. In particular, this study identified an unusually high prevalence of keratoconus as an indication for paediatric keratoplasty in New Zealand.

High success rates at one-year post-operatively, in terms of both keratoplasty survival and visual outcome were identified, especially in cases of keratoplasty for acquired corneal conditions.

Section 2 References

1. Edwards M, Clover GM, Brookes N, et al. Indications for corneal transplantation in New Zealand: 1991-1999. Cornea 2002; 21:152-155.

2. Pollock G. A practical guide to eye banking. In Vajpayee RB, ed. Corneal Transplantation. 1st Ed. New Delhi: Jaypee Brothers Medical Publishers Ltd, 2002: 22-31.

3. European Eye Bank Association Directory. 7th Ed. 1991.

4. Moyes AL, Holland EJ, Palmon FE, Dvorak JA, Doughman DJ. Tissue Utilization at the Minnesota Lions' Eye Bank. Cornea 1995; 14: 571-577.

5. Armitage WJ, Eastly DL. Factors influencing the suitability of organ-cultured corneas for transplantation. Invest Ophthalmol Vis Sci 1997; 38: 16-24

6. Gain P, Thuret G, Chiquet C, et al. Cornea procurement from very old donors: post organ culture cornea outcome and recipient graft outcome. Br J Ophthalmol 2002; 86: 404-411.

7. Moyes AL, Holland EJ, Palmon FE, Dvorak JA, Doughman DJ. Tissue Utilization at the Minnesota Lions' Eye Bank. Cornea 1995; 14(6): 571-577.

8. Williams KA, Muehlberg SM, Lewis RF, et al. Influence of advanced recipient and donor age on the outcome of corneal transplantation. Australian Corneal Graft Registry. Br J Ophthalmol 1997; 81: 835-9.

9. Chu W, Dahl P, O'Neill MJ. Benefits of specular microscopy in evaluating eye donors aged 66 and older. Cornea 1995; 14: 568-70, 634.

10. Forster RK, Fine M. Relation of donor age to success in penetrating keratoplasty. Arch Ophthalmol 1971; 85: 42-7.

11. Chang SD, Pecego JG, Zadnik K, Danneffel MB, Mutti DO, Mannis MJ. Factors influencing graft clarity. Cornea 1996; 15: 577-81.

12. Jenkins MS, Lempert SL, Brown SI. Significance of donor age in penetrating keratoplasty. Ann Ophthalmol 1979; 11:974-6.

13. Boisjoly HM, Tourigny R, Bazin R, et al. Risk factors of corneal graft failure. Ophthalmology 1993; 100: 1728-35.

14. Probst LE, Halfaker BA, Holland EJ. Quality of corneal donor tissue in the greater-than-75-year age group. Cornea 1997; 16: 507-11.

15. Wilson SE, Bourne WM. Corneal preservation for penetrating keratoplasty. Kaufman HE, Barron BA, McDonald MB. The Cornea 2nd Ed. Butterworth-Heinemann 1997; 33: 781-803.

16. Yee RW, Matsuda M, Schultz RO, Edelhauser HF: Changes in the normal corneal endothelial pattern as a function of age. Curr Eye Res 1985; 4:671.

17. Culbertson WM, Abbot RL, Forster RK. Endothelial cell loss in penetrating keratoplasty. Ophthalmology 1982; 89: 600-4.

18. Mattern RM, Heck EL, Cavanagh HD. The impact of tissue utilization of screening donor corneas by specular microscopy at the University of Texas Southwestern Medical Centre. Cornea 1995; 14: 562-7.

19. Bourne WM. Morphologic and functional evaluation of endothelium of transplanted human corneas. Trans Am Ophthalmol Soc 1983; 81: 403.

20. Williams KA, Muehlberg SM, Bartlett CM, Esterman A, Coster DJ. The Australian Corneal Graft Registry Report. Adelaide,1999.

21. The New Zealand Health Information Report. NZ Government Statistics, 2000.

22. Statistics New Zealand Report. NZ Government Statistics, 2001

23. Tullo AB, Dyer PA. Corneal Transplantation in Britain. Br Med J 1995;310:1347

24. Ehlers N. Corneal banking and grafting. The background to the Danish Eye Bank System, where corneas await their patients. Acta Ophthalmol Scand 2002; 80: 572-578.

25. Chu W. The Past 25 Years in Eye Banking. Cornea. 2000; 19(5): 754-765

26. Szaflik J, Liberik I, Brix M. Corneal Storage Methods. Transplantation Proceedings 2000; 32(6): 1424-1425.

27. Doughman DJ. Prolonged donor cornea preservation in organ culture: long term clinical evaluation. Trans Am Ophthalmol Soc. 1980; 78: 567.

28. Doughman DJ, Van Horn D, Harris JE, et al. The ultrastructure of the human organ cultured cornea. Arch Ophthalmol 1974; 92: 516.

29. Leveille AS, McMullan FD, Cavanagh HD. Endophthalmitis following penetrating keratoplasty. Ophthalmology 1983; 90: 38-9.

30. Pardos GJ, Gallagher MA. Microbial contamination of donor eyes. A retrospective study. Arch Ophthalmol 1982; 100: 1611-3.

31. Cameron JA, Antonios SR, Cotter JB, et al. Endophthalmitis from contaminated donor corneas following penetrating keratoplasty. Arch Ophthalmol 1991; 109: 54-9.

32. Kloess PM, Stulting RD, Waring GO III, et al. Bacterial and fungal endophthalmitis after penetrating keratoplasty. Am J Ophthalmol 1993; 115: 309-16

33. Antonios SR, Cameron JA, Badr IA, et al. Contamination of donor cornea: post-penetrating keratoplasty endophthalmitis. Cornea 1991; 10: 217-20.

34. EBAA Medical Advisory Board Agenda. For the Meeting of the EBAA Medical Advisory Board; June 24 1999; Newport Beach, CA.

35. Borderie VM, Laroche L. Microbiologic study of organ-cultured donor corneas. Transplantation 1998; 66:120-3.

36. Poole TG, Insler MS. Contamination of donor cornea by gentamycin-resistent organisms. Am J Ophthalmol 1984; 97:560-4.

37. Mathers WD, Lemp MA. Corneal rim culture. Cornea 1987; 6:231-3.

38. Albon J, Armstrong M, Tullo A. Bacterial Contamination of Human Organ-cultured Corneas. Cornea 2001; 20: 260-3.

39. Wiffen SJ, Weston BC, Maguire LJ, Bourne WM. The value of routine donor corneal rim cultures in penetrating keratoplasty. Arch Ophthalmol 1997; 115: 719-724.

40. Everts RJ, Fowler WC, Chang DH, Reller BL. Corneoscleral rim cultures. Cornea 2001; 20:586-589.

41. Stenson S, Newman R, Freyne P, Rosenthal S. Contamination of M-K stored keratoplasty donor material. Ophthalmic Surg 1980; 11: 395-7.

42. Baer JB, Nirankari VS, Glaros DS. Streptococcus endophthalmitis from contaminated donor corneas after keratoplasty. Arch Ophthalmol 1988; 106: 517.

43. Insler MS, Urso LF. Candida albicans endophthalmitis after penetrating keratoplasty. Am J Ophthalmology 1987; 104: 57.

44. European Eye Bank Association Directory 12th Ed. 2004.

45. Williams KA, White MA, Badenoch PR, et al. Donor cornea procurement: six year review of the role of the eye bank in South Australia. Aus NZ J Ophthalmology 1990; 18: 77-89.

46. Brady SE, Rapuano CJ, Arentsen JJ, et al. Clinical indications for and procedures associated with penetrating keratoplasty, 1983-1988. Am J Ophthalmol 1989;108:118-22.

47. New Zealand Population Consensus Report. NZ Government Statistics, 2001.

48. Chen WL, Hu FR, Wang IJ. Changing indications for penetrating keratoplasty in Taiwan from 1987 to 1999. Cornea 2001;20:141-144.

49. Al-Yousuf N, Mavrikakis I, Mavrikakis E, et al. Penetrating keratoplasty: indications over a 10 year period. Br J Ophthalmol 2004;88:998-1001.

50. Maeno A, Naor J, Lee HM, et al. Three decades of corneal transplantation: indications and patient characteristics. Cornea 2000;19:7-11.

51. Cosar CB, Sridhar MS, Cohen EJ, et al. Indications for penetrating keratoplasty and associated procedures, 1996-2000. Cornea 2002;21:148-151.

52. Robin JB, Gindi JJ, Koh K, et al. An update of the indications for penetrating keratoplasty, 1979 through 1983. Arch Ophthalmol 1986;104:87-9.

53. Legeais J, Parc C, D'Hermies F. Nineteen years of penetrating keratoplasty in the Hotel-Dieu Hospital in Paris. Cornea 2001;20:603-6.

54. Poinard C, Tuppin P, Loty B, et al. The French national waiting list for keratoplasty created in 1999: patient registration, indications, characteristics, and turnover. J Fr Ophthalmol 2003;26:911-19.

55. Frucht-Perry J, Shtibel H, Soloman A, et al. Thirteen years of penetrating keratoplasty in Israel. Cornea 1997;16:16-20.

56. Cursiefen C, Kuchle M, Naumann GOH. Changing indications for penetrating keratoplasty : Histopathology of 1250 corneal buttons. Cornea 1998;17:468-70.

57. Mkanganwi N, Nondo SI, Guramatunhu S. Indications for corneal grafting in Zimbabwe. Central African J Med 2000;46:300-2.

58. Chan CM, Wong TY, Yeong SM, et al. Penetrating keratoplasty in the Singapore National Eye Centre and donor acquisition in the Singapore Eye Bank. Annals Acad Med, Singapore 1997;26:395-400.

59. Flowers CW, Chang KY, McLeod SD, et al. Changing indications for penetrating keratoplasty, 1989-1993. Cornea 1995;14:583-8

60. Williams KA, Muehlberg SM, Bartlett CM, Esterman A, Coster DJ. Australian Graft Registry Report. Adelaide, 2000.

61. Brooks AM, Weiner JM. Indications for penetrating keratoplasty: a Clinicopathologic review of 511 corneal specimens. Aus NZ J Ophthalmol 1987;15:277-81.

62. Claesson M, Armitage WJ. Corneal grafts at St John Eye Hospital, Jerusalem, January 2001-November 2002. Br J Ophthalmol 2004;88:858-860.

63. Pearson AR, Soneji B, Sarvananthan N, et al. Does ethnic origin influence the incidence of severity of keratoconus? Eye 2000;14:625-8.

64. Tay KH, Chan WK. Penetrating keratoplasty for keratoconus. Annals Acad Med, Singapore 1997;26:132-7.

65. Tuft SJ, Moodaley LC, Gregory WM, et al. Prognostic factors for the progression of keratoconus. Ophthalmology 1994;101:439-47.

66. Gasset AR, Hinson WA, Frias JL. Keratoconus and atopic diseases. Ann Ophthalmol 1978; 10:991-4.

67. Khan MD, Kundi N, Saeed N, et al. Incidence of keratoconus in Spring catarrh. Br J Ophthalmol 1988;72:41-3.

68. Totan Y, Hepsen IF, Cekic O, et al. Incidence of keratoconus in subjects with vernal keratoconjunctivitis. Ophthalmology 2001;108, number 4.

69. Owens H, Gamble G. A profile of keratoconus in New Zealand. Cornea 2003;22:122-125.

70. Pomare E, Tutengaehe H, Ramsden I, et al. Asthma in Maori People. N Z Med J 1992;105:469-70.

71. Ihalainen A. Clinical and epidemiological features of keratoconus - genetic and external factors in the pathogenesis of the disease. Acta Ophthalmol Suppl 1986;178:1-64.

72. Zadnik K, Barr JT, Edrington TB, et al. The Collaborative Longitudinal Evaluation of Keratoconus (CLEK) Study. Invest Ophthalmol Vis Sci 1998;39:2537-46.

73. Rabinowitz YS. Keratoconus. Surv Ophthalmol 1998;42:297-319.

74. Dobbins KRB, Price FW, Whitson WE. Trends in indications for penetrating keratoplasty in the Midwestern United States. Cornea 2000;19:813-816.

75. Al-Towerki AE, Gonnah ES, Al Rajhi A, et al. Changing indications for corneal transplantation at the King Khaled Eye Hospital. Cornea 2004;23:584-88.

76. Eye Bank Association of America Statistical Reports 2002. Washington, DC: EBAA, 2002.

77. NZ Statistics. Quick facts-people. NZ Government Statistics, 2001.

78. Lois N, Kowal VO, Cohen EJ, et al. Indications for penetrating keratoplasty and associated procedures, 1989-1995. Cornea 1997;16:623-629.

79. Krachmer JH, Purcell JJ, Jr.,Young CW, et al. Corneal endothelial dystrophy: a study of 64 families. Arch Ophthalmol 1978;96:2036-9.

80. Lee SY, Pavan-Langston D. Role of acyclovir in the treatment of herpes simplex viral keratitis. Int Ophthalmol Clin 1994;34:9-18.

81. Uchio E, Hatano H, Mitsui K, et al. A retrospective study of herpes simplex keratitis over the last 30 years. Jpn J Ophthalmol 1994;38:196-201.

82. Vail A, Gore SM, Bradley BA, Easty DL, Rogers CA, Armitage WJ. Conclusions of the corneal transplant follow-up Study. Br J Ophthalmol 1997;81:631-636.

83. Dandona L, Naduvilath TJ, Janarthanan M, Ragu K, Rao GN. Survival analysis and visual outcome in a large series of corneal transplants in India. Br J Ophthalmol 1997;81:726-731.

84. Vail A, Gore SM, Bradley BA, Easty DL, Rogers CA. Corneal graft survival and visual outcome: a multicenter study. Ophthalmology 1994;101:120-7.

85. Bradley BA, Vail A, Gore SM, Rogers CA, Armitage WJ, Nicholls SM, Easty DL. Penetrating keratoplasty in the United Kingdom: an interim analysis of the corneal transplant follow-up study. Clinical Transplants 1993;293-315.

86. Chan CM, Wong TY, Yeong SM, Lim TH, Tan DT. Penetrating keratoplasty in the Singapore National Eye Centre and donor cornea acquisition in the Singapore Eye Bank. Annals of the Academy of Medicine 1997; 26:395-400.

87. Williams KA, Muehlberg SM, Lewis RF, Coster DJ. How successful is corneal transplantation? A report from the Australian Corneal Graft Register. Eye 1995;9:219-27

88. Gain P, Thuret G, Chiquet C, Rizzi P, Pugniet JL, Acquart S, et al. Corneal procurement from very old donors: post organ culture cornea outcome and graft failure. Br J Ophthalmol 2000;86:404-11.

89. Muraine M, Sanchez C, Watt L, Retout A, Brasseur G. Long-term results of penetrating keratoplasty. A 10-year-plus retrospective study. Graefes Arch Clin Exp Ophthalmol 2003;241:571-6.

90. Teenan DW. Sim KT. Hawksworth NR. Outcomes of corneal transplantation: a corneal surgeon vs the general ophthalmologist. Eye 2003;17:727-30.

91. Borderie VM, Schee S, Touzeau O, Vedie F, Carvajal-Gonzalez S, Laroche L. Donor organ cultured corneal tissue selection before penetrating keratoplasty. Br J Ophthalmol 1998;82:382-88.

92. Yorston D, Wood M, Foster A. Penetrating keratoplasty in Africa: graft survival and visual outcome. Br J Ophthalmology 1996;80:890-94.

93. Thompson RW, Price MO, Bowers PJ, Price FW. Long-term Graft Survival after Penetrating Keratoplasty. Ophthalmology 2003;110:1396-1402.

94. Price MO, Thompson Jr RW, Price Jr FW. Risk factors for various causes of failure in initial corneal grafts. Arch Ophthalmol 2003;121:1087-93.

95. Claesson M, Armitage WJ, Fagerholm P, Stenevi U. Visual outcome in corneal grafts: a preliminary analysis of the Swedish Corneal Transplant Register. Br J Ophthalmol 2002; 86:174-180.

96. Saunders PR, Sibley LM, Richards JS, Holland SP, Chow DL, Courtright P. Outcome of corneal transplantation: can a prioritization system predict outcome? Br J Ophthalmol 2002;86:57-61.

97. Boisjoly HM, Tourigny R, Bazin R, et al. Risk factors of corneal graft failure. Ophthalmology 1993;100:1728-35.

98. Maguire MG, Stark WJ, Gottsch JD, et al, and the Collaborative Corneal Transplantation Studies Research Group. Risk factors for corneal graft failure and rejection in the collaborative corneal transplantation studies. Ophthalmology 1994;101: 1536-47.

99. Williams KA, Muehlberg SM, Lewis RF, Coster DJ. Influence of advanced recipient age and donor age on the outcome of corneal transplantation. Br J Ophthalmol 1997;81:835-839.

100. Volker-Dieben HJ, D'Amaro J, Kok-Van Alphen CC. Hierarchy of prognostic factors for corneal graft survival. Aus NZ J Ophthalmol 1987;15:11-18.

101. Abbott RL, Forster RK. Determinants of graft clarity in penetrating keratoplasty. Arch Ophthalmol 1979; 97:1071-5.

102. Palay DA, Kangas TA, Stulting RD, Winchester K, Litoff D, Krachmer JH. The effects of donor age on the outcome of penetrating keratoplasty in adults. Ophthalmology 1997;104:1576-9.

103. Vail A, Gore SM, Bradley BA, Easty DL, Rogers CA, Armitage WJ. Influence of donor and histocompatibility factors on corneal graft outcome. Transplantation 1994;58:1210-6.

104. Andersen J, Ehlers N. The influence of donor age and post mortem time on corneal graft survival and thickness when employing banked donor material (A five-year follow-up). Acta Ophthalmologica 1988;66:313-7.

105. Sit M, Weisbrod DJ, Naor J, Slomovic AR. Corneal Graft Outcome Study. Cornea 2001;20:129-133.

106. Price Jr FW, Whitson WE, Collins KS, et al. Five-year corneal graft survival: a large, single-centre patient cohort. Arch Ophthalmol 1993;111:799-805.

107. Ehlers N, Olsen T. Long-term results of corneal grafting in keratoconus. Acta Ophthalmol 1983;61:918-26.

108. Bersudsky V, Blum-Hareuveni T, Rehany U, Rumelt S. The profile of repeated corneal transplantation. Ophthalmology 2001;108:461-69.

109. MacEwen CJ, Khan ZU. Anderson E. MacEwen CG. Corneal re-graft: indications and outcome. Ophthalmic Surg 1988;19:706-12.

110. Lim L, Persudovs K, Coster DJ. Penetrating keratoplasty for keratoconus: visual outcome and success. Ophthalmology 2000;107:1125-31.

111. Kirkness CM, Ezra E, Rice NSC, Steele ADM. The success and survival of repeat corneal grafts. Eye 1990;4: 58-64.

112. Kenyon KR, Starck T, Hersh PS. Penetrating keratoplasty and anterior segment reconstruction for severe ocular trauma. Ophthalmology 1992;99:396-402.

113. Doren GS, Cohen EJ, Brady SE. Arentsen JJ. Laibson PR. Penetrating keratoplasty after ocular trauma. Am J Ophthalmol 1990;110:408-11.

114. Pineros O, Cohen EJ, Rapuano CJ. Laibson PR. Long-term results after penetrating keratoplasty for Fuchs' endothelial dystrophy. Arch Ophthalmol 1996;114:15-8.

115. Fishbaugh J. Visual acuity, residual astigmatism, and graft clarity following penetrating keratoplasty for keratoconus. Insight 1991;16:24-7.

116. Mohan M, Panda A, Kumar TS. Results of penetrating keratoplasty in vascularised corneas. Ann Ophthalmol 1990;22:235-8.

117. Inoue K, Amano S, Oshika T, Tsuru T. Risk factors for corneal graft failure and rejection in penetrating keratoplasty. Acta Ophthalmol Scand 2001;79:251-5.

118. Hill JC. The relative importance of risk factors used to define high-risk keratoplasty. Ger J Ophthalmol 1996;5:36-41.

119. Madar TH, Stulting RD. The high risk penetrating keratoplasty. Ophthalmol Clin N Am 1991;4:411-26.

120. Rumelt S, Bersudsky V, Blum-Hareuveni T, Rehany U. Preoperative and postoperative glaucoma in repeated corneal transplantation. Cornea 2002;21:759-65

121. Ing JJ, Ing HH, Nelson LR, et al. Ten-year postoperative results of penetrating keratoplasty. Ophthalmology 1998;105:1855-65.

122. Tay KH. Chan WK. Penetrating keratoplasty for keratoconus. Ann Acad Med 1997;26:132-7.

123. Vail A. Gore SM. Bradley BA. Easty DL. Rogers CA. Armitage WJ. Clinical and surgical factors influencing corneal graft survival, visual acuity, and astigmatism. Ophthalmology 1996;103:41-9.

124. Sanfilippo F, MacQueen JM, Vaughn WK, Foulks GN. Reduced graft rejection with good HLA-A and B matching in high risk corneal transplantation. NEJM 1986;315:29-35.

125. Crawford GJ, Stulting RD. Waring GO 3rd. Van Meter WS. Wilson LA. The triple procedure. Analysis of outcome, refraction, and intraocular lens power calculation. Ophthalmology 1986;93:817-24.

126. Akpek EK, Alton-Yaycioglu R, Karadayi K, Christen W, Stark WJ. Long term outcome of combined penetrating keratoplasty and intraocular lens implantation. Ophthalmology 2003;110:1017-22.

127. Epstein RJ, Seedor JA, Dreizen NG et al. Penetrating keratoplasty for herpes simplex keratitis and keratoconus: allograft rejection and survival. Ophthalmology 1987;94:935-942.

128. Wilson SE, Kaufman HE. Major review: graft failure after penetrating keratoplasty. Surv Ophthalmol 1990;34:325-56.

129. Yamagami S, Suzuki Y, Tsuru T. Risk factors for graft failure in penetrating keratoplasty. Acta Ophthalmol 1996;74: 584-8.

130. Stulting RD, Sumers KD, Cavanagh HD, et al. Penetrating keratoplasty in children. Ophthalmology 1984; 91:1222-30.

131. Dana MR, Moyes AL, Gomes JAP, et al. The indications for and outcome in pediatric keratoplasty. Ophthalmology 1995; 102:1129-38.

132. Cowden JW. Penetrating keratoplasty in infants and children. Ophthalmology 1990; 97:324-9.

133. Aasuri MK, Prashant G, Gokhle N, et al. Penetrating keratoplasty in children. Cornea 2000; 19:140-44.

134. Stulting RD. Penetrating keratoplasty in children. In: Brightbill FS, ed. Corneal surgery: theory, technique and tissue, 3rd ed. St Louis: Mosby, 1999;66: 526-537.

135. Vajpayee RB, Ramu M, Panda A, et al. Oversized grafts in children. Ophthalmology 1999; 106:829-32.

136. Dada T, Sharma N, Vajpayee RB. Indications for pediatric keratoplasty in India. Cornea 1999; 18:296-8.

137. Pearson A, Soneji B, Sarvananthan N, et al. Does ethnic origin influence the incidence or severity of keratoconus? Eye 2000; 14:625-8.

138. Tay KH, Chan WK. Penetrating keratoplasty for keratoconus. Annals Acad Med, Singapore 1997; 26:132-7.

139. Tuft SJ, Moodaley LC, Gregory WM, et al. Prognostic factors for the progression of keratoconus. Ophthalmology 1994; 101:439-47.

140. Legeais JM, Jobin D, Pouliquen Y. Keratoplasties chez l'enfant, J Fr Ophthalmology 1990; 13:116-20.

141. Erlich CM, Rootman DS, Morin JD. Corneal transplantation in infants, children and young adults: experience of the Toronto Hospital for Sick Children 1979-1988. Can J Ophthalmology 1991; 26: 206-10.

142. Parmley VC, Stonecipher KG, Rowsey JJ, et al. Peters' anomaly: a review of 26 keratoplasties in infants. Ophthalmic Surg 1993; 24:31-5.

143. Williams KA, Roder D, Esterman A, et al. Factors predictive of corneal graft survival. Report from Australian Graft Registry. Ophthalmology 1992; 99:403-14.

144. Yang LL, Lambert SR, Lynn MJ, et al. Long-term results of corneal graft survival in infants and children with Peter's anomaly. Ophthalmology 1999; 106: 833-48.

145. Waring GO III, Laibson PR. Keratoplasty in infants and children. Trans Am Acad Ophthalmol Otolaryngol 1977; 83:283-96.

146. Musch DC, Meyer RF. Risk of endothelial rejection after bilateral penetrating keratoplasty. Ophthalmology 1989; 96:1139-43.

147. Voolker-Dieben HJ, Kok-van Alphen CC, Lansbergen Q, et al. Different influences on corneal graft survival in 539 transplants. Acta Ophthalmol 1982; 60:190-202.

Section 3

Analysis of the Corneal Endothelium in the Normal Eye and Following Penetrating Keratoplasty

Chapter 9

The Effects of Corneal Parameters on the Assessment of Endothelial Cell Density in Normal Young Adults

9.1 Abstract

Purpose

The possible impact of corneal parameters on endothelial cell density (ECD) has largely been ignored in the normal eye. The aim of this study was to investigate the possible impact of corneal parameters (corneal thickness, corneal diameter, and corneal curvature) on ECD values in the normal eye of young adults.

Methods

Sixty two normal subjects (42 female, 20 male) were included in the study. The age range was 21 to 30 years with the mean age 25 years (SD 3 years). *In vivo* confocal microscopy was performed to evaluate mean ECD, mean cell area (MCA), coefficient of variation for cell area (COVA), and the proportion of hexagonal cells. The Orbscan system was used to measure corneal thickness, horizontal corneal diameter, and corneal curvature.

Results

The mean ECD was 3169 cells/mm² (SD 309 cells/mm²) and the mean percentage hexagonality was 53% (SD 5%). The mean central corneal thickness (CCT) was 529 µm (SD 43 µm). The central ECD was significantly correlated to the CCT (Pearson's r = 0.26, p = 0.04). Horizontal corneal diameter (r = 0.19, p = 0.14), anterior corneal curvature (r = - 0.07, p = 0.6) and posterior corneal curvature (r = - 0.07, p = 0.6) were not significantly correlated to ECD. Percentage hexagonality was not significantly correlated to the corneal parameters measured.

Conclusion

This study suggests that corneal thickness may have an important relationship with ECD in the normal young adult, with lower ECD values expected in thinner corneas. This is in concordance with previous studies involving children and elderly populations. The study also suggests that, at least for young adults, corneal diameter and corneal curvature does not have a significant impact on the ECD.

9.2 Introduction

The corneal endothelium consists of a single layer of cuboidal, hexagonal cells, which line the posterior corneal surface.[1, 2] As outlined in section 1, the cornea is supplied with a relatively fixed population of endothelial cells (numerical density 3500 to 4000 cells/mm²) at birth.[1] Although mitosis can occur in young endothelial cells, it is infrequent in the adult, and injured cells are not replaced. It is well established that there is a gradual decrease in endothelial cell density (ECD) and a corresponding increase in polymegathism and pleomorphism with advancing age.[1, 3-9] Clinically the assessment of ECD and morphology can provide valuable information in relation to the functional reserve of the corneal endothelium. Previous chapters have highlighted the importance of endothelial cell density in relation to indications for, and suitability of, corneas for penetrating keratoplasty and hence the importance of such parameters for those interested in corneal transplantation.

Surprisingly, there have been few reports in the literature of the possible impact of corneal parameters on ECD in the normal eye. Müller et al[10] have reported on the effects of corneal parameters on the assessment of ECD in the elderly eye. They identified that in an older population, thinner and/or steeper corneas and longer axial lengths were strongly correlated with lower central ECD values. However, they identified no significant correlation between horizontal corneal diameter and ECD in this population. In contrast, in a study involving children (age range 5 to 15 years) a significant independent correlation was identified

between increasing horizontal corneal diameter and lower central ECD values.[11] A significant correlation between thinner corneas and lower ECD values has also been reported in children.[12] However, there have been no previous reports that have assessed the effects of such corneal parameters on ECD in normal young adults.

The aim of this study was to investigate the possible impact of corneal thickness, anterior and posterior corneal curvature, and horizontal corneal diameter on the measurement of ECD values in normal young adults (age range 21-30 years).

9.3 Methods

9.3.1 Subjects

There were 62 normal subjects (42 female, 20 male) in the study group. Only the right eye of each subject was analysed. The age range of subjects was 21 to 30 years (mean age 25 years, SD 2.7 years). Normal subjects were defined as those who had no history of contact lens use, no past ocular disease or trauma, no systemic disease which may affect the cornea, no current ocular symptoms, and no abnormality on biomicroscopic examination. The subjects were recruited from students and staff members of the University of Auckland medical school. Consent was given by all subjects and ethical approval for this study was obtained from the local Auckland Ethics Committee.

9.3.2 Measurements

The Orbscan II slit scanning corneal topography system (Orbscan, Bausch and Lomb, Salt Lake City, UT, USA) was used to measure corneal thickness, corneal topography, horizontal corneal diameter and anterior chamber depth. Before measurement, the subject's head was aligned with the instrument and a head strap was placed around the back of the head. The subject was advised to keep both eyes open and fixate on the target. By viewing the live image of the eye on the monitor, the examiner aligned the two fixation markers reflected by the instrument on the corneal surface before performing the scan. Three scans were performed per cornea and the mean value for central cornea thickness, the horizontal corneal diameter, the spherical equivalent (using the least square method of determining the best fit sphere), and the eccentricity of the anterior and posterior surface was recorded.

In vivo confocal microscopy of the cornea was performed using slit-scanning technology (Confoscan 2, Fortune Technologies America, Greensboro, NC, U.S.A.).The subject was asked to fixate on a target, and the examination was performed with a 40× non-applanating, immersion lens that covers an area of approximately 0.1 mm^2. A drop of Viscotears (Carbomer 940 2 mg/g, CIBA Vision, Australia) on the objective lens served as an immersion and contact substance. For all examinations, a standard setting of four passes was used, with a scanning range of between 700 µm and 800µm (throughout the z-axis). One exam was performed on the centre of each cornea and up to 300 images were obtained for each exam.

Based on the best visibility of endothelial cells, three representative frames from each scan were chosen for analysis. All captured images were analysed using the NAVIS (Nidek Advanced Vision Information System) proprietary software. The chosen frame size, or region of interest (ROI) was 0.035 mm^2. Using manually adjusted automated cell counts, as many clearly visible cells as possible were analysed within each frame. The values for ECD, mean cell area, coefficient of variation for area and length, and percentage of hexagonal cells within each of the three frames were recorded and the mean values for each cornea were calculated.

9.3.3 Statistical analysis

Statistical analysis was performed in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. The SPSS V 12 for windows (Statistical Product and Service Solutions, Inc., Chicago, IL) software package program was used. Descriptive statistics (mean, standard deviation, median and range) were calculated for each set of data. Differences between data sample means were determined by Student's *t* test and one-way analysis of variance. Pearson's correlation coefficients were calculated to analyze the relationship between the parameters. A probability level of 0.05 or less was considered statistically significant.

9.4 Results

9.4.1 Endothelial cell density

The mean endothelial cell density (ECD) for all subjects was 3169 cells/mm² (SD 309 cells/mm²) and the range was 2450 to 3802 cells/mm². There was no significant gender related difference in ECD (mean ECD in females 3159 cells/mm² (SD 313 cells/mm²), mean ECD in males 3187 cells/mm² (SD 308 cells/mm²), p = 0.75). The ECD characteristics overall and for each gender are presented in Table 9:1. The analysis of each cornea included between 94 and 169 clearly visible cells (mean 134, SD 16).

	Whole group		Male		Female	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
ECD (cells/mm²)	3168 (309)	2450-3802	3187 (308)	2512-3761	3159 (313)	2450-3802
MCA (µm²)	318 (33)	263-408	317 (32)	266-397	320 (34)	263-408
COVA	32 (4.1)	23.5-43.2	30.5 (4.7)	24.5-43.2	32.3 (3.7)	23.5-40
COVL	13.7 (1.4)	9.9-16.6	13.1 (1.6)	9.9-16.6	14.0 (1.2)	10.8-16.1
% hexagonality	52.7 (5.3)	42.9-68.1	54.9 (6.6)	42.9-68.1	51.4 (4.3)	43.9-63.1

Table 9:1 ***Corneal endothelial cell characteristics overall and for each gender (ECD = endothelial cell density, MCA = mean cell area, COVA = coefficient of variation for cell area, COVL = coefficient of variation for cell length).***

Analysis of the percentage hexagonality provides a measure of cellular pleomorphism. The mean percentage hexagonality overall was 53% (SD 5%) with the range 43 to 68%. Males (55%) had a significantly higher proportion of hexagonal cells compared to females (51%) ($p = 0.02$). A negative and statistically significant correlation was identified between the proportion of hexagonal cells and the COVA ($r = -0.7$, $p < 0.01$) and COVL ($r = -0.9$, $p < 0.01$), indicating a strong association between loss of hexagonal cell shape and variation in cell size.

9.4.2 Corneal thickness

The mean central corneal thickness (CCT) was 529 µm (SD 43 µm) and the range was 429 µm to 627 µm. There was no significant difference in CCT between males (mean 530 µm, SD 50 µm) and females (mean 529 µm, SD 30 µm) ($p = 0.9$). When the mean central ECD values were compared with central corneal thickness values, a modest positive and statistically significant correlation was identified (Pearson's $r = 0.26$, $p = 0.04$) (figure 9:1). This indicates that lower ECD values were measured in thinner corneas. Although there was a clear trend for mean CCT to be negatively correlated with the proportion of hexagonal cells (indicating thinner corneas have a higher proportion of hexagonal cells), this trend did not reach statistical significance ($r = -0.24$, $p = 0.06$).

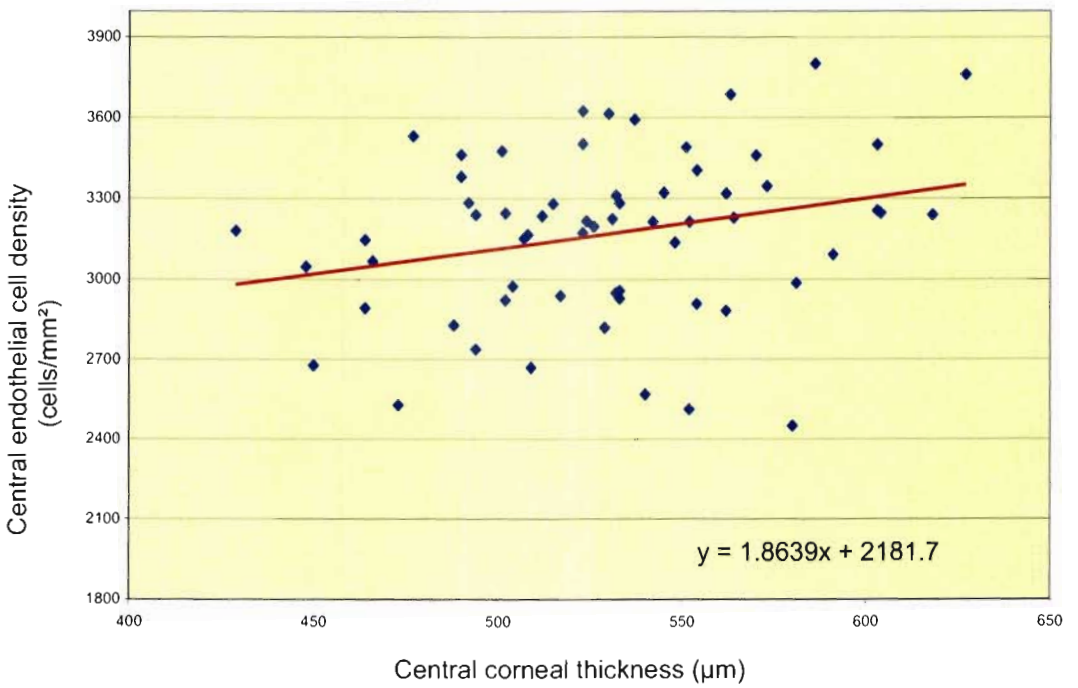


Figure 9:1 ***Relationship between measured central corneal thickness and central endothelial cell density***

9.4.3 Horizontal corneal diameter

The mean horizontal corneal diameter (HCD) overall was 11.7 mm (SD 0.4 mm) and the range was 10.8 to 13.0 mm. There was no statistically significant difference in mean HCD between males (mean 11.6, SD 0.3) and females (mean 11.8, SD 0.4) ($p = 0.2$). No statistically significant correlation between mean HCD and central ECD was identified (Pearson's $r = 0.19$, $p = 0.14$) (figure 9:2). Analysis of percentage hexagonality and HCD revealed no significant relationship ($r = -0.09$, $p = 0.45$).

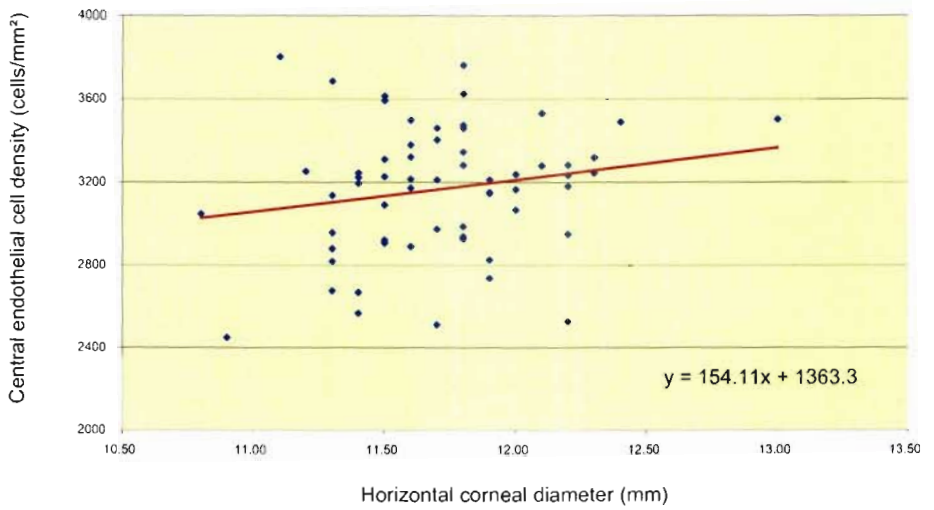


Figure 9:2 ***Relation between horizontal corneal diameter and central endothelial cell density***

9.4.4 Corneal curvature

The mean overall anterior corneal curvature (equivalent of spherical radius of curvature) was 7.9 mm (SD 0.2) and the range was 7.5 mm to 8.7 mm. The mean anterior eccentricity was 0.52, indicating that the mean anterior surface for all corneas assessed had the shape of a prolate ellipse (flattening peripherally). No statistically significant difference in mean anterior curvature between males and females was identified ($p = 0.6$). There was no statistically significant correlation identified between mean anterior corneal curvature and central ECD (Pearson's $r = -0.07$, $p = 0.6$)(figure 9:3).

The mean posterior corneal curvature was 6.5 mm (SD 0.2 mm) and the range was 6.0 to 7.9 mm. The mean posterior eccentricity was 0.50, indicating the

mean posterior surface had a prolate shape. No statistically significant difference in posterior corneal curvature between males and females was identified (p = 0.6). There was no statistically significant correlation between mean posterior corneal curvature and central ECD (r = - 0.07, p = 0.6) (figure 9:4).

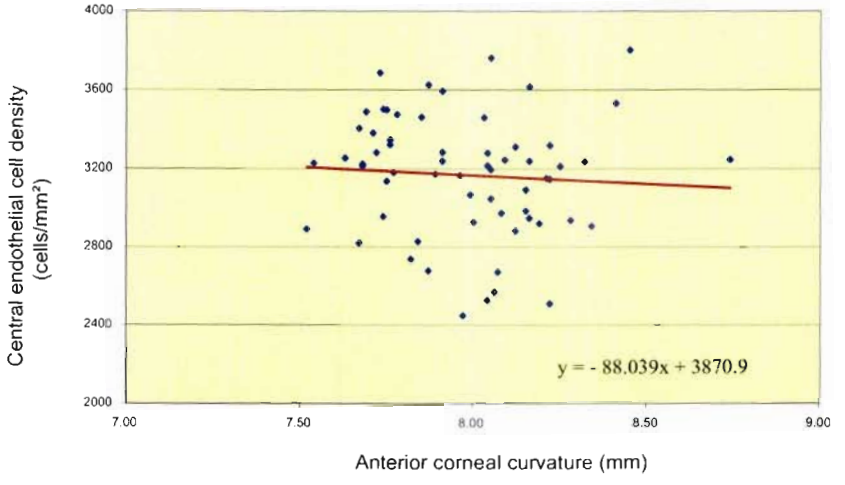


Figure 9:3 ***Relationship between anterior corneal curvature and central ECD***

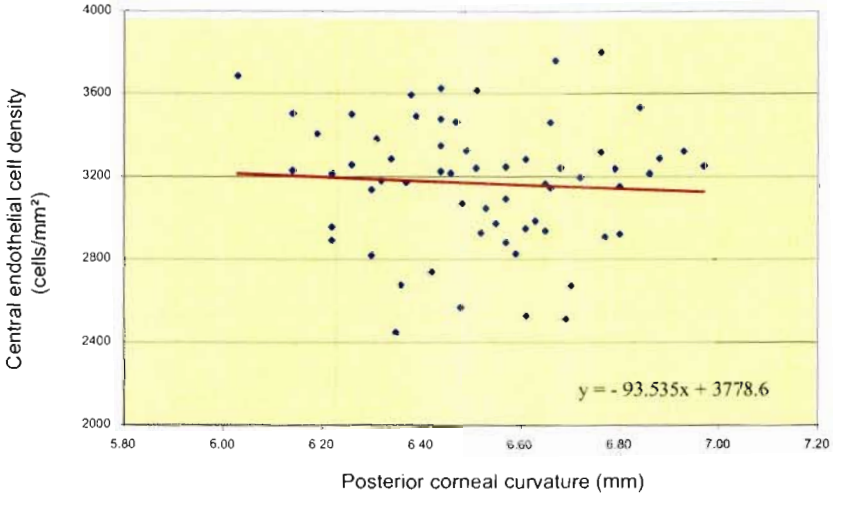


Figure 9:4 ***Relationship between posterior corneal curvature and central ECD***

9.4.5 Anterior chamber depth

The overall mean anterior chamber depth (ACD) was 3.6 mm (SD 0.3 mm) and the range was 3.0 to 4.9 mm. No statistically significant difference in mean ACD between males (mean 3.7 mm, SD 0.4 mm) and females (mean 3.5 mm, SD 0.2 mm) was identified ($p = 0.05$). There was no statistically significant correlation identified between mean ACD and central ECD in this study ($r = 0.15$, $p = 0.3$). A summary of the Pearson's correlation coefficients for all corneal parameters and endothelial values is presented in Table 9:2.

	CCT	HCD	ABFS	PBFS	ACD
ECD	**r = 0.26 p = 0.04**	r = 0.19 p = 0.14	r = - 0.07 p = 0.60	r = - 0.07 p = 0.60	r = 0.15 p = 0.26
MCA	r = - 0.23 p = 0.08	r = - 0.21 p = 0.10	r = 0.07 p = 0.60	r = 0.05 p = 0.73	r = - 0.16 p = 0.22
COVL	r = 0.19 p = 0.15	r = 0.19 p = 0.14	r = 0.08 p = 0.53	r = 0.17 p = 0.20	r = - 0.06 p = 0.64
COVA	r = 0.18 p = 0.17	**r = 0.27 p = 0.03**	r = - 0.08 p = 0.50	r = 0.07 p = 0.59	r = 0.09 p = 0.44
% Hexagonality	r = - 0.23 p = 0.06	r = - 0.10 p = 0.45	r = - 0.02 p = 0.88	r = - 0.14 p = 0.28	r = 0.06 p = 0.65

Table 9:2 ***Summary of Pearson's correlation coefficients for corneal and endothelial parameters (ECD = endothelial cell density, MCA = mean cell area, COVL = coefficient of variation for cell length, COVA = coefficient of variation for cell area, CCT = central corneal thickness, HCD = horizontal corneal diameter, ABFS = anterior best fit sphere, PBFS = posterior best fit sphere, ACD = anterior chamber depth). Statistically significant relationships highlighted in bold.***

9.5 Discussion

The mean value for endothelial cell density (ECD) in this current study (3169 cells/mm²) was in concordance with that previously reported in the literature for young adults (range 2680 to 3700 cells/mm²).[13-18] When compared with mean ECD values reported for children (3400 to 4300 cells/mm²) and for elderly eyes (2324 to 3175 cells/mm²), a clear trend of decreasing mean ECD with advancing age is observed.[9-11, 13, 15, 19-23] Consistent with the majority of other published reports, there was no significant difference in mean ECD based on gender in this study.[5, 9, 14, 15, 24, 25]

The mean central corneal thickness (CCT) measurement in this study (529 µm) was slightly less that that reported by Sanchis-Gimeno et al[26] (554 ± 16 µm), who studied the corneal thickness of 1000 young (range 20 to 30 years) emmetropic subjects using the Orbscan II slit scanning corneal topography system. Comparison of CCT with other published reports is limited due to differences in measurement technique. Although the accuracy, precision and repeatability of the Orbscan system has been accepted for use in research, it is recognized that it overestimates CCT compared to the more commonly used technique of ultrasound pachymetry.[10, 27, 28]

In concordance with previously published reports there was no significant difference in CCT based on gender in this study.[29-31] The relationship between CCT and age has been extensively studied. The majority of authors report a

gradual but insignificant decrease in CCT with advancing age after infancy.[32-36] In contrast, other researchers have reported no change or an increase in CCT with advancing age.[30, 37]

The relationship between significant endothelial dysfunction and increased corneal thickness is well established,[3, 38-40] and recognized causes of increased corneal thickness include anterior segment disease and previous intraocular surgery.[21, 36, 41-44] However, the relationship between corneal thickness and ECD in the normal eye throughout life has yet to be established, with few reports in the literature investigating a possible relationship. Studies investigating the influence of corneal thickness on ECD in the elderly eye have reported conflicting results. Muller et al[10] in a study of pre-operative cataract surgery patients (mean age 75 years, SD 11 years) identified a significant correlation between central ECD and central, superior and temporal corneal thickness. The authors concluded that in an older population, lower ECD values would be expected in thinner corneas. In contrast, Ventura et al[21] failed to identify a correlation between corneal thickness and ECD either pre-operatively or post-operatively in a study of elderly patients (mean age 71 years, SD 15 years) undergoing cataract surgery. Only one study investigating the relationship between corneal thickness and ECD in children was identified in the literature. Muller[12] reported a significant correlation between thinner corneas and lower central ECD values in growing children (mean age 10 years, SD 3 years). Interestingly, the results of this current study suggest that a similar relationship between central ECD and CCT may also exist in normal

healthy, young, adult corneas. Chang et al[14] studied the corneas of young myopic adults (mean age 22 years, SD 4 years). They identified that longer axial length was associated with flatter corneal curvature, decreased corneal thickness and decreased ECD. However, they identified no direct correlation between corneal thickness and corneal curvature with endothelial cell density. There are no other published reports investigating the relationship between corneal thickness and ECD in young adults.

The mean horizontal corneal diameter in this study was identical to that reported by Rüfer et al[45] in their study of 390 normal subjects using the Orbscan II scanning topography system (both 11.7 ± 0.4 mm). No significant difference based on gender was identified in either study.[45] Several studies have investigated the change in corneal diameter with age. The majority of investigators have reported that corneal diameter reaches adult size by the end of the third year of life, with no significant age-related increase in corneal diameter thereafter.[11, 46-48] However, a few investigators have suggested that corneal diameter may continue to change into late childhood.[49, 50] Previous reports have identified that corneal diameter has an important effect on ECD in children, with a significant independent negative correlation between horizontal corneal diameter and central ECD.[11, 51, 52] The enlarged posterior corneal surface associated with an increased corneal diameter may require spreading of endothelial cells to cover the greater surface area, therefore resulting in a corresponding decrease in ECD. An increase in corneal diameter during infancy

or early childhood may therefore be a factor in the recognized decrease in ECD that occurs during this time. The effect of horizontal corneal diameter on central ECD in the elderly eye was studied my Muller et al,[11] with no significant correlation identified. In the current study no significant relationship between horizontal corneal diameter and central ECD was identified for normal young adults. This suggests that the effect of corneal diameter on the measurement of ECD in the normal eye may be limited to infants and children only.

The effect of corneal curvature on the measurement of ECD is yet to be established. A significant correlation between steeper corneas and lower ECD values has previously been recognized in the elderly eye.[10] The authors commented that there may be an artifactual element to this result, as the true optical effects of increased corneal curvature on the measurement of endothelial cell area using specular or *in vivo* confocal microscopy has yet to be elucidated.[10] In this current study no significant correlation between both anterior or posterior corneal curvature and ECD was identified. This is in concordance with the findings of Chang et al[14] who identified no significant relationship between corneal curvature and ECD in myopic young adults. No studies appear to have specifically reported on the influence of corneal curvature on the measurement of ECD in infants or children.

In addition to the measurement of ECD, the percentage hexagonality (percentage of six sided cells) can also be used as an indicator of the health of

the corneal endothelium. The mean percentage hexagonality in this current study (53% ± 5%) was less than that identified in two recent published reports that analyzed mean percentage hexagonality for the same age range as this study (both 61% ± 7%).[15, 53] Percentage hexagonality of the corneal endothelium has been reported to gradually decrease with advancing age.[4, 8, 15, 53] There was no correlation between percentage hexagonality and ECD in this study. However, a significant correlation was identified between decreasing percentage hexagonality and increased variation in cell area. A correlation between increasing percentage hexagonality and decreasing corneal thickness was identified in this study, but the correlation marginally failed to reach statistical significance ($p = 0.057$). Percentage hexagonality was not related to the other corneal parameters assessed or to anterior chamber depth.

9.6 Conclusion

The current study suggests that corneal thickness may have an important influence on the measurement of ECD in the normal young adult, with lower ECD values expected in thinner corneas. The results of this study are therefore in concordance with earlier studies involving children and the elderly that have demonstrated a significant correlation between corneal thickness and ECD. Corneal thickness decreases gradually with age and this may contribute to the apparent decline in ECD recognized with advancing age in the normal eye. The effect of corneal diameter on ECD appears to be limited to children, with no

correlation between these parameters identified in this study. This was in concordance with a previous study involving elderly eyes.

The current study suggests that, at least for young adults, corneal curvature and anterior chamber depth do not have an effect on the measurement on ECD. The possible relationship between corneal parameters and endothelial cell density needs to be further evaluated with larger studies that encompass all age groups.

Chapter 10

Confocal Microscopy Analysis of Endothelial Morphology and Function in the Short and Long term following Penetrating Keratoplasty

10.1 Abstract

Purpose

A cross sectional study using *in vivo* confocal microscopy to evaluate endothelial morphology and function, in relation to corneal pachymetry and clarity, in the short and longer term following penetrating keratoplasty (PKP).

Methods

Prospective, cross-sectional study design assessing two study groups: Group 1 subjects - 3 months following PKP (n = 20) and Group 2 subjects - 10 to 14 years (mean 12 years) following PKP (n = 16). For both groups *in vivo* confocal microscopy was performed to evaluate mean endothelial cell density (ECD), mean cell area (MCA), coefficient of variation for cell area (COVA), and the proportion of hexagonal cells. The Orbscan II slit-scanning topographer was used to measure corneal thickness, corneal curvature, horizontal corneal diameter, keratometric astigmatism and anterior chamber depth.

Results

For Group 1 subjects the mean ECD was 2425 cells/mm² (SD 564 cells/mm²), the MCA was 431 µm² (SD 106 µm²), the COVA was 39.2 (SD 9.1), and the mean percentage of hexagonal cells was 45% (SD 7.1%). For Group 2 subjects the mean ECD was 1394 cells/mm² (SD 156 cells/mm²), the MCA was 722 µm² (SD 82 µm²), the COVA was 56.3 (SD 12.3), and the mean percentage of hexagonal cells was 29% (SD 6%). In Group 2 the mean ECD was significantly less, the COVA was significantly greater, and the proportion of hexagonal cells

was significantly less compared to Group 1 ($p < 0.001$). Corneal thickness was significantly greater in Group 2 compared to Group 1 ($p = 0.04$).

Conclusion

In vivo confocal microscopy can be used successfully to study the corneal endothelium following PKP. The results of this study identify an apparent accelerated loss of endothelial cells in transplanted corneas that persists for several years following PKP. There is also a corresponding increase in cell size, variation in cell size, and variation in cell shape with time following PKP. An increase in corneal thickness was identified in the long term following PKP that likely reflects a decrease in corneal barrier and endothelial pump function.

10.2 Introduction

The endothelial cells of the human cornea are responsible for maintaining the normal, relatively dehydrated state of the tissue, thereby assuring corneal transparency.[54] This is achieved through the metabolic pump function of the endothelial cells, an active process that is controlled by Na^+/K^+ ATPase and involves the generation of a bicarbonate ion gradient across the corneal endothelium.[55-58]

The cornea is supplied with a relatively fixed number of endothelial cells at birth, and it is well-documented that there is a gradual decline in endothelial cell density (ECD) with advancing age.[4, 59, 60] Studies have indicated a minimum ECD of 400-500 cells/mm² is required to control stromal hydration and maintain corneal transparency.[16, 61] The decrease in ECD is associated with a corresponding increase in size variation (polymegathism) and shape variation (pleomorphism), as the remaining cells spread to maintain the barrier properties of the corneal endothelium.[61-63]

It has been demonstrated that endothelial cell loss may be exacerbated by certain corneal diseases and after intraocular surgery.[4, 64] Previous studies have reported that following penetrating keratoplasty (PKP) the rate of endothelial cell loss may be especially marked.[65, 66] This increased rate of decline in ECD may have a significant effect on long term corneal transplant survival.[5, 16, 61]

In this cross sectional study, *in vivo* confocal microscopy was used to evaluate endothelial morphology and function in the short and long term following PKP. As highlighted in the previous chapter, *in vivo* confocal microscopy is a powerful tool which allows observations of the living human eye at the cellular level (figure 10:1).[38] High *resolution en face* optical sections of the corneal endothelial layer are provided for analysis. A sophisticated quantitative image analysis system allows detailed assessment of the endothelial cell layer.[38] There have been few previous reports that use *in vivo* confocal microscopy to examine transplanted corneas in patients.[66] In this study *in vivo* confocal microscopy was used to evaluate ECD, mean endothelial cell area, variation in cell length and area, and cell shape ('percentage hexagonality') of recipient corneas following PKP.

Slit scanning topography was also used in this study to evaluate corneal thickness and corneal shape following PKP (figure 10:2). Corneal thickness is an important parameter that can be used to indirectly evaluate corneal barrier and endothelial pump function.[39] Other corneal parameters measured were anterior and posterior corneal curvature, horizontal corneal diameter, keratometric astigmatism, and anterior chamber depth.

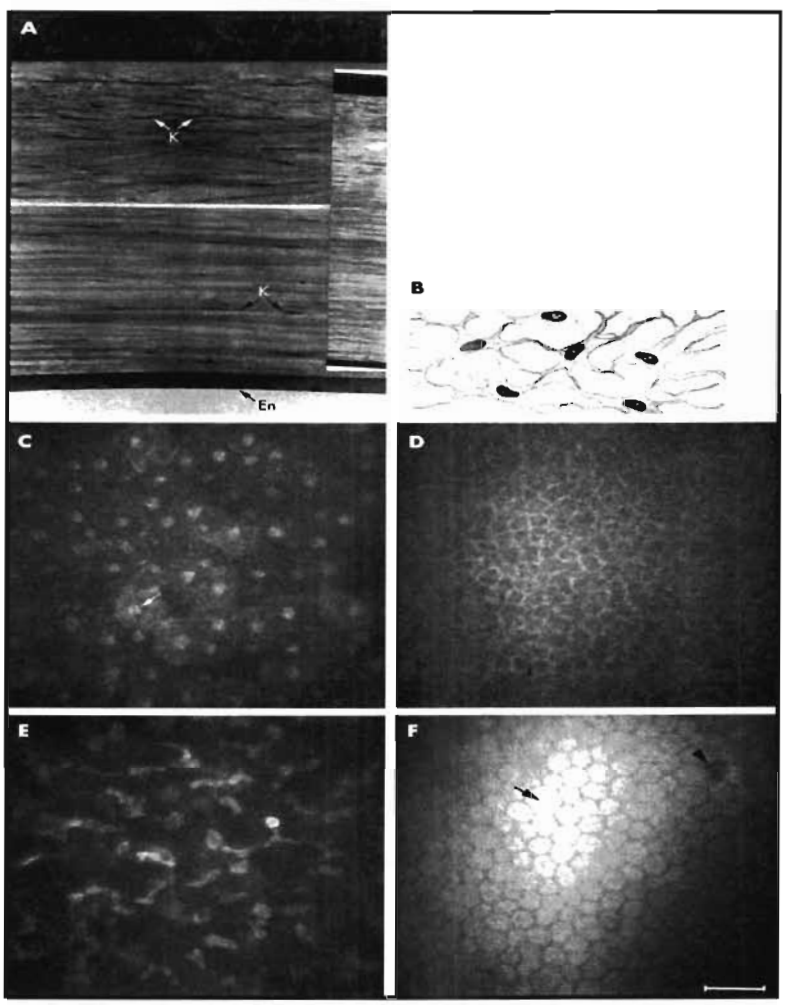


Figure 10:1 ***(A) Corneal histology: meridional section of the human anterior (top) and posterior (bottom) cornea stained with haematoxylin and eosin. Inset = full section. DM = Descemet's membrane; En endothelium; K = keratocytes. (B) Schematic representation of the human keratocyte network. (C-F) In vivo confocal microscopy of the central human cornea. Bar = 50 µm. (C) Surface epithelial cells and their nuclei (D) Basal epithelium. (E) Keratocyte nuclei in the anterior stroma. (F) Endothelium. Cell nuclei (arrow) and endothelial guttae (arrowhead) are occasionally visible.***

(From: Jalbert I, Stapleton F, Papas E, et al. In vivo confocal microscopy of the human cornea. British Journal of Ophthalmology 2003;87:225-236)

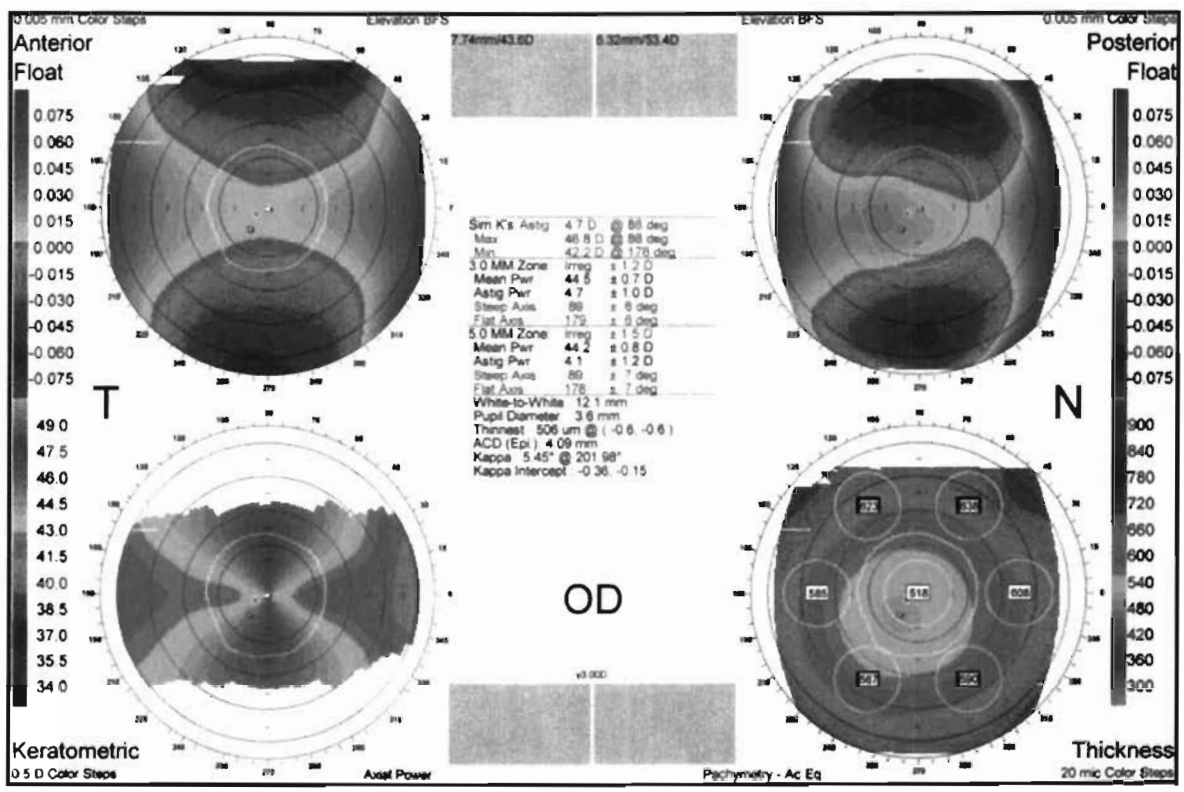


Figure 10:2 ***Standard Orbscan II topography system Quad map print out with anterior float map (top left), posterior float map (top right), keratometric map (bottom left) and pachymetry map (bottom right).***

10.3 Methods

10.3.1 Subjects

Group 1 subjects (short term outcome) were recruited from Auckland Public Hospital beginning in January 2004. All patients who were booked for PKP following standard clinical assessment were invited to participate in the study. Consecutive patients were approached until the sample size of 20 was reached. The demographic and clinic data of subjects is presented in Table 10:1. There was no difference from current standard practice in the pre-operative, surgical, and post-operative management of patients. All PKP's were performed by one of four consultant ophthalmologists who sub-specialise in corneal surgery. All clinical investigations for the study were performed 3 month post-operatively.

Group 2 subjects (long term outcome) were recruited using the New Zealand National Eye Bank (NZNEB) database. The NZNEB was established in 1991 and maintains an electronic database with information collected on all corneal transplant recipients. The NZNEB has supplied all donor tissue used by corneal surgeons at Auckland Public Hospital from 1991 onwards. The inclusion criterion for this study was 'all patients who underwent PKP for keratoconus at Auckland Public Hospital between 1991 and 1994 (corneal transplants between 10 and 14 years old)'. Exclusion criteria were: (1) patients not living in the Auckland geographical region, (2) patients who required regrafting, (3) patients who had additional surgical procedures in the grafted eye, and (4) patients with documented corneal transplant failure or irreversible rejection. A total of 68

patients met the required criteria and were invited to participate in the study. A final sample size of 16 transplanted corneas (15 subjects) was recruited. The mean post-operative time following PKP was 12 years (SD 1.7 years) and the range was 10 to 14 years. The demographic and clinical data of subjects is presented in Table 10:1.

	Group 1 Subjects	**Group 2 Subjects**
Total number	20	16
Age (yrs)		
Mean (SD)	46 (13)	47 (9)
Range	22 - 76	35 – 68
Gender		
Male	12	7
Female	8	8
Clinical indications for PKP	Keratoconus (n = 12) Viral keratitis (n = 3) Fuchs dystrophy (n = 2) Regraft (n = 2) Bullous keratopathy (n =1)	Keratoconus (n = 16)

Table 10:1 ***Demographic and clinical information for Group 1 and Group 2 subjects***

All donor corneal tissue was provided by the NZNEB. The NZNEB uses light microscopy accompanied by intravital staining with trypan blue to assess the endothelial layer. As noted in previous chapters, an ECD greater than or equal to 2500 cells/mm^2 is the threshold required for a cornea to be accepted for transplantation. Ethical approval for this study was obtained from the local

Auckland Ethics Committee and informed written consent was obtained from all subjects prior to their enrolment in the study.

10.3.2 Measurements

The Orbscan II slit scanning corneal topography system (Orbscan, Bausch and Lomb, Salt Lake City, UT, USA) was used to measure corneal thickness, corneal topography, horizontal corneal diameter and anterior chamber depth. Before measurement, the subject's head was aligned with the instrument and a head strap was placed around the back of the head. The subject was advised to keep both eyes open and fixate on the target. By viewing the live image of the eye on the monitor, the examiner aligned the two fixation markers reflected by the instrument on the corneal surface before performing the scan. Three scans were performed per cornea and the mean value for central cornea thickness, the horizontal corneal diameter, the spherical equivalent (using the least square method of determining the best fit sphere), the eccentricity of the anterior and posterior surface, mean keratometric astigmatism and anterior chamber depth was measured.

In vivo confocal microscopy of the cornea was performed using a slit-scanning technology (Confoscan 2, Fortune Technologies America, Greensboro, NC, U.S.A.).The subject was asked to fixate on a target, and the examination was performed with a 40× non-applanating, immersion lens that covers an area of approximately 0.1 mm^2. A drop of Viscotears (Carbomer 940 2 mg/g, CIBA Vision, Australia) on the objective lens served as an immersion and contact

substance. For all examinations, a standard setting of four passes was used, with a scanning range of between 700 µm and 800µm (throughout the z-axis). One exam was performed on the centre of each cornea and up to 300 images were obtained for each exam.

Based on the best visibility of endothelial cells, three representative frames from each scan were chosen for analysis. All captured images were analysed using the NAVIS (Nidek Advanced Vision Information System) proprietary software. The chosen frame size, or region of interest (ROI) was 0.035 mm^2. Using manually adjusted automated cell counts, as many clearly visible cells as possible were analysed within each frame. The values for ECD, mean cell area, coefficient of variation for area and length, and percentage of hexagonal cells within each of the three frames were recorded and the mean values for each cornea were calculated.

10.3.3 Statistical analysis

Statistical analysis was performed in consultation with a medical statistician from the Epidemiology Department of the University of Auckland. The SPSS V 12 software package was used. Descriptive statistics (mean, standard deviation, median and range) were calculated for each set of data. Other statistical methods used were the Fishers exact test to compare proportions between groups and the Student's *t* test to compare means between groups. The level of statistical significance was $P < 0.05$ unless stated otherwise.

10.4 Results

10.4.1 Short term outcome

The mean ECD was 2425 cells/mm² with a standard deviation (SD) of 564 cells/mm². The median ECD was 2532 cells/mm² and the range was 1536 to 3277 cells/mm². The mean endothelial cell area was 431 µm² (SD 106 µm²) with the range 305 to 653 µm². The coefficient of variation of cell area was 39.2 (SD 9.1). The mean percentage of hexagonal cells was 45% (SD 7.1%) with the range 25 to 58%.

The mean central corneal thickness was 560 µm (SD 50 µm) with the range 460 to 700 µm. The mean overall anterior corneal curvature was 8.3 mm (SD 0.9 mm), with the median 8.1 mm and range 6.3 to 10 mm. The mean anterior eccentricity was 0.27 (SD 0.31), indicating that the mean anterior cornea assessed followed the shape of a prolate ellipse (flattens peripherally). The mean posterior corneal curvature was 6.6 mm (SD 0.7 mm), with the median 6.5 mm and range 5.1 to 8.6 mm. The mean posterior eccentricity was 0.18 (SD 0.21) (prolate shape). The mean horizontal corneal diameter was 11.7 mm (SD 0.6 mm), the mean anterior chamber depth was 3.7 mm (SD 0.7mm), and the mean keratometric astigmatism was 6.0 D (SD 3.2 D).

10.4.2 Long term outcome

The mean ECD was 1394 cells/mm² (SD 156 cells/mm²). The median ECD was 1411 cells/mm² and the range was 1182 to 1712 cells/mm². The mean

endothelial cell area was 722 μm^2 (SD 82 μm^2) with the range 591 to 852 μm^2. The coefficient of variation of cell area was 56.3 (SD 12.3). The mean percentage of hexagonal cells was 29% (SD 6%) with the range 21 to 40%.

The mean central corneal thickness was 590 µm (SD 40 µm) with the range 530 to 670 µm. The mean overall anterior corneal curvature was 7.10 mm (SD 0.77 mm), with the median 7.0 mm and range 6.0 to 8.4 mm. The mean anterior eccentricity was 0.41 (SD 0.51) (prolate shape). The mean posterior corneal curvature was 6.6 mm (SD 0.7 mm), with the median 6.5 mm and range 5.1 to 8.6 mm. The mean posterior eccentricity was 0.40 (SD 0.43) (prolate shape). The mean horizontal corneal diameter was 11.7 (SD 0.5), the mean anterior chamber depth was 3.6 mm (SD 0.7 mm), and the mean keratometric astigmatism was 7.1 dioptres (SD 3.0 dioptres).

The mean ECD was significantly greater ($p < 0.001$) and the mean cell area significant less ($p < 0.001$) in Group 1 compared to Group 2. The coefficient of variation for cell area was significantly less in Group 1 compared to the Group 2 ($p < 0.001$). The mean percentage of hexagonal cells was significantly greater in Group 1 compared to Group 2 ($p < 0.001$). The mean corneal thickness was significantly less in Group 1 compared to Group 2 ($p = 0.04$). The mean anterior corneal curvature ($p = 0.01$) and posterior corneal curvature ($p = 0.02$) was significantly greater in Group 1 compared to Group 2. There was no significant

difference in horizontal corneal diameter (p = 0.46), anterior chamber depth (p = 0.5), or mean keratometric astigmatism (p = 0.2) between the groups.

10.5 Discussion

There have been few previous reports in the literature that describe the use of clinical corneal *in vivo* confocal microscopy to evaluate the endothelium of transplanted human corneas. In this study *in vivo* confocal microscopy was used to analyze the corneal endothelium following PKP. There were two study groups; one group included subjects who had recently undergone PKP and the second group included age-matched subjects that had undergone PKP several years previously. *In vivo* confocal microscopy was successfully completed in both groups, enabling detailed analysis of the corneal endothelium to be performed.

The confocal microscopy analysis demonstrated that in the longer term, following PKP (with an NZNEB minimal count of 2500 cell/mm^2), there was a significantly lower mean endothelial cell density and a corresponding significantly higher mean endothelial cell area compared to the short term following PKP. In addition, there was a significant increase in cellular polymegathism (reflected in a greater coefficient of variation for cell area) and cellular pleomorphism (reflected in the decreased proportion of hexagonal cells) in the long term. These results are in concordance with other published reports that have used specular microscopy to evaluate the corneal endothelium following PKP.[25, 65-71] The findings of this study and other published reports are consistent with a significantly higher rate of

endothelial cell loss and more rapid development of abnormal morphological features in transplanted corneas compared to normal corneas.[25, 65-71]

There are several factors that may influence ECD and endothelial cell loss following PKP. In the short term, the two most important factors are the quality of donor tissue preservation and the amount of trauma during surgery.[66] The adequacy of corneal storage can be assessed by comparing the ECD in the donor cornea after PKP to that before preservation.[72] Studies have identified that donor corneas lose 10% or more of their central endothelial cell population by 2 months after PKP.[73-77] This includes endothelial cells lost during the storage period, the transplantation, and the subsequent 2 months. This rate of cell loss was similar for both organ culture storage and preservation with Optisol GS at 4°C.[73-77]

Surgical trauma was previously thought to have a significant effect on ECD following PKP.[78, 79] Mechanical rubbing of the endothelium by the iris was considered the most likely reason.[66] Due to the introduction of viscoelastics, which prevent contact between the iris and the endothelium, surgical trauma is thought to be of lesser importance now.[66] Other potential factors include the occurrence of early cell-mediated rejection episodes and other post-operative complications.[65]

Previous reports have identified that in addition to the initial loss of endothelial cells from preservation and keratoplasty, the endothelial cell density continues to

decline for years following PKP at a higher than normal rate.[65, 66, 70] In normal eyes that have not undergone intraocular surgery, the rate of endothelial cell loss is 0.6% per year.[60, 80] Following cataract surgery it is 2.5% and following PKP it is reported to be greater than 4%.[70, 81, 82] In this current study the average rate of endothelial cell loss was calculated to be 4.3% per year. Assuming a critical ECD of 400-500 cells/mm² is required for corneal transplant function, it is estimated that the minimum donor ECD should therefore be 2000 cells/mm² for a corneal transplant to survive 20 years.[65]

The reason for the increased long term cell loss following PKP remains unknown. Although episodes of endothelial rejection are known to increase endothelial cell loss, the increased rate of cell loss is present in corneal transplants that are not known to have had an endothelial rejection episode.[70] Armitage et al[65] have postulated that there may be a common mechanism for long-term cell loss after cataract surgery and PKP. They postulate that disruption of the blood-ocular barrier and the subsequent inflammatory response causes in a pro-apoptotic environment in the anterior chamber that may potentiate endothelial cell loss. Li et al[83] also reported that there may be disruption of the control mechanisms that render endothelial cells less susceptible to apoptosis. Kaji et al[84] proposed an alternative mechanism, where accumulation of advanced glycation end products in Descemet's membrane affects the attachment of endothelial cells and may contribute to the loss of endothelial cells with age and following intraocular surgery.

Corneal thickness following PKP can be evaluated using a number of methods including ultrasound pachymetry, optical slit-lamp pachymetry and specular microscopy.[85-88] Although the majority of published reports use ultrasound pachymetry, in this study the Orbscan II corneal topography system was used to measure corneal thickness following PKP. Yaylali et al[89] reported that the relative accuracy and precision of the Orbscan system is similar to ultrasonic pachymetry, although they identified that the measurement with the Orbscan system was 23-28 µm greater than that observed by ultrasound pachymetry. Possible explanations for the observed difference included the Orbscan system being non-contact method, and that it may also measure the hydrated mucous gel covering the corneal surface.[89, 90]

This study identified that the mean central corneal thickness was significantly greater in the long term following PKP compared to the short term. This is consistent with the results of several longitudinal studies that have reported a gradual increase in corneal thickness with time following PKP (once the initial corneal oedema has resolved).[66, 67, 70, 71, 91] The increase in corneal thickness is thought to reflect the corresponding decrease in overall corneal barrier and endothelial function with time after PKP.[3, 39, 40, 66, 67, 70, 71, 91]

The Orbscan II system was able to measure several other corneal parameters including anterior and posterior corneal curvature, keratometric astigmatism, horizontal corneal diameter and anterior chamber depth. Both the anterior and

posterior corneal curvature was significantly greater in the short term following PKP compared to the long term. This may reflect the effect of sutures on corneal curvature in the early post-operative period. There was no significant difference with respect to the other corneal parameters between the short and long term groups.

10.6 Conclusion

This study demonstrated that *in vivo* confocal microscopy can be used successfully to study the corneal endothelium both in the short and long term following PKP with a donor minimum cell count of 2500cells/mm^2. This study demonstrates an early reduction to a mean of less than 2500 cells per mm^2 at three months post transplantation followed by an accelerated loss of endothelial cells in transplanted corneas, which appears to persist at a rate of approximately 4.3% per year for several years following PKP. There is also a corresponding increase in cell size, variation in cell size, and variation in cell shape with time following PKP.

This study also demonstrated that the Orbscan II topography system can be used to reliably measure corneal thickness and corneal shape following PKP and correlate this with ECD and other endothelial cell parameters. In concordance with other published reports, an increase in corneal thickness was identified in the long term following PKP, and reflects an overall decrease in corneal barrier and endothelial pump function.

Section 3 References

1. Shaw E, Rao G, Arthur E. The functional reserve of the corneal endothelium. Trans Am Acad Ophthalmol Otolaryngol 1978;85:640.

2. Kuwabara T. Current concepts in anatomy an histology of the cornea. Contact lens Intraoc Lens Med J 1978;4:101.

3. Waring G, Bourne W, Edelhauser H, et al. The corneal endothelium. Normal and pathologic structure and function. Ophthalmol 1982;89:531-90.

4. Bourne M, Kaufman H. Specular microscopy of human corneal endothelium in vivo. Am J Ophthalmol 1976;81:319-23.

5. Yee R, Matsuda M, Schultz R, et al. Changes in the normal corneal endothelial cellular pattern as a function of age. Curr Eye Res 1985;4:671-8.

6. Sturrock G, Sherrard E, Rice N. Specular microscopy of the corneal endothelium. Br J Ophthalmol 1978;62:809-14.

7. Cheng H, Jacobs P, McPherson K, et al. Precision of cell density and endothelial cell loss with age. Arch Ophthalmol 1985;103:1478-81.

8. Laing R, Sandstrom M, Berrospi A, et al. Changes in the corneal endothelium as a function of age. Exp Eye Res 1976;22:587-94.

9. Laule A, Cable M, Hoffman C, et al. Endothelial cell population changes of human cornea during life. Arch Ophthalmol 1978;96:2031-5.

10. Muller A, Craig J, Grupcheva C, et al. The effects of corneal parameters on the assessment of endothelial cell density in the elderly eye. Br J Ophthalmol 2004;88:325-30.

11. Muller A, Doughty M. Assessment of corneal endothelial cell density in growing children and its relationship to horizontal corneal diameter. Optom Vis Sci 2002;79:762-70.

12. Muller A. Assessment of eye growth related changes in the corneal endothelium of children and young teenagers. Glasgow Caledonian University PhD Thesis, 2000.

13. Doughty M, Muller A, Zaman M. Assessment of the Reliability of Human Corneal Endothelial Cell Density Estimates using a Noncontact Specular Microscope. Cornea 2000;19:148-58.

14. Chang S, Tsai I, Hu F, et al. The cornea in young myopic eyes. Brit J Ophthalmol 2001;85:916-20.

15. Rao S, Sen P, Fogla R, et al. Corneal Endothelial Cell Density and Morphology in Normal Indian Eyes. Cornea 2000;19:820-3.

16. Bigar F. Specular microscopy of the corneal endothelium: optical solutions and clinical results. Dev Ophthalmol 1982;6:1-94.

17. Wilson R, Roper-Hall M. Effect of age on the endothelial cell count in the normal eye. Br J Ophthalmol 1982;66:513-5.

18. Doughty M. Prevalence of "non hexagonal" cells in the corneal endothelium of young caucasian adults, and their inter-relationships. Ophthalmic Physiol Opt 1998;18:415-22.

19. Snellingen T, Rao G, Shrestha J, et al. Quantitative and morphological characteristics of the human corneal endothelium in relation to age, gender, and ethnicity in cataract populations of South Asia. Cornea 2001;20:55-8.

20. Johnston R, Hasany S, Rootman D. Endothelial cell analysis of corneas from donor eyes with an intraocular lens: a comparative study. Cornea 1997;16:531-3.

21. Ventura A, Walti R, Bohnke M. Corneal thickness and endothelial density before and after cataract surgery. Br J Ophthalmol 2001;85:18-20.

22. Azen S, Smith R, Burg K, et al. Variation in central and vertical corneal endothelial cell density in normal subjects. Acta Ophthalmol 1981;59:94-9.

23. Price N, Barbour D. Corneal endothelial cell density in twins. Br J Ophthalmol 1981;65:812-4.

24. Matsuda M, Yee R, Edelhauser H. Comparison of the corneal endothelium in an American and a Japanese population. Arch Ophthalmol 1985;103:68-70.

25. Inoue K, Kimura C, Amano S, et al. Corneal endothelial changes twenty years after penetrating keratoplasty. Jap J Ophthalmol 2002;46:189-92.

26. Sanchis-Gimeno J, Lleo-Perez A, Alonso L, et al. Anatomic study of the Corneal Thickness of Young Emmetropic Studies. Cornea 2004;23:669-73.

27. Marsich M, Bullimore M. The repeatability of corneal thickness measures. Cornea 2000;19:792-5.

28. Chakrabarti H, Craig J, Brahma B, et al. Comparison of corneal thickness measurements using ultrasound and Orbscan slit scanning topography in normal and post-LASIK eyes. J Cataract Refract Surg 2001;27:1-6.

29. Price F, Koller D, Price M. Central corneal pachymetry in patients undergoing laser in situ keratomileusis. Ophthalmol 1999;106:2216-20.

30. Cosar C, Sener A. Orbscan corneal topography system in evaluating anterior structures of the human eye. Cornea 2003;22:118-21.

31. Bron A, Creuzot-Garcher C, Goudeau-Boutillon S, et al. Falsely elevated IOP due to increased corneal thickness. Graefes Arch Clin Exp Ophthalmol 1999;237:220-4.

32. Korey M, Gieser D, Kass M, et al. Central endothelial cell density and central corneal thickness in ocular hypertension and primary open angle glaucoma. Am J Ophthalmol 1982;94:610-16.

33. Olsen T. Light scattering from human cornea. Invest Ophthalmol Vis Sci 1982;23:81-6.

34. Herse P, Yao W. Variation of corneal thickness with age in young New Zealanders. Acta Ophthalmol 1993;71:360-4.

35. Lam A, Douthwaite W. The corneal thickness profile in Hong Kong Chinese. Cornea 1998;17:384-8.

36. Olsen T. Corneal thickness and endothelial damage after intercapsular cataract extraction. Acta Ophthalmol 1980;58:424-33.

37. Rapuano C, Fishbaugh J, Strike D. Nine point corneal thickness measurements and keratometry readings in normal corneas using ultrasound pachymetry. Insight 1993;18:16-22.

38. Jalbert I, Stapleton F, Papas E, et al. In vivo confocal microscopy of the human cornea. Br J Ophthalmol 2003;87:225-36.

39. O'Neal M, Polse K. In vivo assessment of mechanism controlling corneal hydration. Invest Ophthalmol Vis Sci 1985;26:849-56.

40. Cheng H, Bates A, Wood L, et al. Positive correlation of corneal thickness and endothelial cell loss. Serial measurements after cataract surgery. Acta Ophthalmol 1988;106:920-2.

41. Burns R, Bourne W, Brubaker R. Endothelial function in patients with corneal guttata. Invest Ophthalmol Vis Sci 1981;20:77-85.

42. Erickson P, Doughty M, Comstock T, et al. Endothelial cell density and contact lens induced corneal swelling. Cornea 1998;17:152-7.

43. Amon M, Menapace R, Radax U, et al. Endothelial cell density and corneal pachymetry after no stitch, small incision cataract surgery. Doc Ophthalmol 1992;81:301-7.

44. Ravalico G, tognetto D, Palomba M, et al. Corneal endothelial function after extracataract cataract extraction and phacoemulsification. J Cataract Refract Surg 1977;23:1000-5.

45. Rufer F, Schroder A, Erb C. White to white corneal diameter: Normal values in healthy humans obtained with Orbscan II Topography System. Cornea 2005;24:259-61.

46. Hymes C. The postnatal growth of the corneaand palpebral fissure and the projection of the eyeball in early life. J Comp Neurol 1929;48:415-40.

47. Sorsby A, Benjamin B, Sheridan M. Refraction and its components during the growth of the eye from the age of three. London: Her Majesty's Stationary Office 1961.

48. Peter R. Uber die Korneagrosse und ihre Vererbung. von Graefes Arch Ophthalmol 1924;115:29-48.

49. Bao S. Measurement of horizontal corneal diameter in 717 preschool children. Chung Hua Yen Ko Tsa Chih 1981;17:286-8.

50. Bruckner R, Batschelet E, Hugenschmidt F. The Basal Longitudinal Study on aging: ophthalmo-gerontological research results. Doc Ophthalmol 1986;64:235-310.

51. Muller A, Doughty M, Wright L. Reassessment of the corneal endothelial cell organisation in children. Br J Ophthalmology 2000;84:692-6.

52. Muller A, Doughty M, Watson L. A retrospective pilot study to assess the impact of strabismus surgery on the corneal endothelium in children. Ophthalmic Physiol Opt 2002;22:38-45.

53. Padilla D, Sibayan S, Gonzales C. Corneal endothelial cell density and morphology in normal filipino eyes. Cornea 2004;23:129-35.

54. Stocker F. The endothelium of the cornea and its clinical implications. Trans Am Ophthalmol Soc 1953;51:669-78.

55. Hodson S. Evidence for a bicarbonate dependent sodium pump in corneal endothelium. Exp Eye Res 1971;11:20-9.

56. Maurice D. The location of the fluid pump in the cornea. J Physiol 1972;221:43-54.

57. Dikstien S, Maurice D. The metabolic basis of the fluid pumps in the cornea. J Physiol 1974;221:29-31.

58. Geroski H, Matsuda M, Yee R, et al. Pump function of the human corneal endothelium. Effect of age and corneal guttata. Ophthalmol 1985;92:759-63.

59. Carlson K, Bourne W, McLaren J, et al. Variations in human corneal endothelial cell morphology and permeability to fluorescein with age. Exp Eye Res 1988;47:27-41.

60. Moller-Pedersen T. A comparative study of human corneal keratocyte and endothelial cell density during aging. Cornea 1997;20:560-9.

61. Mishima S. Clinical investigations on the corneal endothelium. Am J Ophthalmol 1982;93:1-29.

62. Laing R, Sandstrom M, Berropsi A. Changes in the corneal endothelium as a function of age. Exp Eye Res 1976;22:587-94.

63. Murata T, Hinokuma R, Matsumoto K, et al. A clinical study in human corneal endothelium. Acta Soc Ophthalmol Jap 1979;81:319-23.

64. Bourne W, O'Fallon W. Endothelial cell loss during penetrating keratoplasty. Am J Ophthalmol 1978;85:760-6.

65. Armitage W, Dick A, Bourne W. Predicting endothelial cell loss and long term corneal graft survival. Invest Ophthalmol Vis Sci 2003;44:3326-31.

66. Bourne W. Cellular changes in transplanted human corneas. Cornea 2001;20:560-69.

67. Patel S, Hodge D, Bourne W. Corneal endothelium and postoperative outcomes 15 years after penetrating keratoplasty. Am J Ophthalmol 2005;139:311-19.

68. Dooren BV, Mulder P, Nieuwendaal C, et al. Endothelial cell density after posterior lamellar keratoplasty. Am J Ophthalmol 2004;138:211-7.

69. Vasara K, Setala K, Ruusuvaara P. Follow-up study of human corneal endothelial cells, photographed in vivo before enucleation and 20 years later in grafts. Acta Ophthalmol 1999;77:273-6.

70. Ing J, Ing H, Nelson L, et al. Ten-year postoperative results of penetrating keratoplasty. Ophthalmol 1998;105:1855-65.

71. Matsuda M, Bourne W. Long term morphological changes in the endothelium of transplanted corneas. Arch Ophthalmol 1985;103:1343-6.

72. Bourne W. The endothelial cell assay method for the evaluation of corneal preservation. In: Cavanagh H, ed. The Cornea. Transactions of the world congress on the cornea III. New York: Raven Press, 1988.

73. Borderie V, Kantelip B, Delbose B, et al. Morphology, histology, and ultrastructure of human C31 organ cultured corneas. Cornea 1995;14:300-10.

74. Hjortdal J, Ehlers N, Anderson C. Some metabolic changes during human corneal organ culture. Acta Ophthalmol 1989;67:295-300.

75. Pels E, Schuchard Y. Organ culture preservation of human corneas. Doc Ophthalmol 1983;56:147-53.

76. Bourne W, Nelson L, Maguire L, et al. Comparison of Chen Medium versus Optisol GS for human corneal preservation: results of transplantation. Cornea 2000;20:683-686.

77. Redbrake C, Salla S, Frantz A. Changes in human donor corneas preserved for longer that four weeks. Cornea 1998;17:62-5.

78. Dohlman C. Pathophysiology of graft failure. In: Ciba Foundation Symposium 15. Amsterdam: Assoc Scientific Publishers, 1973.

79. Bourne W, O'Fallon W. Endothelial cell loss during penetrating keratoplasty. Am J Ophthalmol 1978;85:760-7.

80. Bourne W, Nelson L, Hodge D. Central corneal endothelial cell changes over a ten year period. Invest Ophthalmol Vis Sci 1997;38:779-82.

81. Bourne W, Nelson L, Hodge D. Continued endothelial cell loss ten years after lens implantation. Ophthalmol 1994;101:1014-23.

82. Zacks C, Abbott R, Fine M. Long term changes in corneal endothelium after keratoplasty. A follow-up study. Cornea 1990;9:92-7.

83. Li X, DeMarco B, Mayhew E, et al. Aqueous humor borne factor upregulates Bcl-2 expression in corneal endothelial cells. Curr Eye Res 1998;17:970-8.

84. Kaji Y, Amano S, Usui T, et al. Advance glycation end products in Descemets membrane and their effect on corneal endothelial cells. Curr Eye Res 2001;23:469-77.

85. Remon L, Cristobal J, Castillo J, et al. Central and peripheral corneal thickness in full-term newborns by ultrasonic pachymetry. Invest Ophthalmol Vis Sci 1992;33:3080-3.

86. Argus W. Ocular hypertension and central corneal thickness. Ophthalmol 1995;102:1810-12.

87. Salz J, Azen S, Berstein J, et al. Evaluation and comparison of sources of variability in the measurement of corneal thickness with ultrasonic and optical pachymeters. Ophthalmic Surg 1983;14:1750-4.

88. Klyce S, Maurice D. Automatic reading of the corneal thickness in vitro. Invest Ophthalmol Vis Sci 1976;15:550-3.

89. Yayali V, Kaufman S, Thompson H. Corneal thickness measurements with the Orbscan topography system and ultrasound pachymetry. J Cataract Refract Surg 1997;23:1345-50.

90. Prydal J, Artal P, Woon H, et al. Study of human precorneal tear film thickness and structure using laser interometry. Invest Ophthalmol Vis Sci 1992;33:2006-11.

91. Seitz B, Langenbucher A, Nguyen N, et al. Graft endothelium and thickness after penetrating keratoplasty, comparing mechanical and excimer laser trephination: a prospective randomised study. Graefes Arch Clin Exp Ophthalmol 2001;239:12-7.

Section 4

Conclusions

Chapter 11

Conclusions

11.1 Introduction

The series of studies comprising this thesis was developed to answer a number of key inter-related questions with regard to eye banking and corneal transplantation in New Zealand.

1. To evaluate the source and management of corneal tissue procured by the New Zealand National Eye Bank.

2. To evaluate currents trends and ethnicity differences in indications for penetrating keratoplasty (PKP) in New Zealand.

3. To evaluate survival and visual outcome following penetrating keratoplasty in New Zealand.

4. To evaluate the indications and outcome of paediatric corneal transplantation in New Zealand.

5. To evaluate endothelial cell density in the normal eye and the effects of corneal parameters on its measurement.

6. To evaluate endothelial morphology and function in the short and long term following penetrating keratoplasty.

For the purposes of discussion each of the above areas will be reviewed in turn.

11.2 The source and management of corneal tissue procured by the New Zealand National Eye Bank

The purpose of this study was to evaluate donor demographics and source, donor tissue processing and storage, biological contamination, and the utilization and distribution of corneal tissue procured by the New Zealand National Eye Bank. A comparison with international methods of donor tissue processing and storage was performed.

Conclusions:

a) The New Zealand National Eye Bank (NZNEB) is the major source of donor tissue for corneal transplantation in New Zealand supplying at least 90% of all tissue.

b) The age distribution of donors in New Zealand is comparable to that reported internationally. The majority of donors were elderly and the mean age of donors is increasing each year.

c) Advanced donor age was not related to corneal suitability for transplantation. Donor age alone is not indicative of poor tissue quality and appropriately screened tissue from elderly donors may therefore be of use, particularly for recipients of similar age, although the NZNEB does not have a specific policy of age matching tissue.

d) There is a male preponderance of donors to the NZNEB and this is related to the higher prevalence of male deaths within the age range donors are accepted by the NZNEB. In particular, this reflects a high mortality rate from trauma and cardiovascular disease for young New Zealand males.

e) The major donor procurement source to the NZNEB was the Coroner's service followed by public hospitals and multi-organ donors. The supply from the Coroner's service is decreasing but that from public hospitals and multi-organ donors is increasing.

f) The causes of donor death in New Zealand are comparable to other eye banks. Cardiovascular disease followed by trauma and cerebrovascular disease were the most common causes of donor death. Donors that died of cardiovascular disease or cerebrovascular disease were more likely to have corneas suitable for transplantation, which may be due to the higher proportion of donors in these groups who died suddenly with less co-morbidity.

g) The change from hypothermic to organ culture storage as standard NZNEB policy has significantly increased donor tissue storage duration. The advantages of increased storage duration to the NZNEB have included the following: improved corneal tissue utilization; the performance of corneal transplantation as a planned elective surgical procedure; greater reserve of tissue for emergency procedures; and additional time for improved microbiological and serological screening, and tissue compatibility matching if required.

h) Microbiological screening and donor tissue decontamination is a priority of the NZNEB. Biological contamination has steadily and significantly decreased over the last decade. Reasons for this improvement include: the change to organ culture; improved facilities and equipment; and more experienced and technically skilled staff.

i) The most common bacterial contaminant was coagulase-negative staphylococci followed by *Staphylococcus aureus*, and the most common fungal contaminant was *Candida* sp. This was in concordance with reports from other eye banks.

j) The corneal utilization rate of the NZNEB is higher that that reported by other eye banks and is increasing each year. This is due to the thorough pre-screening of potential donors (for age and medical contraindications) prior to the procurement of their tissue. This is made possible by the close relationship between the NZNEB and its donor sources. The most common reasons for discarding tissue were microbiological contamination, abnormal serology, and failed endothelial assessment.

This study provided important information in relation to eye banking in New Zealand. In particular, significant trends were identified with respect to donor demographics, donor procurement source, improved donor tissue processing and storage, decreased biological contamination, and increased utilization of corneal tissue.

11.3 Current trends and ethnicity differences in indications for penetrating keratoplasty in New Zealand

In this study the NZNEB database was analyzed with respect to clinical indications for PKP in New Zealand. The results from an earlier NZNEB study were used for comparison to allow current trends in indications to be identified. Ethnicity differences in indications for PKP were also analysed.

Conclusions:

a) Keratoconus remains the most common indication for PKP in New Zealand, accounting for a significantly greater proportion of PKPs than other published reports. Keratoconus was the most common indication in all ethnicity groups.

b) PKP for keratoconus was particularly high in the Maori and Polynesian communities of New Zealand. Maori and Polynesian patients were also significantly younger at the time of PKP for keratoconus. This suggests that there may be a higher prevalence, disease severity, and/ or more rapid progression of keratoconus in New Zealand, particularly amongst the Maori and Polynesian populations. A high familial rate and a higher prevalence of asthma, atopic disease and eye rubbing are likely to be important contributing factors.

c) Pseudophakic or aphakic bullous keratopathy is the second most common indication for PKP in New Zealand. A decreasing trend was identified that was in concordance with other published reports. Improvements in cataract

surgery technique, intraocular lens technology, use of viscoelastics, and a reduction in anterior chamber intraocular lens use have all been cited as reasons for the decreasing trend.

d) Regraft remains the third most common indication for PKP in New Zealand. In concordance with other published reports there was an increasing trend identified. It is likely that the gradually expanding pool of PKP recipients has led to a corresponding increase in the number of regraft procedures being performed in New Zealand.

e) Fuchs' endothelial dystrophy is the fourth most common indication for PKP, with an increasing trend identified. PKP for Fuchs' dystrophy was significantly more common in females. Almost all PKP's for Fuchs' dystrophy were performed in Caucasian Europeans with no cases reported in Maori or Polynesian populations.

f) Viral keratitis is the fifth most common indication for PKP with a decreasing trend identified. The most likely reason for this is the significant advances in the medical management of herpetic eye disease. However, Maori were over-represented in the ethnicity distribution of PKP for viral keratitis. This possibly reflects poorer medical management in this group rather than a greater prevalence of disease.

This study revealed significant trends in relation to indications for penetrating keratoplasty in New Zealand. These trends are likely to continue and will result in significant changes in indications for PKP in the future. This study identified important differences in indications based on ethnicity background, highlighted by

the particularly high incidence of PKP for keratoconus in the Maori and Polynesian populations.

11.4 The survival and visual outcome of penetrating keratoplasty in New Zealand

The purpose of this study was to evaluate potential donor, recipient, surgical and post-operative factors that may influence survival and visual outcome of penetrating keratoplasty in New Zealand.

Conclusions:

a) The overall one-year survival rate identified was comparable to that of other published reports. Irreversible rejection followed by primary endothelial failure and vascularisation were identified as the most common reasons for failure of the corneal transplant. Overall visual outcome was also similar to that of other published reports, with 60% of recipients achieving a one-year post-operative BCSVA of 6/18 or better.

b) Donor factors (donor age, donor source, donor cause of death, death to preservation interval, endothelial cell density, donor lens status, and storage duration) were not significantly associated with decreased corneal transplant survival or visual outcome in this study. This confirms what has previously been reported with respect to donor-related risk factors.

c) Pre-operative diagnosis was significantly associated with survival outcome. Keratoconus and Fuchs' endothelial dystrophy have the best survival outcome, while trauma and regraft have the poorest survival outcome. For regraft, survival rate further decreased according to the number of previous grafts.

d) Pre-operative diagnosis was significantly associated with visual outcome. Keratoconus and Fuchs' endothelial dystrophy were associated with the most successful visual outcome. In contrast, non-infective keratitis, bullous keratopathy and trauma were identified as independent risk factors for poor visual outcome.

e) Pre-existing vascularisation of the recipient cornea was identified as a significant independent risk factor for decreased corneal transplant survival. Furthermore, the decrease in survival rate was proportional to the number of quadrants of pre-existing vascularisation.

f) The presence of active anterior segment inflammation at the time of PKP was identified as one of the most significant independent risk factors for decreased corneal transplant survival. This is in concordance with other published reports. This study also confirmed that a history of pre-operative glaucoma is a significant independent risk factor for decreased corneal transplant survival.

g) After accounting for the differences in pre-operative diagnosis between age groups, this study identified no significant decrease in survival rate with advancing recipient age. Again this is in concordance with other studies that

report no association or only a marginal association between advancing recipient age and survival rate. There appears to be no association between recipient gender and survival rate.

h) Recipient factors that were identified as independently associated with poor visual outcome were pre-existing vascularisation, active anterior segment inflammation at the time of PKP, a history of pre-operative glaucoma, and advancing recipient age.

i) Both very small and large graft sizes were identified as independent risk factors for decreased corneal transplant survival. The literature reports contradictory findings with respect to graft size and corneal transplant survival.

j) The performance of additional surgical procedures at the time of PKP was analysed. Anterior vitrectomy at the time of PKP significantly decreased survival rate at the univariate level but the effect was no longer apparent in multivariate analysis. The majority of published reports identify anterior vitrectomy to have a significant effect on survival rate. In concordance with other published reports, the performance of additional lens surgery was not significantly associated with decreased corneal transplant survival in this study.

k) This study confirmed the association previously recognized between post-operative episodes of reversible rejection and decreased corneal transplant survival. Only one episode of reversible rejection was required to

significantly decrease survival rate. The survival rate further decreased with increasing episodes of reversible rejection.

The large and comprehensive New Zealand National Eye Bank database has enabled detailed statistical analysis of a large series of PKPs to be performed, with several independent risk factors identified that significantly influenced survival and visual outcome. This information will be invaluable to patients and surgeons with respect to determining prognosis and clinical decision making.

11.5 Paediatric corneal transplantation in New Zealand

The purpose of this study was to evaluate patient characteristics, indications, surgical details, and outcome of paediatric keratoplasty in New Zealand.

Conclusions:

a) Paediatric keratoplasty accounts for 3% of all corneal transplants performed in New Zealand. There were significantly more males undergoing paediatric keratoplasty compared to females.

b) Indications for paediatric keratoplasty were divided in to congenital (16%), acquired non-traumatic (74%), and acquired traumatic conditions (10%). The most common congenital indications were Peters' anomaly and congenital hereditary endothelial dystrophy. The most common acquired non-traumatic indication was keratoconus followed by viral keratitis. The most common acquired traumatic indication was penetrating trauma.

c) The indications for paediatric keratoplasty in New Zealand differed significantly from that reported in the literature, in which congenital indications account for a significantly greater proportion. Of particular note was that keratoconus accounted for 67% of paediatric keratoplasties in New Zealand compared to 0-11% in other published reports.

d) Penetrating keratoplasty accounted for almost all corneal transplants in the paediatric age group (95%) with lamellar keratoplasty accounting for the remainder.

e) The survival rate for paediatric keratoplasty was 82% at one year post-operatively. The survival rate for acquired conditions was significantly greater than that for congenital conditions. This was in concordance with other published reports. Factors associated with decreased survival rate in the literature included performance of an additional surgical procedure at the time of keratoplasty, pre-operative glaucoma, associated ocular conditions and corneal vascularisation.

f) The visual outcome for paediatric keratoplasty is significantly better when performed for acquired compared to congenital indications. A higher prevalence of amblyopia and associated ocular abnormalities in the congenital group has been cited as the reason for the less successful visual outcome in this group.

Analysis of the NZNEB database provided valuable information in relation to paediatric keratoplasty in New Zealand. In particular, this study highlighted an unusually high prevalence of keratoconus as an indication for keratoplasty. In

addition, a high one-year survival rate and good visual outcome was identified, especially in cases of keratoplasty for acquired conditions.

11.6 The evaluation of endothelial cell density in the normal eye and the effects of corneal parameters on its measurement

The possible impact of corneal parameters on ECD in the normal eye has largely been ignored. The effects of corneal parameters on ECD in children and in the elderly eye have previously been reported, but no studies have analysed the effects in normal young adults. The effect of corneal thickness, corneal diameter and corneal curvature on ECD in normal young adults was therefore studied.

Conclusions:

a) The mean value for ECD was in concordance with that previously reported for young adults. When compared with mean ECD values reported for children and for elderly eyes, a clear trend of decreasing mean ECD with advancing age is observed. Consistent with the majority of other published reports, there was no significant difference in mean ECD between males and females.

b) Central corneal thickness (CCT) was identified to have an association with ECD in young adults, with lower ECD values apparent in thinner corneas. This has been previously reported in growing children and in studies involving the elderly eye.

c) Horizontal corneal diameter (HCD) did not have a significant effect on the measured ECD in young adults. This is in concordance with studies involving the elderly eye. HCD has previously been reported to be an important determinant of ECD measurement in children, with larger diameters resulting in lower ECD values.

d) The effect of corneal curvature on the measurement of ECD is yet to be clearly established. This study identified no significant correlation, although a previous study involving elderly eyes reported a significant correlation, with lower ECD values expected in steeper corneas. No previous reports involving children were identified.

e) Percentage hexagonality is another indicator of the health of the corneal endothelium and was assessed in this study. There was no significant relationship identified between percentage hexagonality and the corneal parameters assessed.

The results of this study, and those of other published reports, indicate that corneal thickness may have a significant association with ECD throughout life. It also appears that horizontal corneal diameter may have an important effect on ECD in children but not in adults. The influence of corneal curvature remains largely unknown and further investigation is required.

8.7 The evaluation of endothelial morphology and function in the short and long term following penetrating keratoplasty

There have been few previous reports that describe the use of clinical corneal confocal microscopy to evaluate the endothelium of transplanted human corneas. In this study confocal microscopy was used to analyze the corneal endothelium in the short and long term following penetrating keratoplasty (PKP). The Orbscan II corneal topography system was used to assess corneal thickness in the short and long term following PKP.

Conclusions:

a) The study demonstrated that clinical confocal microscopy and Orbscan slit scanning topography can be successfully performed and used as research tools in both the short and long term following PKP.

b) The *in vivo* confocal microscopy analyses demonstrated that in the longer term following PKP the mean ECD was significantly lower and the mean endothelial cell size significantly greater compared to the short term following PKP. There was also a significant increase in cell size and shape variation in the long term following PKP. These findings suggest increased instability of the corneal endothelium with time following PKP.

c) A significantly higher rate of endothelial cell loss and more rapid development of abnormal endothelial cells appear to occur in transplanted corneas compared to normal corneas.

d) The mean central corneal thickness was significantly greater in the long term following PKP compared to the short term. This is consistent with the results of several longitudinal studies that have reported a gradual increase in corneal thickness with time following PKP (once the initial corneal oedema has resolved). The increase in corneal thickness is thought to reflect the corresponding decrease in overall corneal barrier and endothelial function with time after PKP.

This study demonstrated that confocal microscopy and slit scanning topography can be successfully used to analyze endothelial morphology and function in the short and long term following PKP. The results of this study, in concordance with other published reports, identified an accelerated loss of endothelial cells and more rapid development of abnormal endothelial cells in transplanted corneas.

Section 5

Appendices

Appendix 1: Papers and presentations from this thesis

Papers

1. Patel HY, Brookes NH, Moffatt LS, Sherwin T, Ormonde S, McGhee CNJ. The New Zealand National Eye Bank Study 1991-2003: A Review of the Source and Management of Corneal Tissue. *Cornea* 2005;24(5):576-582

2. Patel HY, Ormonde S, Brookes NH, Moffatt LS, McGhee CNJ. The Indications and Outcome of Paediatric Corneal Transplantation in New Zealand: 1991-2003. *Br J Ophthalmol* 2005;89(4):404-8

3. Patel HY, Ormonde S, Brookes NH, Moffatt LS, Sherwin T, McGhee CNJ. The New Zealand National Eye Bank 1993-2001: Survival and visual outcome one-year following corneal transplantation. (Submitted *Cornea*)

4. Patel HY, Ormonde S, Brookes NH, Moffatt LS, Sherwin T, McGhee CNJ. Clinical indications for corneal transplantation in New Zealand – recent update on trends over last 4 years compared to data 1991-99. (Submitted *Cornea*).

5. Patel HY, Patel DV, McGhee CNJ. The effects of corneal parameters on the assessment of endothelial cell density in normal young adults. (Submitted *Br J Ophthalmol*).

Presentations

1. A review of the source and management of donor corneal tissue in New Zealand. *Presented at RANZCO New Zealand branch meeting 2004.*

2. Paediatric corneal transplantation in New Zealand 1991-2003. *Presented at RANZCO New Zealand branch meeting 2004.*

3. Survival and visual outcome following corneal transplantation in New Zealand. *Presented at RANZCO annual scientific conference Melbourne 2004.*